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Nitrogen enrichment during decomposition of mangrove leaf litter in an east African coastal lagoon (Kenya): Relative importance of biological nitrogen fixation

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Abstract. *In situ* decomposition of senescent leaves of two abundant mangrove species (*Rhizophora mucronata* Lamarck and *Ceriops tagal* (Perr) C.B. Rob), enrichment of nitrogen and activity of dinitrogen fixing bacteria during decomposition were investigated during both rainy and dry seasons in a tropical coastal lagoon (Gazi, Kenya). Rates of leaf decomposition were higher for *R. mucronata* than for *C. tagal* and were highest, for both species, during rainy season. Rates of decomposition, expressed as percentage dry mass loss, over a decomposition period of 50 days was: *C. tagal* (rainy season), 69%; *C. tagal* (dry season), 27%; *R. mucronata* (rainy season), 98%; and *R. mucronata* (dry season), 48%. High rainfall and diurnal tidal inundation appear to enhance the leaf decomposition process. Maximum rates of nitrogen fixation were 380 nmol N_2 h^{-1} g^{-1} dw for *C. tagal* (rainy season), 78 nmol N_2 h^{-1} g^{-1} dw for *C. tagal* (dry season), 390 nmol N_2 h^{-1} g^{-1} dw for *R. mucronata* (rainy season) and 189 nmol N_2 h^{-1} g^{-1} dw for *R. mucronata* (dry season). Although N_2 fixation rates were highest during rainy season, total nitrogen immobilised in the leaves was highest during the dry season. Biological nitrogen fixation can account for between 13 to 21% of the maximum nitrogen immobilised in the decaying mangrove leaves. Nitrogen fixation, as a source of allochthonous nitrogen, sustains a nitrogen input to the mangrove ecosystem, which adds significantly to the nitrogen input through leaf litterfall.

Introduction

Mangroves are important sites of primary production and contribute considerable quantities of organic matter, primarily as leaves, to adjacent waters and sediments (Benner et al. 1986). Only a small percentage of mangrove leaves is consumed directly by terrestrial grazing animals (Lee et al. 1990), whereas mangrove detritus constitutes a large carbon reservoir potentially available to the estuarine food web (Benner & Hodson 1985; Tam et al. 1990). The pioneering work of W.E. Odum and E.J. Heald in southern Florida in the late 1960's documented the importance of detritus-based food chains for

near shore secondary production in mangrove-dominated estuaries (Odum & Heald 1975; Robertson et al. 1992).

The C:N ratio of mangrove litter has been considered as an indicator of its nutritional value (Fell et al. 1984). Furthermore it has been well documented that the nutritional value of mangrove litter increased through nitrogen enrichment during microbial decomposition (Fell et al. 1984; Newell et al. 1984; Van der Valk & Attiwill 1984; Mann & Steinke 1992). Microbial carbon respiration can contribute to the relative nitrogen enrichment of plant litter as suggested by Morris & Lajtha (1986). White & Howes (1994) estimated that the microbial biomass can contribute 20 to 25% of the leaf litter nitrogen of *Spartina*. In addition, the following mechanisms for nitrogen accumulation in detritus were proposed (Rice 1982): (1) after initial leaching from fresh detritus, microbial enzymes depolymerize the detritus substrate producing reactive carbohydrates, phenols, small peptides, and amino acids; (2) the carbohydrates and phenols condense with polypeptides and amino acids to produce nitrogenous geopolymers; (3) microbes associated with detritus assimilate nitrogen from an external source for protein (including exoenzyme) synthesis; (4) exoenzymes exuded by microbes condense with reactive carbohydrates and phenols in the humifying detritus. If the condensation products are not easily solubilized in sea water, the detritus particle becomes enriched with humic nitrogen. A central question is: what are the possible significant sources of external nitrogen for leaf litter nitrogen enrichment? One of the sources of external nitrogen to leaf detritus can be the water column (Rice & Tenore 1981; Rice 1982; Steinke & Charles 1986). In addition, biological nitrogen fixation has been suggested as a possible source of nitrogen (Fell et al. 1984; Van der Valk & Attiwill 1984; Twilley et al. 1986; Boto & Robertson 1990). Several authors have reported the presence of different types of nitrogen-fixing micro-organisms in sediments and decaying leaves of mangroves (Zuberer & Silver 1978; Mann & Steinke 1992). Gotto and Taylor (1976) attributed two thirds of nitrogenase activity associated with decomposing *R. mangle* leaves to photosynthetic bacteria and non-heterocystous bluegreen algae. Facultative or strictly anaerobic heterotrophic bacteria account for the remaining nitrogenase activity.

Using natural carbon isotope abundance Rao et al. (1994) showed that mangrove leaves represent a major source of organic carbon to the mangrove sediments of Gazi. Moreover, they observed a tenfold decrease in C:N ratio from senescent leaves (C:N = 200) to the sediments (C:N = 22), suggesting significant nitrogen enrichment during leaf decomposition. Hemminga et al. (1994) observed that seston from Gazi lagoon consists largely of mangrove derived particulate organic matter having a low C:N ratio (6.5 to 10) relative to senescent mangrove leaves, and they suggest that the mangrove derived

organic particles have gone through intensive processing during outwelling from the mangrove and trapping in the seagrass zone.

The studies of Rao et al. (1994) and Hemminga et al. (1994) have emphasized the importance of mangrove derived detritus in Gazi lagoon and stress the need for assessing the role and fate of mangrove litter in the lagoonal ecosystem. The aims of the present study were to quantify nitrogen enrichment during leaf decomposition, to assess the contribution of biological nitrogen fixation (as a microbial pathway of incorporating inorganic nitrogen from the atmosphere), to assess the seasonal variability of these processes and to quantify the nitrogen input through mangrove litterfall into the lagoonal ecosystem.

Material and methods

Site description

Gazi is situated 50 km south of Mombasa, Kenya (Figure 1). Large parts of the lagoon are covered with seagrass beds and a mangrove swamp of 6.6 km^2. Two major tidal creeks are leading out of the mangrove forest and only the western creek receives fresh water from the river Kidogoweni. Eight mangrove species are found, with dominant species being *Rhizophora mucronata* locally mixed with *Bruguiera gymnorrhiza* and *Ceriops tagal*. Stands of *Sonneratia alba* and *Avicennia marina* are found along the creek fringes (Gallin et al. 1989). On higher areas, *Ceriops tagal* appears in monospecific stands (Hemminga et al. 1994; Rao et al. 1994). The area is influenced by two monsoonal regimes: from October to March by the northeast monsoon and from March to October by the southeast monsoon (McClanahan 1988). Rainfall is highest in the period from May to July during southeast monsoon (Table 1 and McClanahan 1988; Ruwa 1993).

Two fieldwork plots (20 m by 20 m) were selected to conduct litterfall measurements and decomposition experiments. The plots were chosen amidst vegetation zones representative of the Gazi mangrove. The first one is a pure *Ceriops tagal* plot developed on sandy sediment and inundated only during spring tide events. These events extend over six days and occur twice a month. The second plot is located within a pure *Rhizophora mucronata* vegetation zone developed on muddy sediment and is inundated twice a day. The tidal amplitude is approximately 3 m. The location of both plots is shown in Figure 1.

