Structural changes and associated microbial activity accompanying decomposition of mangrove leaves in Mgeni Estuary

T.D. Steinke*, A.D. Barnabas and R. Somaru

Estuarine and Marine Group, Department of Botany, University of Durban-Westville, Private Bag X54001, Durban, 4000 Republic of South Africa

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Leaves of *Avicennia marina* (Forssk.) Vierh. and *Bruguiera gymnorrhiza* (L.) Lam. were placed in litter bags and allowed to decompose *in situ* before collection at 7, 14, 21, 42, 64 and 85 days. Material from each collection was examined for structural changes using light microscopy and SEM. Samples of each collection were also cultured for fungi and bacteria. Examination of senescent leaves still attached to the trees revealed the presence of phylloplane micro-organisms. Decomposition in both species followed a similar pattern although the leaves of *A. marina* decomposed more rapidly than those of *B. gymnorrhiza*. By day 7 there were no obvious changes in leaf structure, initial decomposition of the leaf base was the only apparent change in structure on day 14; by day 21 portions of the cuticular layer had separated from the leaf tissue; on day 42 most of the leaves were split longitudinally, separating the adaxial and abaxial surfaces; in the day-63 material, fragmentation of the leaves was evident; further fragmentation and 'skeletonization' of leaves was observed after 85 days of decomposition. Both bacteria and fungi were important in the decomposition process. Leaves of both mangroves were found to harbour 20–25 species of fungi. This work appeared to confirm the significance of micro-organisms in the estuarine food web.

Blare van *Avicennia marina* (Forssk.) Vierh. en *Bruguiera gymnorrhiza* (L.) Lam. is in plantafvalsakke geplaas en toegelaat om *in situ* te vergaan voordat dit na 7, 14, 21, 42, 64 en 85 dae herwin is. Materiaal van elke versameling is vir strukturele veranderinge met behulp van 'n ligmikroskoop en die aftaselelektronmikroskoop ondersoek. Monsters van elke versameling is ook blootgestel aan die groei van fungi en bakterieë. 'n Ondersoek van verouderde blare wat nog aan die plante aanwesig is, het die aanwesigheid van blaargedraagde mikro-organismes getoon. Afbraak by beide spesies volg 'n soortgelyke patroon, alhoewel die blare van *A. marina* vinniger verrot as dié van *B. gymnorrhiza*. Op dag 7 was daar geen sigbare veranderinge in blaarstruktuur nie, aanvanklike afbraak van die blaarbasis was die enigste duidelike verandering in struktuur op dag 14; teen dag 21 het gedeeltes van die kutikula begin los raak van die res van die blaarweefsel; op dag 42 het die meeste blare oorlangs in ad- en abaksiale helftes verdeel; in die dag-63-materiaal is fragmentasie van die blare sigbaar; verdere fragmentasie en 'skeletvorming' van blare is na 85 dae van verrotting waargeneem. Bakterieë sowel as fungi het 'n belangrike rol in die afbraakproses gespeel. Blare van beide mangliete huisves tussen 20 en 25 fungi as spesies. Hierdie werk bevestig die belangrikheid van mikro-organismes in die voedselweb in strandmere.

Keywords: Decomposition, mangrove litter, micro-organisms, microscopy

*To whom correspondence should be addressed

Introduction

Mangrove swamps are recognized as highly productive ecosystems having a high rate of primary production. A large quantity of organic matter, particularly fallen leaf material, enters the estuarine system, where it forms the basis for a complex food web (Golley *et al.* 1962; Odum & Heald 1972; Snedaker 1978; Teas 1976). Most of the energy from primary production becomes available to support consumer production only after it has been fragmented and processed through decomposer pathways (Newell 1982). Decomposing mangrove leaf material is therefore of significance in the estuarine food chain and this presented a need to investigate the nature of leaf decomposition and its transformation into detritus particles. In the present study, therefore, decomposing leaves of *Avicennia marina* (Forssk.) Vierh. and *Bruguiera gymnorrhiza* (L.) Lam. were examined to follow the physical sequence of processes occurring during decomposition and to assess the occurrence of microbial colonization concomitant with decomposition.

Materials and Methods

Dry matter yields

Yellow, senescent leaves, the abscission of which was judged to be imminent, were picked from trees on 23 February 1983 and taken to the laboratory within an hour. Leaves of *A. marina* and *B. gymnorrhiza* were sorted into groups of approximately equal numbers (12 and 8 for each species respectively), weighed to equal mass and placed in nylon bags with a very fine, but irregular mesh (*c.* 1-mm aperture). Samples with the same number and mass of leaves for dry mass determinations, were weighed, dried in an oven at 60°C for 1 week and then re-weighed. The litter bags, which were tied to marker posts in the Beachwood stream (Mgeni Estuary) rested on the mud and were completely submerged during the entire experimental period.

The mangrove communities in this area have been described by Padia (1980). Water temperatures 100 mm below the surface varied from 28.5°C in the summer to 18°C towards the end of the experiment, although more extreme temperatures may have occurred. Large differences in salinity were recorded, particularly during the rainy season. Values were as low as 0‰ and as high as 34‰ although generally the salinity was approximately 12‰.

Litter bags were retrieved at six harvests; three bags of each species were collected after 7, 14, 21, 42, 64 and 85 days. The bags were transported to the laboratory in sterile plastic containers. There the leaves were washed gently in sterilized distilled water to remove surface sediment. After sampling for microscopical observation and isolation of fungi and bacteria (see below), the remaining leaf material was oven-dried at 60°C to determine loss of dry mass. At each harvest one leaf per bag was placed in sterile distilled water and both adaxial and abaxial surfaces examined microscopically. Notes were made of any features of interest before these leaves were oven-dried and weighed. The remaining leaf material was used for sampling for microscopy and microbial isolation. Leaf samples of *A. marina* and *B. gymnorrhiza* received the same treatment.