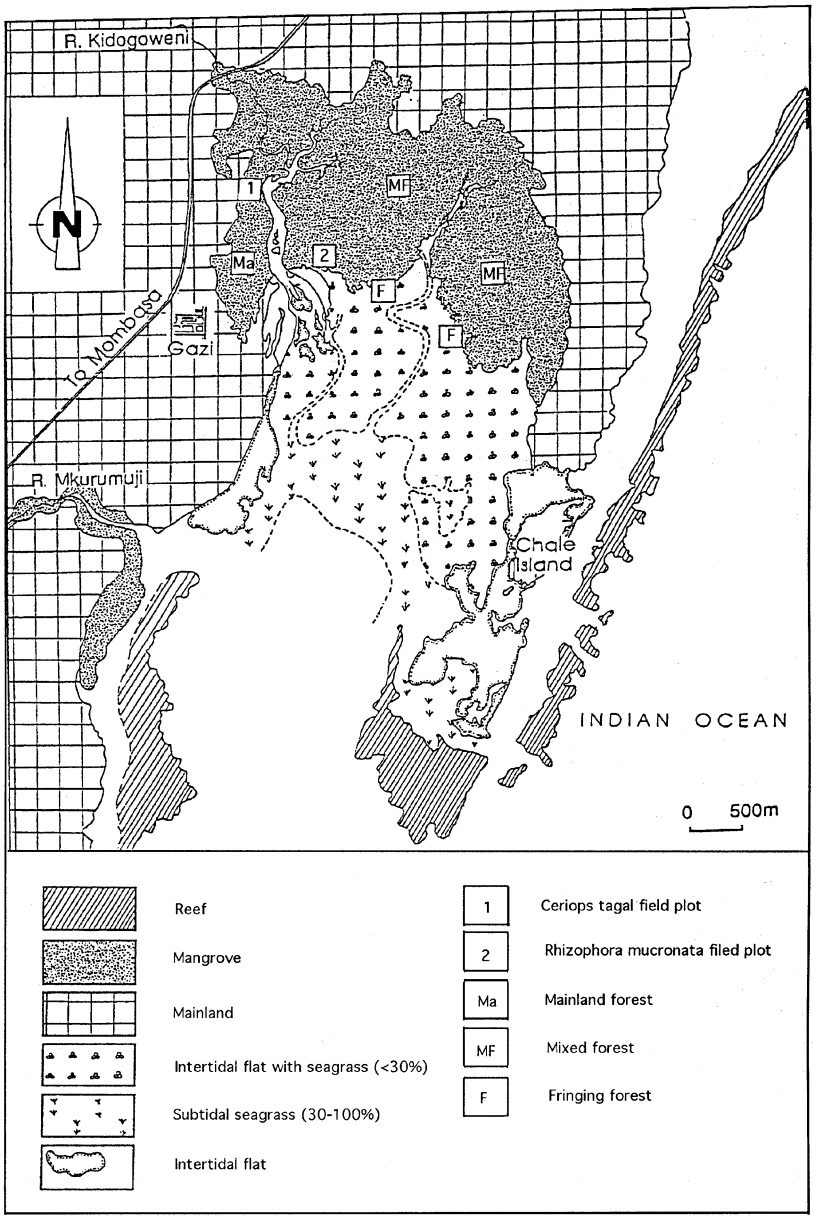

Figure 1. Map of the Gazi Bay area, adapted from Slim & Gwada 1993.

Leaf decomposition

Mangrove leaf decomposition was studied with the litter bag technique (Brock 1984; Newell et al. 1984). Senescent leaves of *R. mucronata* and *C. tagal*

Table 1. Mean monthly temperature (°C) and rainfall (mm) data of Mombasa meteorological station for the southeast monsoon period in 1991 and 1992 (B. Ohowa, personal communication).

Months	Temp. 1991 °C	Temp. 1992 °C	Rainfall 1991 mm	Rainfall 1992 mm
May	25.8	25.7	468	230
June	24.7	24.7	133	74
July	24.2	23.4	112	127
November	26.8	–	52	–
December	27.2	–	59	–
January	–	27.4	–	2
February	–	28.1	–	0

–: no data

(yellow leaves still attached to branches but ready to abscise) were collected in the two field plots, air dried for 7 days in the laboratory, and subsequently cut into cross halves. Three half *Rhizophora* leaves or five half *Ceriops* leaves were weighed to within 0.001 g (leaf weights ranged between 1.11 and 4.11 g) and packed into nylon litter bags (10 cm × 20 cm) with mesh size of 2 mm. This mesh size is too small for significant loss of small leaf particles but is large enough to allow for microbial colonisation and passage of small benthic invertebrates (Gallardo & Merino 1992). The remaining leaf halves were kept for analysis of initial C and N content. In the selected *Rhizophora* and *Ceriops* plots, 36 to 54 litter bags were tied with nylon rope to the aerial roots of the trees. Empty litter bags were set out as well to serve as blanks for the nitrogen fixation measurements.

A first series of experiments was conducted during the rainy season (May till July 1991), and a second one during the dry season (December 1991 till February 1992). A third experiment was designed to investigate differences between *Rhizophora* leaves that were continuously submerged in the creek and samples kept in the *Rhizophora* plot and affected by daily tidal inundation. Finally, a fourth experiment was carried out during rainy season (June 1992) in the *Rhizophora* plot to study leaf decomposition during the first 15 days with a higher time resolution.

Triplicate litter bag samples were collected randomly, together with one blank sample, at different time intervals from day 1 till the end of the experiments. In the Mombasa laboratory they were kept one night in the dark, at laboratory temperature (25 °C) and humidity before being assessed for nitrogen fixation. Prior to nitrogen fixation measurements, the litter bags were gently washed with tap water to remove attached sediment particles (Newell et al. 1984; Twilley et al. 1986). After nitrogen fixation measurements, leaves were

removed from the litter bags, dried at 80 °C to constant weight, weighed and stored for later C and N analysis.

C and N content analysis

The dry leaves were ground into fine powder after pre treatment with liquid nitrogen to increase their brittleness. Total organic carbon and nitrogen were measured with a Carlo Erba NA 1500 elemental analyser on weighed aliquots of approximately 3 mg. Acetanilide (C = 71.03%, N = 10.36%) was used for standardization.

Nitrogen fixation measurements

Nitrogen fixation activity was measured according to the acetylene reduction technique of Hardy et al. (1968). Within 20 hours after sampling the triplicate litter bags were incubated together in the same gas tight plexiglas enclosure (diameter, 20 cm; height, 25 cm), fitted with a gas sampling device. Incubations were done at 25 °C, in aerobiose. With each run, a blank sample (litterbag without leaves) was incubated separately. Acetylene was injected into the enclosures to a final concentration of 10% in volume. Ethane (100 μl) was injected as an internal standard. Reduction of acetylene into ethylene was monitored during 8 h with hourly analyses of 100 μl gas samples using a Varian model 3300 gas chromatograph. Peak areas were measured with a Varian model 4400 integrator. Acetylene, ethylene and ethane were calibrated using a mixture of pure calibration gases. The acetylene reduction rates were converted to rates of nitrogen fixation using the theoretical conversion factor of three acetylene molecules for one N_2 molecule (Turner & Gibson 1980).

Statistics

Statistical analysis through one way ANOVA at 95% confidence interval and further analysis through Fisher PLSD (Protected Least Square Design) and Scheffe F-test were performed by means of a Macintosh computing package “Statview II”.

Results

Leaf decomposition

The coefficient of variation on the average dry mass loss for triplicate samples varied during the dry season from 1 to 39%, and during the rainy season from

Table 2. Decomposition of *Ceriops tagal* leaves during the rainy season (May to July 1991). Dry mass remaining (%); No = original nitrogen content and Nt = nitrogen content at sampling time (mg g^{-1} dry mass); C:N atomic ratios; N immobilised (%) and nitrogen fixation (nmol N_2 h^{-1} g^{-1} dry mass).

Sampling (days)	6	13	19	25	32	39	47
DM remaining (%)*	52	50	66	46	41	56	31
$\pm\ \sigma$	26	28	13	10	2	1	13
No (mg g^{-1})*	1.67	1.90	2.40	2.30	2.30	1.74	2.27
$\pm\ \sigma$	0.21	0.62	0.42	0.40	1.21	0.52	0.76
Nt (mg g^{-1})*	3.90	4.43	3.06	4.43	4.70	4.84	4.30
$\pm\ \sigma$	0.78	0.58	0.12	0.29	0.78	0.59	0.38
C:No	290	267	205	214	268	296	233
$\pm\ \sigma$	35	80	38	32	193	103	110
C:Nt	141	112	148	115	117	98	100
$\pm\ \sigma$	26	6	14	4	25	19	25
N immob. (%)	121	117	84	89	84	134	59
N-fixation nmol N_2 h^{-1} g^{-1}	4	–	132	89	380	59	69

–: no data
*: average value for 3 litterbags

2 to 100% (Tables 2, 3, 4, 5). The 100% variability is observed for *Rhizophora* leaves and results from the very rapid loss of initial material (76% dry mass loss after 10 days, Table 4). In the rainy season (May to July 1991) we observed that the dry mass of *C. tagal* and *R. mucronata* decreased by 69% and 98% in 50 days respectively (Tables 2 and 4). In the dry season (December 1991 to February 1992) the leaves of *C. tagal* and *R. mucronata* lost respectively 27% and 48% of their dry mass in 50 days (Tables 3 and 5). During both seasons, decomposition rates of *R. mucronata* leaves were significantly higher ($p < 0.05$) than decomposition rates of *C. tagal*.

The decomposition rates of *R. mucronata* tidally submerged leaves kept on the forest floor, were significantly higher ($p < 0.05$) than leaves continuously submerged (Figure 2). During the rainy season 1992 leaf decomposition rate of *R. mucronata* was significantly ($p < 0.05$) lower than during the rainy season 1991 that had higher rainfall. After 15 days of decomposition the dry mass loss was 80% for 1991 and only 35% for 1992 (Figure 3).

Nitrogen immobilisation

All the decomposition experiments show a decrease in C:N atomic ratio in decaying leaves relative to their initial condition (Tables 2, 3, 4, 5). Since the carbon content stays relatively constant (e.g rainy season *C. tagal*, mean

Table 3. Decomposition of *Ceriops tagal* leaves during dry season (November 1991–February 1992). Dry mass remaining (%); No = original nitrogen content and Nt = nitrogen content at sampling time (mg g^{-1} dry mass); C:N atomic ratios; N immobilized (%) and nitrogen fixation (nmol N_2 h^{-1} g^{-1} dry mass).

Sampling (days)	1	3	5	7	11	14	18	25	33	39	46	56	70
DM remaining (%)*	95.3	93.9	93.5	75.6	89.2	80.6	83.4	83.9	83.6	89.4	72.9	78.2	89.3
$\pm\sigma$	0.7	1	1	11	2.8	10	1.8	5.9	3.8	1	3.2	8	2.6
No (mg g^{-1})*	2.57	2.75	2.67	2.65	2.60	2.37	3.17	2.80	2.33	2.57	2.70	2.37	2.75
$\pm\sigma$	0.15	0.07	0.32	0.21	0.36	0.31	0.29	#	0.21	0.35	0.28	0.51	0.21
Nt (mg g^{-1})*	2.77	3.00	2.70	3.53	2.77	3.17	3.25	3.63	3.73	3.17	4.43	4.13	4.90
$\pm\sigma$	0.47	0.26	0.26	0.92	0.35	0.12	0.21	0.51	0.32	0.55	0.21	0.67	0.14
C:No	179	170	179	177	185	197	148	163	188	184	177	191	174
$\pm\sigma$	4	1	27	9	24	25	15	#	6	21	17	28	14
C:Nt	156	150	156	144	155	155	114	124	133	135	111	115	91
$\pm\sigma$	34	21	12	33	5	6	52	14	13	16	3	15	15
N immob. (%)	103	102	95	101	95	108	86	109	134	110	120	136	159
N-fixation nmol N_2 h^{-1} g^{-1}	0.9	40	23	78	4	13	–	6	9	0	17	3	–

-: no data
*: average value for 3 litterbags
#: only one litterbag recovered

Table 4. Decomposition of *Rhizophora mucronata* leaves during the rainy season (May to July 1991). Dry mass remaining (%); No = original nitrogen content and Nt = nitrogen content at sampling time (mg g^{-1} dry mass); C:N atomic ratios; N immobilised (%) and nitrogen fixation (nmol N_2 h^{-1} g^{-1} dry mass).

Sampling (days)	3	10	16	22	29	36	44
DM remaining (%)*	68	24	14	12	3	3	2
$\pm\ \sigma$	5	28	16	12	3	3	5
No (mg g^{-1})*	2.13	2.70	3.10	2.03	2.43	2.10	2.70
$\pm\ \sigma$	0.12	0.20	0.17	0.84	0.68	0.62	0.56
Nt (mg g^{-1})*	4.23	5.05	7.30	7.45	10.10	5.40	8.40
$\pm\ \sigma$	0.90	1.96	3.25	1.48	2.08	#	0.14
C:No	217	190	150	264	201	223	177
$\pm\ \sigma$	11	22	10	93	47	61	42
C:Nt	129	110	69	60	56	83	64
$\pm\ \sigma$	35	38	41	18	15	28	0.7
N immob. (%)	135	45	33	44	12	8	6
N-fixation (nmol N_2 h^{-1} g^{-1})	20	–	390	360	167	3	0

–: no data
*: average value for 3 litterbags
#: only one litterbag recovered

C initial: 404±12 mg g^{-1}, mean C final: 412±63 mg g^{-1}) this decrease in C:N ratio is due to a relative enrichment of nitrogen versus carbon during leaf decomposition.