Microscopy

Leaf samples for light microscopy were taken from the mid-sections of senescent leaves, which had not yet abscised, as well as from decomposing leaves at the first, second, third and fourth harvests, i.e. after 7, 14, 21 and 42 days of submergence. The samples were fixed in FAA and embedded in paraffin wax. Transverse and longitudinal sections were cut on a sledge microtome and double-stained with safranin and fast green.

At each harvest small pieces of lamina from the base, middle and apex of the leaves were excised with alcohol flame-sterilized instruments. These samples were fixed under vacuum in 6% glutaraldehyde, dehydrated in a graded ethanol series, critical point dried, coated with gold and examined with a Philips 500 SEM.

Fungal isolations

All water used for preparation of media and slides was collected from the mangrove swamp, filtered and autoclaved. Two media were chosen for fungal isolations: malt extract agar (MEA) in seawater diluted to 25 and 15‰ and corn meal agar (CMA) in seawater diluted to 15‰. Chloramphenicol (0.02%) was added to all fungal media to inhibit bacterial growth. The pH was adjusted to 7.5.

To determine which phylloplane fungi were present, senescent leaves of both species were picked from the trees; squares of *c.* 2 mm were excised and plated on MEA (15‰).

Isolations from decomposing leaves followed two procedures. One culture method involved leaf-washing, in which a leaf was shaken in 50 ml sterile distilled water. The washing (*c.* 10 ml per plate) was pour-plated or transferred to the agar medium with a sterile pipette. The second method involved plating out small pieces (*c.* 2 mm^2) of leaf, cut with alcohol flame-sterilized instruments from the base, middle and apex of the decomposing leaves. Four leaf squares were placed on each plate, some with the adaxial and others with the abaxial surface uppermost, on the different media. Surface-sterilization of some leaf samples by immersion in mercuric chloride (0.1%) for 1 min was carried out to isolate the endophytic fungi. The plates were incubated at 25°C for 6–7 days and the fungi were recorded. Subsequent examinations were carried out at regular intervals until no new species were recorded. The fungi were subcultured to obtain pure cultures.

Microscope slides were prepared using cotton-blue to stain fungi. Identification of fungi was carried out locally and was possible generally only to generic level.

Bacterial cultures

The media selected for growth of bacteria were: (1) a cellulose medium containg 0.1% cellulose and 1.5% agar; and (2) a general bacto-peptone medium. Both were adjusted to pH 7.5.

At each harvest the following sampling procedures were followed:

1. leaf-washing method (as described for fungi);
2. surface swabbing, where sterile cotton swabs were used to pick up bacteria from the leaf surface.
3. leaf maceration, where small pieces of leaf, dipped in 70% ethanol for 30 sec, were transferred to 0.85% sterile saline and macerated with 2 ml distilled water in a sterile mortar and pestle; this macerated material was used as the inoculum.

All petri dishes were incubated at 25°C. Bacteria from culture plates were gram-stained and examined. No identifications were possible.

Results and Discussion

Dry matter yields

The decrease in mass of leaves of both species is shown in Figure 1. Leaves of *A. marina* decomposed more

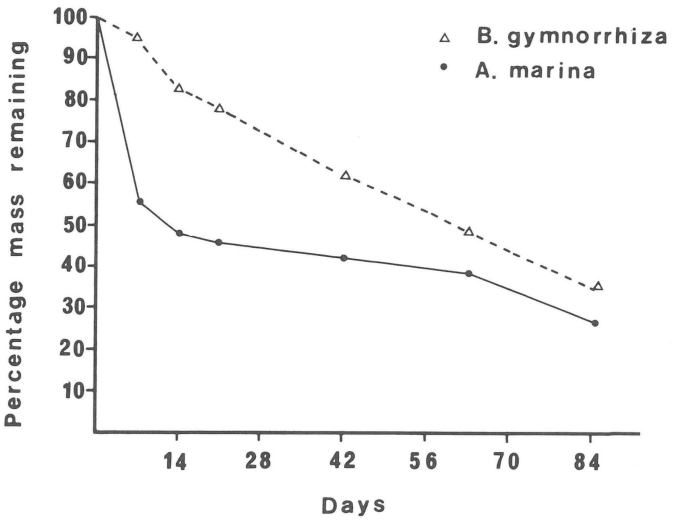


Figure 1 Dry matter losses of mangrove leaves during decomposition.

rapidly than *B. gymnorrhiza* leaves. *A. marina* leaves revealed a rapid decrease in mass in the first 2 weeks, with an early loss of approximately half their mass, followed by a slow decrease for the remainder of the experimental period. Leaves of *B. gymnorrhiza* showed a steady decrease during the experiment. By the end of the experiment (85 days) the leaves of *A. marina* and *A. gymnorrhiza* were reduced to 25% and 35% of their original mass respectively. In comparison, Odum & Heald (1975) found that 9% of the original mass of *Rhizophora mangle* leaves remained after 4 months of submergence, while Steinke & Ward (1987) showed that reduction in mass of *A. marina* leaves was 96% within 3 months.

Stages in leaf decomposition

The general pattern in both mangroves species seemed to be progressive decomposition from the leaf base towards the apex (Figure 2). When examined with the naked eye, material of day 7 showed no obvious changes in leaf structure; initial decomposition of the leaf base was the only apparent change in structure in the day-14 material; by day 21 portions of the cuticular layer separated from the leaf tissue; on day 42 most of the leaves were split longitudinally, separating the adaxial and abaxial surfaces, and the leaves were fragile; in the day-63 material, fragmentation of leaves was evident; further fragmentation and 'skeletonization' of leaves, with the cuticle sometimes remaining unfragmented, was observed after 85 days of decomposition.

Senescent leaves

Scanning electron microscopy revealed that a microbial population built up on the leaves as degradation of structural material progressed. Decomposition appears to begin at the time of senescence. Perforations and cracks in the cuticle appeared at this stage in both species (Figure 3). The attached senescent leaves were found to harbour micro-organisms (Figure 4). Fungal hyphae were observed on the leaf surfaces of both species, while large numbers of spore-like forms were found in and among the non-glandular trichomes of *A. marina* leaves (Figure 5).

1–7 days after submergence

By day 7 debris had accumulated on the leaf surfaces. The cuticle remained largely intact, although perforations in the adaxial cuticular layer were now larger and more frequent (Figure 6). The internal leaf tissue, however, still remained intact. Micro-organisms occurred in low numbers; fungal hyphae were fairly frequent, while a few scattered cocci were present on the cuticle.

7–14 days after submergence

On day 14 there was localized erosion of epidermal and subepidermal layers of cells, which was more prevalent on the abaxial leaf surfaces (Figure 7). In both species a greater degree of colonization by microflora was observed on abaxial than adaxial leaf surfaces. A dense mycelial growth was observed in both species (Figure 8), but *A. marina* leaves appeared to support a greater growth of fungi. Diatoms were also present in abundance. Bacteria had increased rapidly in numbers and could be seen among particles of debris, on the cuticular surface especially where cracks were frequent, and on and around stomata.

14–21 days after submergence

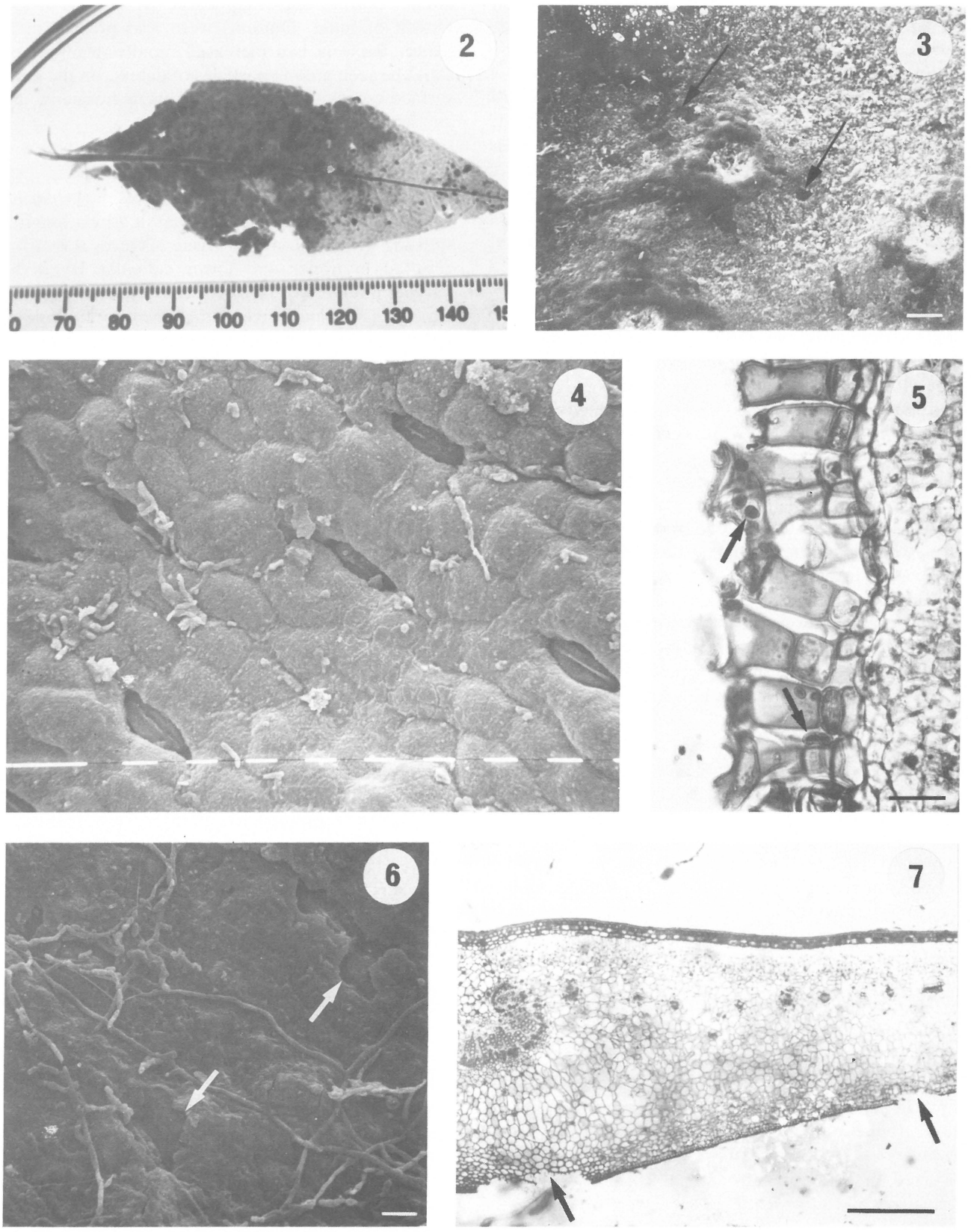
At day 21, adaxial and abaxial surfaces of *A. marina* and *B. gymnorrhiza* leaves were separated by a longitudinal splitting of the mesophyll tissue (Figures 9 & 10). This resulted in upper and lower cuticular layers having varying numbers of layers of cells attached to them. In several *A. marina* leaves either the cuticle alone or the cuticle accompanied by the epidermal layer, peeled away from relatively large areas of the leaf surface. In the midrib region, the cuticle crumpled as it peeled off the surface (Figure 11). There was a marked increase in growth of fungi, while stomata were increasingly invaded by bacteria and the guard cells showed signs of degradation.