The nitrogen change during decomposition is calculated as the ratio of the nitrogen content at sampling time (Nt) corrected for mass loss over the original nitrogen content (No) and expressed as a percentage. Values exceeding 100% indicate immobilisation of nitrogen in the decaying leaves.

During the 1991 rainy season, there was a slight nitrogen immobilisation in the leaves of *C. tagal* during the first 13 days of decomposition. Afterwards, nitrogen was lost except for the samples retrieved at day 39 that show some nitrogen immobilisation. Nitrogen was immobilised in the leaves of *R. mucronata* during the first 3 days of decomposition and was lost during the rest of the decomposition period, indicating that the loss of organic matter due to leaf breakdown overtakes the increase in the nitrogen content of the leaf.

During the dry season, the absolute amount of nitrogen in the *C. tagal* leaves, remained relatively unchanged during the first 25 days of decomposition. Afterwards, nitrogen was immobilised almost throughout the decomposition period with a maximal value of 159% (day 70). *R. mucronata* leaves started to immobilise nitrogen after 5 days of decomposition to reach a

Table 5. Decomposition of *Rhizophora mucronata* leaves during the dry season (November 1991 to February 1992). Dry mass remaining (%); No = original nitrogen content and Nt = nitrogen content at sampling time (mg g^{-1} dry mass); C:N atomic ratios; N immobilised (%) and nitrogen fixation (nmol N_2 h^{-1} g^{-1} dry mass).

Sampling (days)	1	3	5	7	11	14	18	25	33	39	46	54	63	70
DM remaining (%)*	89.8	82.5	85.4	80.1	83.3	77.8	71.5	80.2	81.2	63	51.7	70	48.4	43
$\pm\sigma$	2.7	2.8	2.2	4.5	5.6	6.6	9.7	0.3	4.4	5.7	12.1	16	19	1
No (mg g^{-1})*	2.65	2.89	3.13	2.66	3.26	2.77	2.83	2.61	2.85	3.10	3.23	3.07	3.40	2.80
$\pm\sigma$	0.13	0.35	0.25	0.15	1.01	0.06	0.68	0.34	0.18	0.3	0.71	0.06	0.65	0.14
Nt (mg g^{-1})*	3.14	3.48	4.45	3.96	4.80	5.53	4.36	4.67	7.30	7.51	9.33	10.49	8.58	5.81
$\pm\sigma$	0.92	0.13	0.50	0.72	0.49	0.35	0.05	0.54	2.07	1.49	1.80	1.41	2.28	1.97
C:No	175	167	159	177	157	175	177	187	171	159	153	157	147	174
$\pm\sigma$	5	20	15	6	46	4	34	26	11	13	31	10	33	6
C:Nt	156	145	114	125	107	90	114	108	70	66	56	44	68	93
$\pm\sigma$	42	12	14	20	11	7	8	14	24	16	11	6	17	41
N immob. (%)	106	99	121	119	123	155	110	143	208	153	149	239	122	89
N-fixation (nmol N_2 h^{-1} g^{-1})	51	107	83	125	96	122	–	57	84	32	21	55	37	–

–: no data
*: average value for 3 litterbags

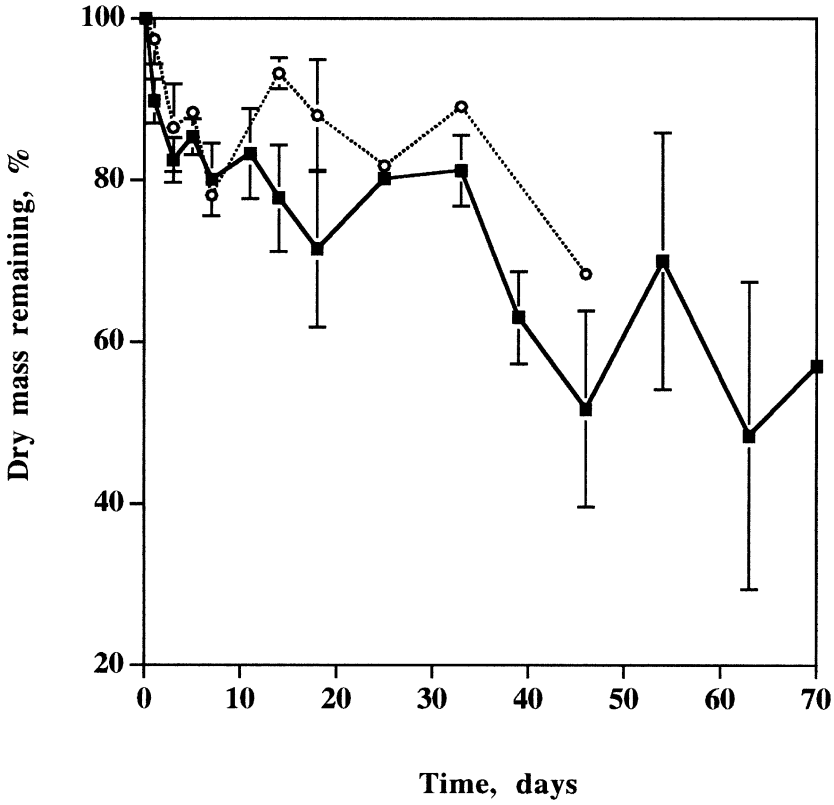

Figure 2. Comparison of dry mass loss of continuously submerged (open circles) with tidally submerged leaves (filled squared) of *Rhizophora mucronata* during dry season.

maximum value of 239% (day 54). Afterwards, the immobilisation of nitrogen decreased and at day 70, nitrogen loss overtook nitrogen gain.

During both seasons, significantly more nitrogen ($p < 0.05$) was immobilised in *R. mucronata* leaves than in *C. tagal* ones. Significantly more nitrogen ($p < 0.05$) was immobilised in *R. mucronata* leaves kept on the forest floor compared to continuously submerged leaves (data not shown).

Nitrogen fixation

Maximum rates of nitrogen fixation were observed during or at the end of the first month of decomposition (Tables 2, 3, 4, 5).