21–42 days after submergence

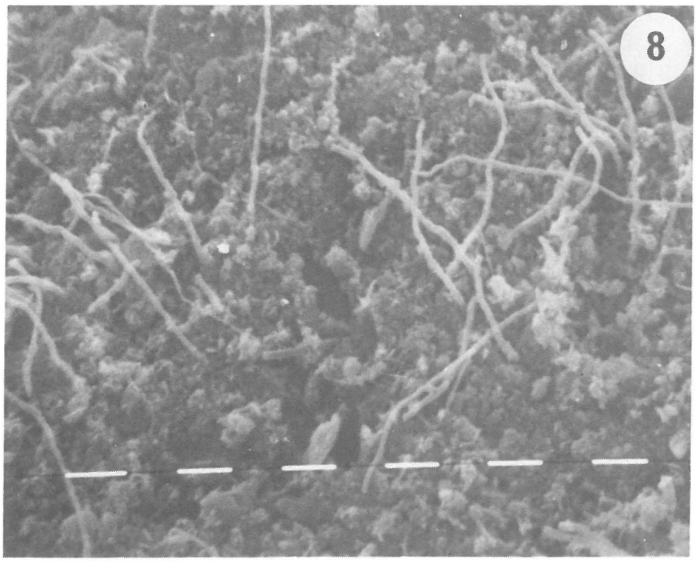
After 42 days there was considerable degradation. Degradation had proceeded to the mesophyll tissues which were exposed by this time. Spongy mesophyll tissue appeared to decompose before palisade mesophyll tissue (Figure 12). Although the cuticle peeled off most of the leaf, in some cases (more frequently in *B. gymnorrhiza* leaves) it remained relatively unfragmented up to the twelfth week. Frequently the cuticle split along the anticlinal walls of the epidermal cells resulting in a mosaic-like layer. Degeneration of the transverse (outer) cell walls began along these splits and proceeded towards the central portion of these walls, reducing them to variously shaped fragments (Figure 13). At this stage the surfaces and split areas were covered with large numbers of fungal hyphae and bacteria (Figure 14). Bacilli were encountered more frequently than cocci. Unicellular and filamentous algae were also frequent at this stage. The outer cell walls of the epidermis became completely eroded, exposing the interior of the cell (Figure 15). While stomata were degenerating, the salt glands appeared to be resistant and persisted after most of the stomata had disintegrated.

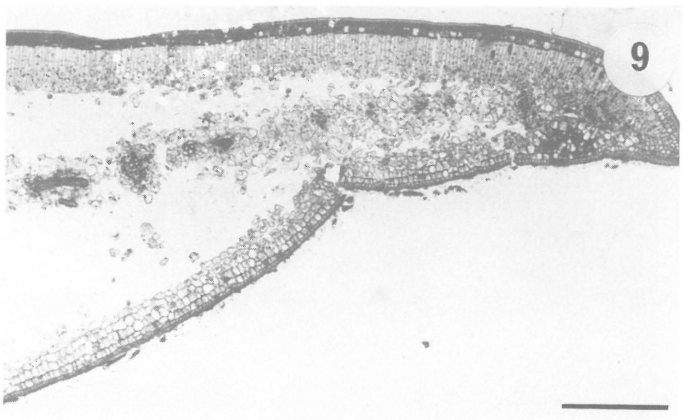
42–64 days after submergence

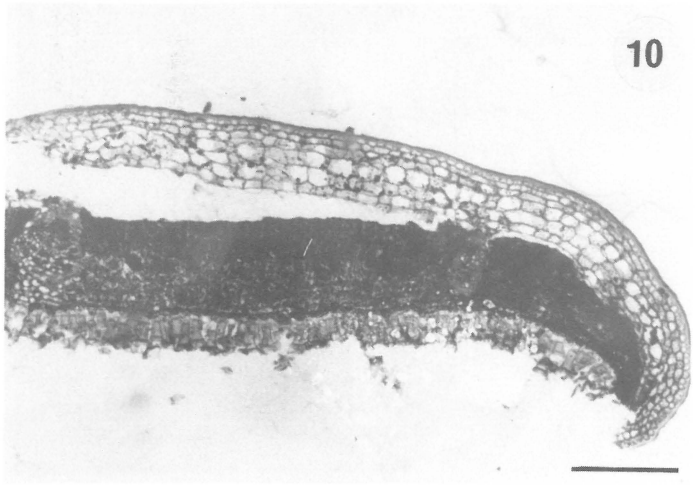
By day 64 decomposition of both *A. marina* and *B. gymnorrhiza* leaves had reached an advanced stage, although the process was slower in the latter species. *A. marina* leaves were more finely fragmented. At this stage the leaves of both species consisted mainly of the veins, palisade mesophyll and scattered remnants of spongy mesophyll. In their very late stages of degradation, the spongy mesophyll cells were identifiable only by their relative position in the leaf (Figure 16). The surfaces of the veins and all remaining layers on the leaf were now covered by masses of micro-organisms. The outer (exposed) walls of the vessels were at varying

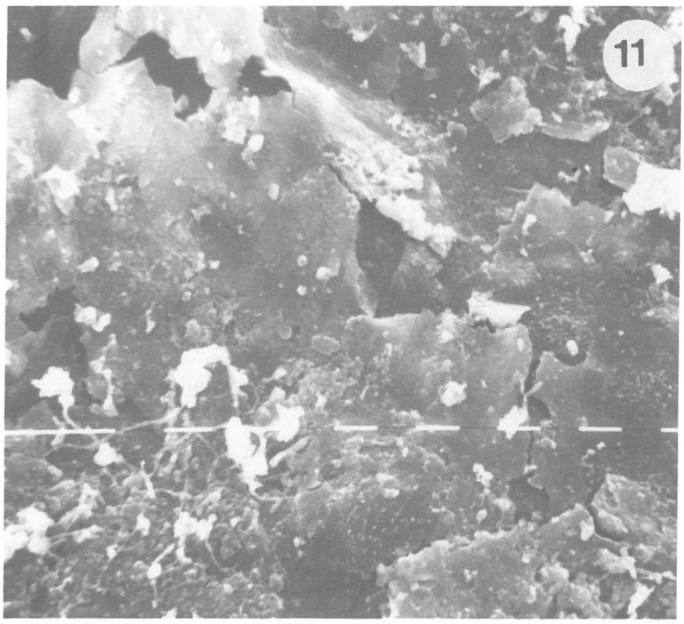


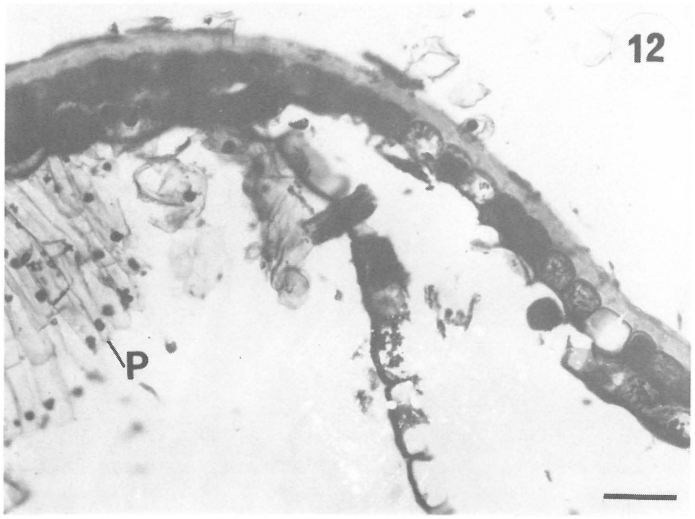
Figures 2–7 Light micrographs (Figures 2, 5 & 7) and scanning electron micrographs (Figures 3, 4 & 6) of senescent and decomposing mangrove leaves. **2**. Six-week sample of *A. marina* showing decomposition from base to apex. **3–5**. Surfaces of senescent leaves of *A. marina* (Figures 3 & 5) and *B. gymnorrhiza* (Figure 4) depicting perforations in cuticle (Figure 7 arrows), microorganisms including fungal hyphae (Figure 4), and spore-like forms among trichomes (Figure 5 arrows). Bars = 10 μm for Figures 3 & 4 and 20 μm for Figure 5. **6**. Large cuticular perforations (arrows) and network of fungal hyphae on surface of a 1-week sample of *B. gymnorrhiza* leaves. Bar = 10 μm. **7**. Some epidermal and subepidermal tissues being eroded (arrows) in a 2-week sample of *B. gymnorrhiza*. Bar = 100 μm.