During the 1991 rainy season, we observed a maximal nitrogen fixation rate of 380 nmol N_2 h^{-1} g^{-1} after 32 days of decomposition for *C. tagal*. *R. mucronata* exhibited peak activity of 390 nmol N_2 h^{-1} g^{-1} after 16 days and a decrease to a zero value after 36 days due to almost complete decomposition

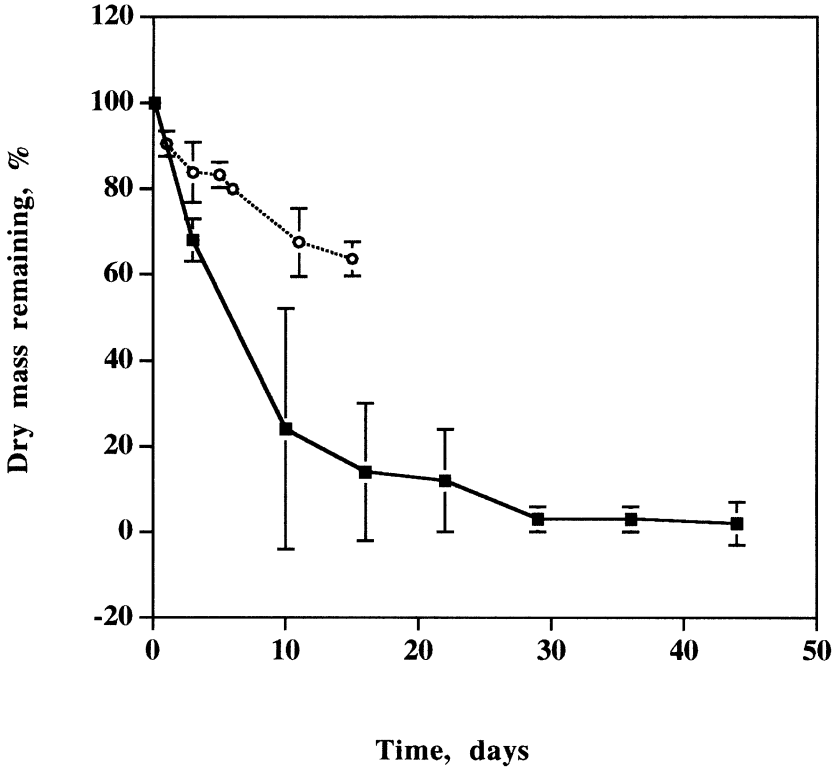

Figure 3. Comparison of dry mass loss of *Rhizophora mucronata* leaves between rainy seasons of 1991 (filled squared) and 1992 (open circles).

of the leaves. During dry season 1991–1992 both mangrove species showed an initial increase in nitrogen fixation activity to reach a maximum value of 78 nmol N_2 h^{-1} g^{-1} for *C. tagal* and of 125 nmol N_2 h^{-1} g^{-1} for *R. mucronata* after 7 days.

Nitrogen fixation rates of *C. tagal* decaying leaves during the rainy season were significantly higher ($p < 0.05$) than during the dry season. No significant seasonal differences in nitrogen fixation rates were observed in *R. mucronata* decaying leaves.

During the dry season, nitrogen fixation rates of *R. mucronata* decaying leaves were significantly higher ($p < 0.05$) than these of *C. tagal*. During two successive rainy seasons (1991 and 1992, data not shown), no significant differences in nitrogen fixation rates were observed for *R. mucronata* decaying leaves. During the dry season, no significant differences in nitrogen fixation rates were observed for *R. mucronata* decaying leaves on the forest floor compared to submerged ones (data not shown).

Discussion

Leaf decomposition

The decay rate of plant litter can be affected by litter quality and by ambient physico-chemical conditions such as temperature, oxygen concentration and humidity (Melillo et al. 1984). Several authors (Melillo et al. 1984; Twilley et al. 1986; Robertson et al. 1992) have reported a close relationship between decay rate and initial leaf nitrogen concentration, with faster degradation associated with higher nitrogen content. Our observations do not confirm this. We observed highest decomposition rates for both species of mangrove during the rainy season when the C:N ratios are highest. Indeed, the mean initial C:N ratios for dry season (178 for *Ceriops* and 167 for *Rhizophora*) were significantly different ($p < 0.05$) from the mean initial C:N ratios for the rainy season (256 for *Ceriops* and 206 for *Rhizophora*). Other factors such as the ratio of lignin to nitrogen has been shown to correlate well with mass loss rates for non-leguminous litter (Constantinides & Fownes 1994). In the present study we did not analyse for lignin content of the mangrove leaves.

Some authors (Twilley et al. 1986; Steinke & Ward 1987; Robertson et al. 1992) have emphazised the importance of the frequency of tidal inundation on the rate of leaf decomposition with higher rates associated with increased tidal frequency. Our results show a similar trend. The *C. tagal* plot is situated at a higher intertidal range and is inundated only during spring tides. Leaf degradation in the *C. tagal* plot is slower than in the *R. mucronata* plot that is situated at a low intertidal range and subject to diurnal tidal inundation. However, for *R. mucronata*, comparison of the decomposition rate of continuously submerged leaves with the rate of leaves kept within the *Rhizophora* plot and submerged twice a day, showed slightly but significantly lower ($p < 0.05$) decomposition rates for continuously submerged leaves (Figure 2). Similar results were obtained in South Africa by Mann & Steinke (1992) for leaves of *A. marina* and in Thailand by Boonruang (1984) for leaves of *R. apiculata*. The latter author attributes the difference to the presence of a larger number of micro-organisms (fungi and bacteria) and grazers within the forest.

Very few of the authors who studied mangrove litter decomposition paid attention to the seasonal variation of decomposition rates. Twilley et al. (1986) and Flores-Verdugo et al. (1987) observed higher degradation rates associated with the rainy season. Steinke and Ward (1987) observed higher degradation rates during the warm season but did not describe rainfall. In our study, decomposition was faster during the rainy season despite a lower air temperature (Table 1). Furthermore, a large difference in decay rates of *R. mucronata* leaves was observed between rainy seasons of two successive years. Leaf decomposition during the period from May till July 1991 was faster than

during June 1992 and rainfall in May and June was about twice as high in 1991 as in 1992, while there were no significant differences in air temperature between both rainy seasons (Table 1). It may be, therefore, that rainfall is more important than air temperature and that higher rainfall enhances leaf decomposition rates. The effect of rainfall on decomposition rates, could result from the subsequent supply of inorganic nutrients through freshwater discharge in the lagoonal waters (Kazungu et al. 1993). Indeed, during the rainy season, nitrate concentrations ranged from 0.2 to 1.7 μmol l^{-1} and ammonium from 0.1 to 0.9 μmol l^{-1} between high and low tide. During dry season, on the contrary, there is no significant freshwater discharge in the system and nitrate and ammonium concentrations remain below 0.2 μmol l^{-1} (Kazungu et al. 1993, and unpublished results). Higher nutrient content of the water column during rainy season together with a higher relative humidity, could sustain a rapid initial microbial colonisation of the leaves.

Nitrogen fixation and nitrogen immobilisation

Maximum nitrogen fixation rates for *Ceriops* amounted to 78 and 380 nmol N_2 h^{-1} g^{-1} for dry and rainy season respectively. For *Rhizophora*, maximum nitrogen fixation rates were 125 and 390 nmol N_2 h^{-1} g^{-1} for dry and rainy season respectively. The values we observed for the dry season are similar to the values reported by others, while our values for the rainy season are larger than those reported elsewhere. Examples of literature values for nitrogen fixation associated with decaying mangrove litter are, for *R. mangle*: 30 nmol N_2 h^{-1} fresh weight g^{-1} (Zuberer & Silver 1978) and for *A. marina*: 12 nmol N_2 h^{-1} g^{-1} (Hicks & Silvester 1985); 347 nmol N_2 h^{-1} g^{-1} (Mann & Steinke 1992); 156 nmol N_2 h^{-1} g^{-1} (Van der Valk & Attiwill 1984).