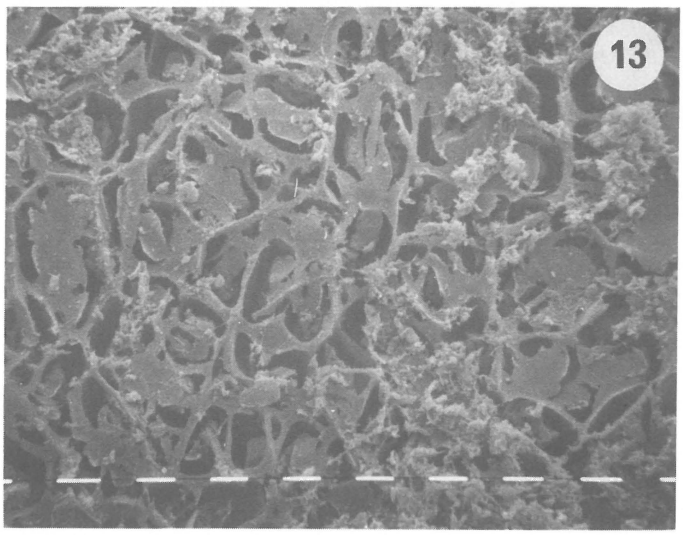




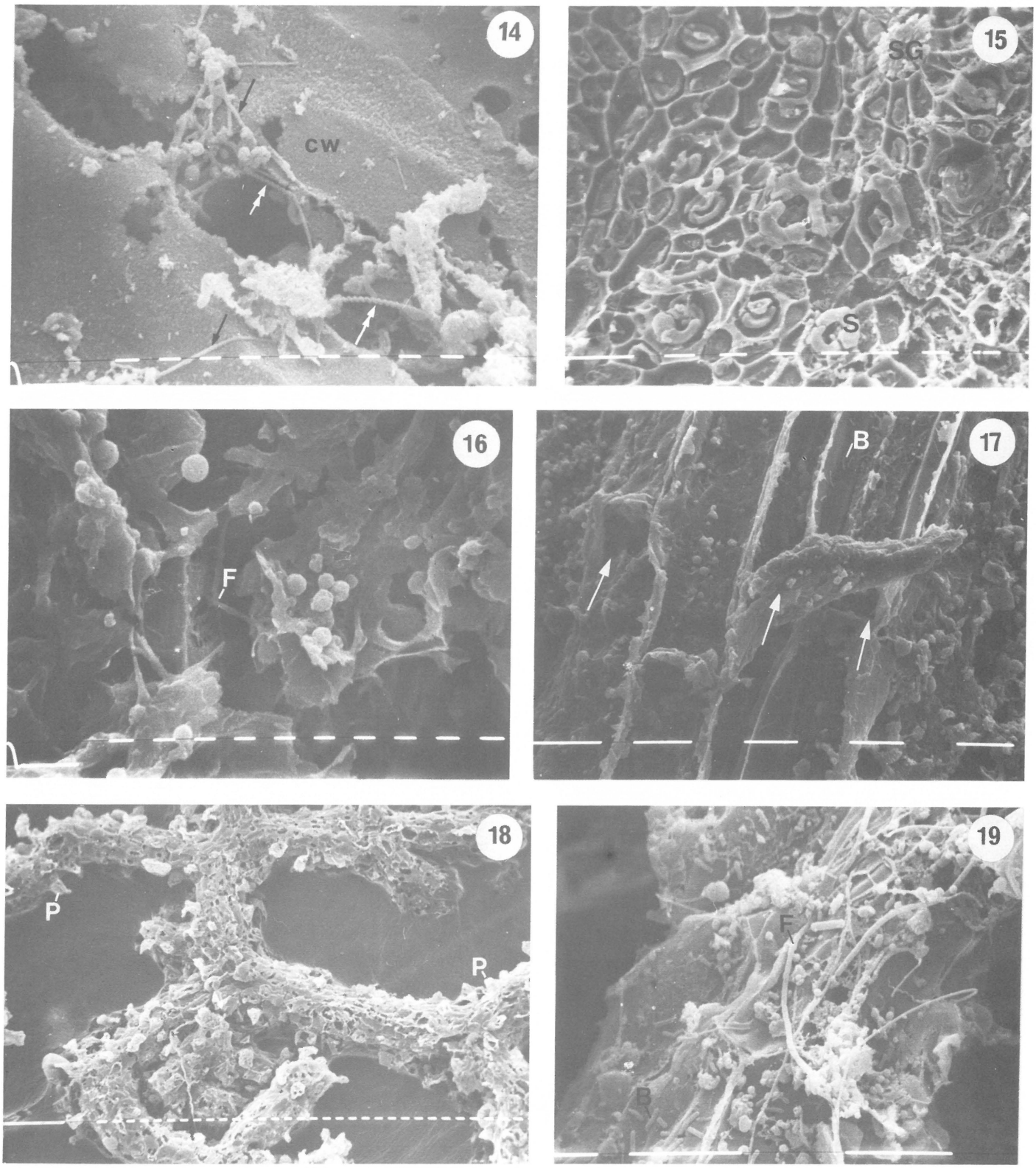








Figures 8–13 Light micrographs (Figures 9, 10 & 12) and scanning electron micrographs (Figures 8, 11 & 13) of decomposing mangrove leaves. **8**. Two-week sample of *B. gymnorrhiza* showing surface erosion and mass of fungal hyphae. Bar = 10 μm. **9 & 10**. Three-week sample of *B. gymnorrhiza* (Figure 9) and *A. marina* (Figure 10) depicting splitting of leaf tissues. Bars = 100 μm. **11**. Breakdown of cuticle in a 3-week sample of *B. gymnorrhiza*. Bar = 10 μm. **12 & 13**. Six-week samples showing further decomposition of leaf tissues of *B. gymnorrhiza* (Figure 12) and degradation of outer wall of epidermal cells of *A. marina* (Figure 13). P palisade mesophyll. Bars = 20 μm for Figure 12 and 10 μm for Figure 13.



Figures 14–19 Scanning electron micrographs of decomposing mangrove leaves. **14 & 15**. Six-week samples of *A. marina* showing spiral bacteria (double arrows) and fungal hyphae (arrows) associated with splits in epidermal cell walls (Figure 14), and epidermal tissue with eroding outer wall of cells (Figure 15). Bars = 1 μm for Figure 14 and 10 μm for Figure 15. S guard cells of stomata; SG salt gland. **16 & 17**. Degradation of mesophyll cells (Figure 16) and some degradation of vessel elements of a small vein (Figure 17 arrows) in 9-week samples of *B. gymnorrhiza*. B bacteria; F fungal hyphae. Bars = 1 μm for Figure 16 and 10 μm for Figure 17. **18 & 19**. Twelve-week samples of *A. marina* showing portion of main leaf veins with attached parenchyma cells (Figure 18), and surface of vein cells with numerous bacteria and fungal hyphae present. B bacteria; F fungal hyphae; P parenchyma cells. Bars = 10 μm.

stages of degeneration, whereas the inner walls were relatively intact. Cross-walls within these elements were clearly visible. Disintegration of the cell walls was frequently initiated around the pits which developed into large holes and led eventually to the fragmentation of these cell walls (Figure 17). Within some of the cells and along their length, bacilli were found to occur in distinct rows. At this stage both bacteria and fungi were abundant.

64–85 days after submergence

After 85 days the most obvious physical change in both species was the disappearance of all the softer tissues, reducing main leaf veins to a skeleton of vascular tissue. Two stages could be distinguished in this process. In the earlier stage parenchyma cells were still atttached along the veins (Figure 18). Large numbers of bacteria and fungi were present (Figure 19) which appeared to be active in breaking down the cell wall into minute fragments. The later stage of decomposition showed the leaves reduced to skeletons without attached cells (Figure 20). Large numbers of bacilli were again seen in regular rows along the length of the cells (Figure 21). These bacilli were found close to holes in the cell wall and it is considered that they might possibly have been responsible for cellulose pitting in these cells.

The sequence of events in leaf decomposition is summarized in Table 1.