To evaluate the contribution of nitrogen fixation to the immobilisation of nitrogen in mangrove leaves when any, we integrated the nitrogen fixation rates over time. Nitrogen fixation values reported in Tables 2, 3, 4, 5, were corrected for mass loss and expressed per day. We arbitrarily decided N_2 fixation to apply only between sunrise and sunset (12 h, Gotto & Taylor 1976). We assumed these corrected nitrogen fixation rates applied throughout each period comprised between midpoints of successive litterbag sampling events. These time-weighted N_2 fixation values were summed, and these integrated values (in μg N g^{-1}) were compared with the difference (N gain) between Nt, corrected for mass loss, and No (Nt and No from Tables 2, 3, 4, 5).

During the 1991 rainy season, after the first days of decomposition, mass losses exceeded the input of external nitrogen in the decaying leaves of both species of mangroves and therefore no nitrogen immobilisation was observed (Figures 4a and 4c).

For the 1991–1992 dry season, at maximum immobilisation of nitrogen in the *C. tagal* leaves (70 days, 1626 μg N g^{-1}), integrated nitrogen fixation reached 208 μg N g^{-1} and could account for 13% of the nitrogen immobilised (Figure 4b). At maximum immobilisation of nitrogen in the leaves of *R. mucronata* (54 days, 4273 μg N g^{-1}), integrated nitrogen fixation reached 887 μg N g^{-1}, and could account for 21% of the nitrogen immobilised in the leaves (Figure 4d). In general, these calculated values are lower than the results of Van der Valk & Attiwill (1984) who estimated that nitrogen fixation could supply 40 to 64% of the nitrogen enrichment of *A. marina* leaves during decomposition.

Nitrogen input to the mangrove ecosystem

From litterfall data for the *Rhizophora* and *Ceriops* plots in Gazi (Slim & Gwada 1993) and initial nitrogen content of leaf litter (this study) we calculated the nitrogen input to the lagoonal ecosystem through leaf shedding to range between 0.86 and 13.98 mg N m^{-2} d^{-1} (Table 6), depending on mangrove species and season. To calculate the additional amount of allochtonous nitrogen resulting from nitrogen fixation during mangrove leaf decay, we multiplied the N_2 fixation values integrated over the decomposition period by the litterfall values (Table 6). Thus the additional amount of allochtonous nitrogen that can be imported into the mangrove sediments, represented between 0.28 and 4.15 mg N m^{-2} d^{-1} (Table 6). However, it is probable that nitrogen fixation is not restricted to the decaying leaf but occurs also on small detrital particles accumulated in the sediments (Table 6). We obtained some preliminary results concerning nitrogen fixation associated with sediments. During the dry season (February 1994) we measured an activity of 2.3 mg N m^{-2} d^{-1} for sediments of the *Ceriops* plot and of 0.8 mg N m^{-2} d^{-1} for sediments of the *Rhizophora* plot. The importance of the allochtonous nitrogen input associated with nitrogen fixation in the sediments is presently under further investigation and natural nitrogen isotope abundance will be analysed to evaluate directly the percentage of incorporated nitrogen derived from biological nitrogen fixation (e.g. Mariotti et al. 1992).

The data above suggest that nitrogen fixation can contribute significantly to the observed nitrogen enrichment of organic detritus between its input as mangrove leaf litter and its accumulation in the sediments. However other sources of allochthonous nitrogen, such as the inputs of nutrient rich freshwater through rainfall, river discharge and ground water seepage, exist and need to be taken into account.

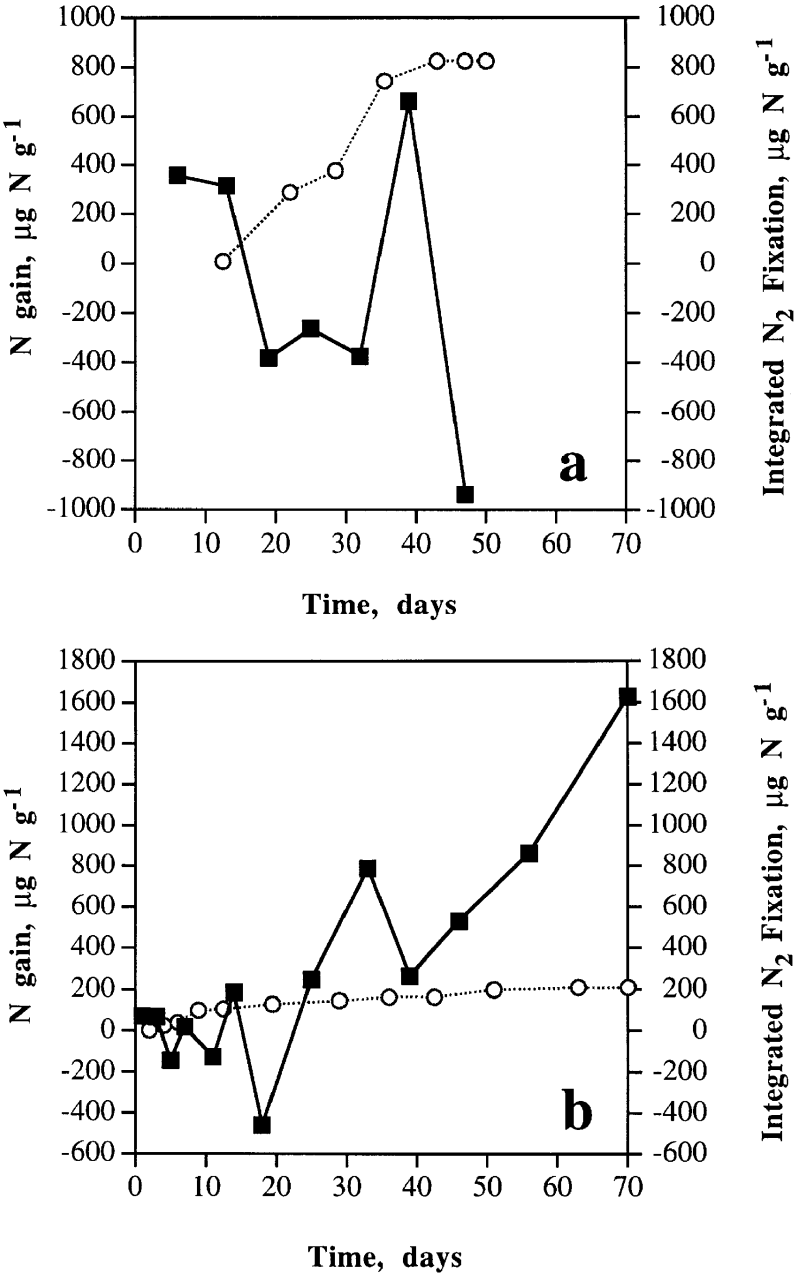

Figure 4. Change of immobilisation of nitrogen in mangrove leaves (μg N g^{-1}; filled squared) and integrated nitrogen fixation rates (μg g^{-1}; open circles) over time for a) *Ceriops tagal* leaves during rainy season; b) *Ceriops tagal* leaves during dry season; c) *Rhizophora mucronata* leaves during rainy season; d) *Rhizophora mucronata* leaves during dry season.