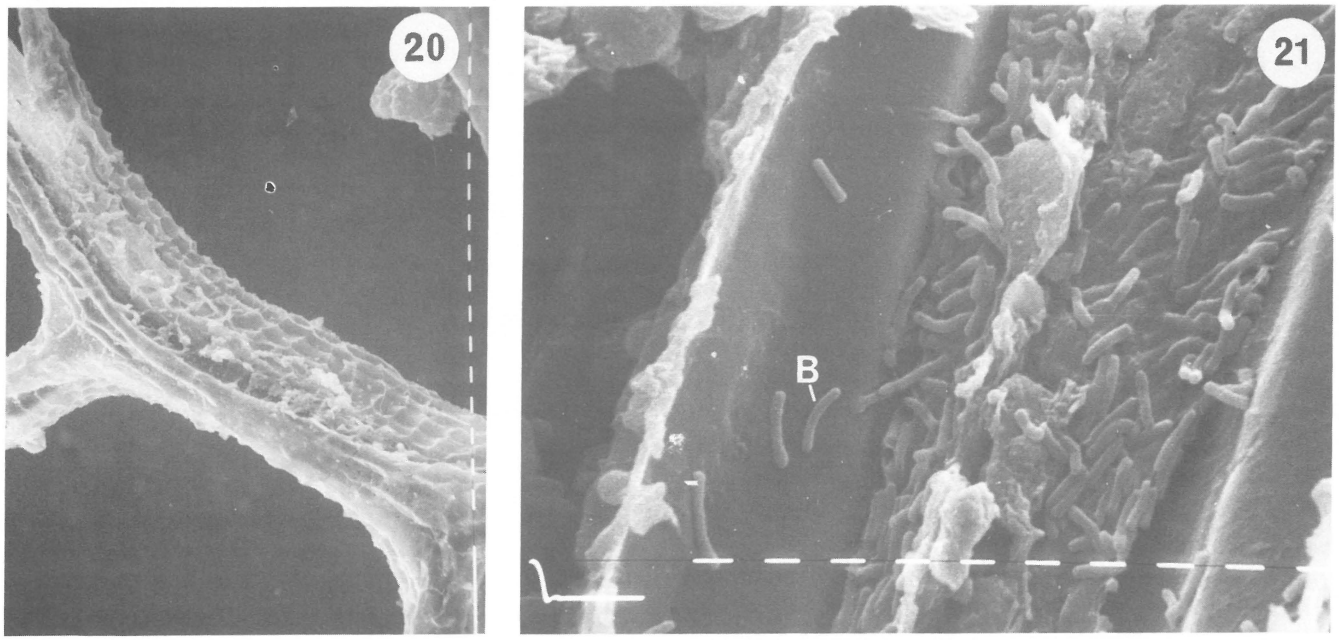
Fungal isolations

Most of the fungi seemed to show no preference for either of the two media used. In general, however, there was a larger number of fungal colonies and more vigorous growth of fungi occurred on MEA at 15‰ salinity. Growth on plates prepared by the leaf-washing method was rapid. More isolations were made from samples taken from the middle portions of the leaves.

The percentage frequency for each species was calculated by expressing the number of isolations per species as a percentage of the total number of isolations of all species. These values indicate the relative presence of the species isolated at the different harvests as well as from senescing leaves (Table 2).

Where data do not appear in the Table, there were no values recorded for those harvests. Leaves of both species were found to harbour 20–25 species of fungi, but some of these were lost during isolation. It is suspected that some of those lost might have been oomycetous genera which have been isolated by other workers during early stages of leaf decomposition (Fell *et al.* 1975). The majority of the fungi which were isolated successfully belonged to the Subdivision Deuteromycotina (fungi imperfecti) (Kohlmeyer & Kohlmeyer 1979). This group contributed more than 80% of the total isolates with the most common genera *Fusarium, Cladosporium* and *Trichoderma* accounting for 50% of the total. In most cases there was agreement between the values for the two mangrove species. Using Newell's (1976) criterion that species with a frequency of occurrence of 5% or more can be considered 'prevalent fungi', only six species isolated in this study occupy this category. Previous work has shown that most of the genera have been recorded from marine environments, although not as many different isolates were obtained from this study (Fell & Master 1973; Newell 1976; Kohlmeyer & Kohlmeyer 1979; Araujo *et al.* 1981). The sequence of occurrence of fungi on the leaves of the two mangrove species is indicated in Figures 22 & 23.

Cladosporium and *Fusarium* persisted throughout the



Figures 20–21 Scanning electron micrographs of 12-week samples of decomposing leaves of *A. marina* showing remains of leaf veins without attached cells (Figure 20) and large numbers of bacteria associated with leaf vein cells (Figure 21). B bacteria. Bars = 10 μm for Figure 20 and 1 μm for Figure 21.

Table 1 Sequence in the decomposition of *A. marina* and *B. gymnorrhiza* leaves

Time (days)	Events
Senescent leaves	Fungi and bacteria in phyllosphere
1–7	Accumulation of debris on leaf surfaces; initial colonization by bacteria
7–14	Localized erosion of leaf surface; in addition to bacteria, colonization of external surface by fungi and diatoms
14–21	Peeling of cuticle; splitting of mesophyll tissue; marked increase in bacteria and fungi
21–42	Erosion of entire leaf surface; colonization of mesophyll by bacteria and fungi
42–64	Fragmentation of leaves; rich micro-flora of cellulolytic bacteria and fungi
64–85	Skeletonization of leaf; microbial population appears to be stabilized