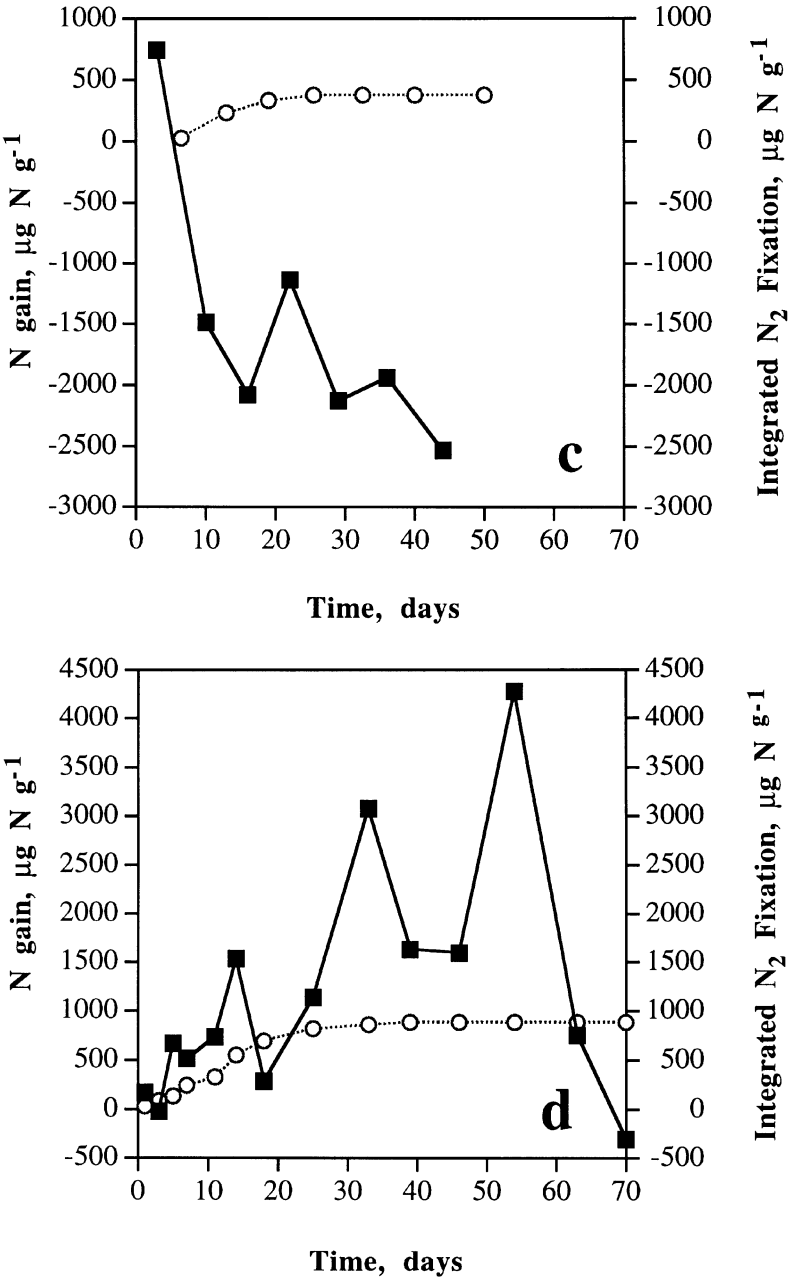

Figure 4. Continued.

Table 6. Comparison of original nitrogen input through leaf shedding with nitrogen input through nitrogen fixation during leaf decay.

Species/ Season	Litterfall* ($g\ m^{-2}\ d^{-1}$)	Mean innitial leaf N content ($mg\ g^{-1}$ dw)	N input through leaf shedding ($mg\ N\ m^{-2}\ d^{-1}$)	Integrated N_2 fixation ($mg\ N\ g^{-1}$)	N input through N_2 fixation ($mg\ N\ m^{-2}\ d^{-1}$)
C. tagal/ Rainy season	0.43	2.0	0.86	0.83	0.36
C. tagal/ Dry season	1.34	2.6	3.48	0.21	0.28
R. mucronata/ Rainy season	3.22	2.4	7.73	0.38	1.22
R. mucronata/ Dry season	4.66	3.0	13.98	0.89	4.15

* data from Slim & Gwada (1993)

Conclusion

In this study of a tropical mangrove ecosystem we focused on the seasonal variation of: (1) *in situ* decomposition of senescent mangrove leaves; (2) immobilisation of nitrogen during leaf decomposition and (3) nitrogen fixation associated with the decaying leaves. Rates of leaf decomposition were higher for *R. mucronata* than for *C. tagal* and were highest, for both species, during the rainy season. High rainfall and a diurnal alternation of drying and wetting of the leaves by tidal inundation appear to enhance leaf decomposition rates. During rainy season, mass loss exceeded the external input of nitrogen after a maximum of 13 days of decomposition. More nitrogen was immobilised in the leaves of both species of mangroves during the dry season than during the rainy season. Rates of nitrogen fixation were highest during the rainy season for *C. tagal*. During the dry season, rates of nitrogen fixation were higher for *R. mucronata* than for *C. tagal*. Biological nitrogen fixation accounted for between 13 and 21% of the maximum nitrogen immobilisation in the decomposing leaves, indicating that nitrogen fixation, although representing a significant source of allochtonous nitrogen to the mangrove ecosystem, is not the major nitrogen source.

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References

Benner R & Hodson RE (1985) Microbial degradation of the leachable and lignocellulosic components of leaves and wood from *Rhizophora mangle* in a tropical mangrove swamp. Marine Ecology Progress Series 23: 221–230

Benner R, Peele ER & Hodson RE (1986) Microbial utilization of dissolved organic matter from leaves of the red mangrove *Rhizophora mangle*, in the Fresh Creek estuary, Bahamas. Estuarine, Coastal and Shelf Science 23: 607–619

Boonruang P (1984) The rate of degradation of mangrove leaves, *Rhizophora apiculata* Bl. and *Avicennia marina* (Forsk) Vierh. at Phuket Island, western peninsular of Thailand. In: Soepadmo E, Rao AN & Mackintosh DJ (Eds) Proceedings of the Asian Symposium on Mangrove Environment, Research and Management, 25–29 August 1980, Kuala Lumpur (pp 200-208). University of Malaya and UNESCO

Boto KG & Robertson AI (1990) The relationship between nitrogen fixation and tidal exports of nitrogen in a tropical mangrove system. Estuarine, Coastal and Shelf Science 31: 531–540

Brock TCM (1984) Aspects of the decomposition of *Nymphoides peltata* (Gmel.) O. Kuntze (Menyanthaceae). Aquatic Botany 19: 131–156

Constantinides M & Fownes JH (1994) Nitrogen mineralization from leaves and litter of tropical plants: Relationship to nitrogen, lignin and soluble polyphenols concentrations. Soil Biology and Biochemistry 26(1): 49–55

Fell JW, Master IM & Wiegert RG (1984) Litter decomposition and nutrient enrichment. In: Moss ST (Ed) The Biology of Marine Fungi (pp 239–251). Cambridge University Press, Cambridge

Flores-Verdugo FJ, Day JW & Briseno-Duenas R (1987) Structure, litterfall, decomposition, and detritus dynamics of mangroves in a Mexican coastal lagoon with an ephemeral inlet. Marine Ecology Progress Series 35: 83–90

Gallardo A & Merino J (1992) Nitrogen immobilization in leaf litter at two mediterranean ecosystems of SW Spain. Biogeochemistry 15: 213–228

Gallin E, Coppejans E & Beeckman H (1989) The mangrove vegetation of Gazi Bay (Kenya). Bulletin de la Société Royale de Botanique de Belgique 122: 197–207

Gotto JW & Taylor BF (1976) N_2 Fixation associated with decaying leaves of the red mangrove (*Rhizophora mangle*). Applied and Environmental Microbiology 31(5): 781–783

Hardy RWF, Holsten RD, Jackson ED & Burns RC (1968) The acetylene-ethylene assay for nitrogen fixation: Laboratory and field evaluation. Plant Physiology 43: 1185–1207

Hemminga MA, Slim FJ, Kazungu J, Ganssen GM, Nieuwenhuize J & Kruyt NM (1994) Carbon outwelling from a mangrove forest with adjacent seagrass beds and coral reefs (Gazi Bay, Kenya). Marine Ecology Progress Series 106: 291–301

Hicks BJ & Silvester WB (1985) Nitrogen fixation associated with the New Zealand mangrove (*Avicennia marina* (Forsk.) Vierh.var.resinifera (Forst. f.) Bakh.). Applied and Environmental Microbiology 49(4): 955–959

Kazungu J, Dehairs F & Goeyens L (1993) Distribution of nutrients and particulate organic material in Gazi Bay. In: Woitchik AF (Ed) Dynamics and Assessment of Kenyan Man-

grove Ecosystems (pp 175–193). Commission of the European Communities, Project TS2-0240-C (GDF), Final Report

Lee KH, Moran MA, Benner R & Hodson E (1990) Influence of soluble components of red mangrove (*Rhizophora mangle*) leaves on microbial decomposition of structural (lignocellulosic) leaf components in seawater. Bulletin of Marine Science 46(2): 374–386

Mann FD & Steinke TD (1992) Biological nitrogen fixation (acetylene reduction) associated with decomposing *Avicennia marina* leaves in the Beachwood Mangrove Nature Reserve. South African Journal of Botany 58(6): 533–536

Mariotti A, Sougoufara B & Dommergues YR (1992) Estimation of nitrogen fixation using the natural abundance method in a plantation of *Casurina equisetifolia* (Forst.). Soil Biology and Biochememistry 24(7): 647–653

McClanahan TR (1988) Seasonality in East Africa's coastal waters. Marine Ecology Progress Series 44: 191–199

Melillo JM, Naiman RJ, Aber JD & Linkins AE (1984) Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. Bulletin of Marine Science 35(3): 341–356

Morris JT & Lajtha K (1986) Decomposition and nutrient dynamics of litter from four species of freshwater emergent macrophytes. Hydrobiologia 131: 215–223

Newell SY, Fell JW, Statzell-Tallman A, Miller C & Cefalu R (1984) Carbon and nitrogen dynamics in decomposing leaves of three coastal marine vascular plants of the subtropics. Aquatic Botany 19: 183–192

Odum WE & Heald EJ (1975) The detritus-based food web of an estuarine mangrove community. In: Cronin LE (Ed) Estuarine Research (pp 265–286). Academic Press Inc., New York

Rao RG, Woitchik AF, Goeyens L, van Riet A, Kazungu J & Dehairs F (1994) Carbon, nitrogen contents and stable isotope abundance in mangrove leaves from an east African Coastal Lagoon (Kenya). Aquatic Botany 47: 175–183

Rice DL (1982) The detritus nitrogen problem: new observations and perspectives from organic geochemistry. Marine Ecology Progress Series 9: 153–162

Rice DL & Tenore KR (1981) Dynamics of carbon and nitrogen during the decomposition of detritus derived from estuarine macrophytes. Estuarine, Coastal and Shelf Science 13: 681–690

Robertson AI, Alongi DM & Boto KG (1992) Food chains and carbon fluxes. In: Robertson AI & Alongi DM (Eds) Tropical Mangrove Ecosystems, Coastal and Estuarine Studies 41 (pp 293–326). American Geophysical Union

Ruwa RK (1993) Zonation and distribution of creek and fringe mangroves in the semi-arid Kenyan coast. In: Lieth H & Al Masoom A (Eds) Towards the Rational Use of High Salinity Tolerant Plants, Vol. 1 (pp 97–105)

Slim FJ & Gwada P (1993) The mangrove vegetation. In: Woitchik AF (Ed) Dynamics and Assessment of Kenyan Mangrove Ecosystems (pp 6–34). Commission of the European Communities, Project TS2-0240-C (GDF), Final Report

Steinke TD & Charles LM (1986) *In vitro* rates of decomposition of leaves of the mangrove *Bruguiera gymnorrhiza* as affected by temperature and salinity. South African Journal of Botany 52(1): 323–328

Steinke TD & Ward CJ (1987) Degradation of mangrove litter in the St. Lucia Estuary as influenced by season and exposure. South African Journal of Botany 53(5): 323–328

Tam NFY, Vrijmoed LLP & Wong YS (1990). Nutrient dynamics associated with leaf decomposition in a small subtropical mangrove community in Hong Kong. Bulletin of Marine Science 47(1): 68–78

Turner GL & Gibson AH (1980) Measurements of nitrogen fixation by indirect means. In: Bergersen FG (Ed) Methods for Evaluating Biological Nitrogen Fixation (pp 111–138). Wiley, New York

Twilley RR, Lugo AE & Patterson-Zucca C (1986) Litter production and turnover in basin mangrove forests in south west Florida. Ecology 67(3): 670–683

Van der Valk AG & Attiwill PM (1984) Acetylene reduction in an *Avicennia marina* community in Southern Australia. Australian Journal of Botany 32: 157–164

White DS & Howes BL (1994) Nitrogen incorporation into decomposing litter of *Spartina alterniflora*. Limnology and Oceanography 39(1): 133–140

Zuberer DA & Silver WS (1978) Biological Dinitrogen Fixation (Acetylene Reduction) associated with Florida Mangroves. Applied and Environmental Microbiology 35(3): 567–575