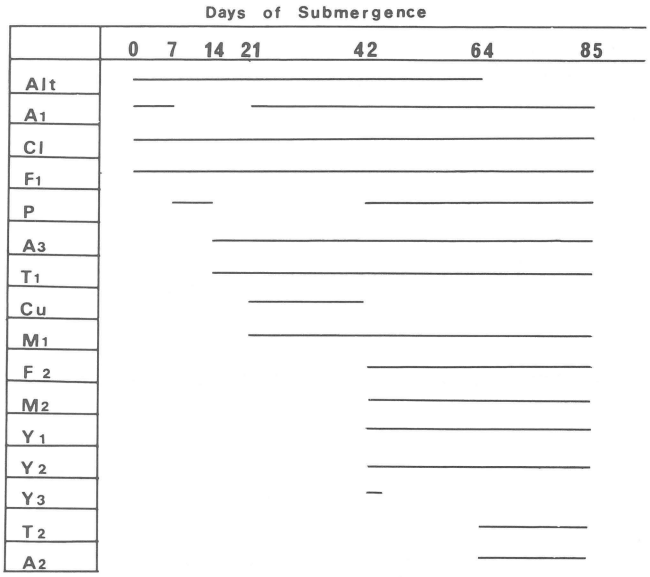


Figure 22 Sequence of fungi on leaves of *A. marina* during decomposition (— = period in which the fungus was recorded; symbols as for Table 2).

decomposition period, most of the others were isolated only later. It would appear that most of the isolates were probably parasitic and saprobic terrestrial species and not obligate or even facultative marine species (Kohlmeyer 1969).

Bacterial cultures

Attempts to isolate cellulolytic bacteria proved unsuccessful. Bacterial colonies grew profusely on most culture plates and, while all sampling procedures were successful, the leaf-washing method appeared to produce the largest number of colonies. Both gram positive and negative cocci and bacilli were isolated.

General Discussion

It is clear from this study that microbial activity was important in the decomposition of leaves of *A. marina* and *B. gymnorrhiza*. There has been criticism of the use of litter bags for studies of decomposition as these bags are claimed to eliminate predation by macro-invertebrates. However, these criticisms are not relevant to this study which had as its object the effect of microbial activity on decomposition.

Macro-invertebrates have been shown to play an important role in the breakdown of mangrove leaf litter to detritus (Boonruang 1978; Malley 1978; Leh & Sasekumar 1985; Robertson 1986). Decomposition is then continued through the action of fungi and bacteria on this leaf detritus (Fell *et al.* 1975; Cundell *et al.* 1979).

The more rapid decomposition of *A. marina* leaves can probably be attributed to differences in morphology, anatomy and chemistry between leaves of these two mangrove species. *B. gymnorrhiza* has glabrous leaf surfaces which are covered with a thick cuticle. In contrast, only the adaxial surface of *A. marina* leaves has a thick cuticle; the lower (abaxial) surface is covered with numerous fine, non-glandular hairs. Fahn & Shimony (1977) showed that most of the non-glandular hair is covered by a very thin cuticle which may even be absent in parts. Pubescent leaves are regarded as superior spore traps (Gregory 1971). That hairs on the abaxial surface act as efficient traps was shown by the numerous spores and fungal hyphae found in between the non-glandular trichomes of *A. marina* leaves. Spores that are trapped in this way would probably not be easily washed off the leaf surface.

The amount of tannin in the leaves could probably influence the rate of decomposition. Loss of tannins from leaves has been shown to coincide with rapid increases in the densities of bacteria on mangrove leaves (Cundell *et al.* 1979; Robertson 1988). Tannin, which is

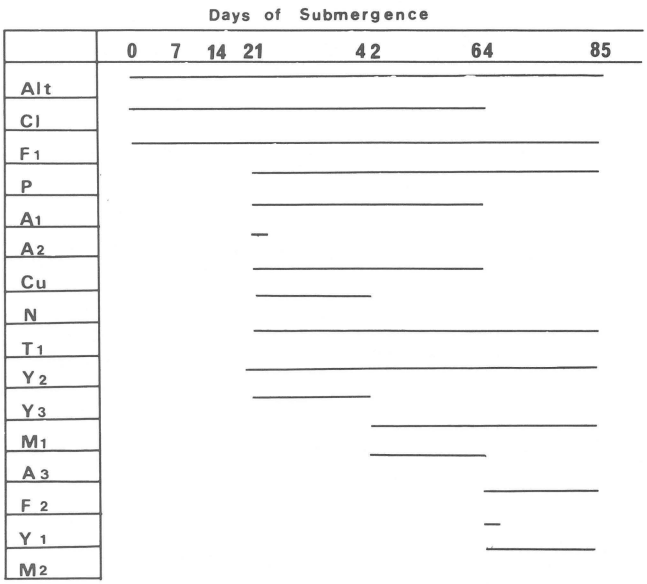


Figure 23 Sequence of fungi on leaves of *B. gymnorrhiza* during decomposition (— = period in which the fungus was recorded; symbols as for Table 2).

Table 2 Percentage frequencies of fungi on leaves of *A. marina* and *B. gymnorrhiza*

Fungi	Mangrove substrates	Days of submergence 0	7	14	21	42	64	85	Total %
Deuteromycotina									
Alternaria sp.	*A. marina*	0.56	0.56	0.56	1.67	1.11	0.56		5.02
(Alt)	*B. gymnorrhiza*	0.61	0.61	0.61	1.21	2.42	0.61	1.21	7.28
Aspergillus niger	*A. marina*	0.56	1.11		2.22	2.22	2.78	2.22	11.11
(A 1)	*B. gymnorrhiza*				1.82	1.21	0.61		3.64
Aspergillus sp.	*A. marina*						1.67	0.56	2.23
(A 2)	*B. gymnorrhiza*				0.61				0.61
Aspergillus sp.	*A. marina*			0.56	2.22	1.11	0.56	0.56	5.01
(A 3)	*B. gymnorrhiza*					2.42	0.61		3.03
Cladosporium sp.	*A. marina*	0.56	2.22	3.89	5.00	2.78	2.22	2.22	18.89
(Cl)	*B. gymnorrhiza*	6.67	4.24	1.82	2.42	1.82	0.61		17.58
Curvularia sp.	*A. marina*				0.56	1.67			2.23
(Cu)	*B. gymnorrhiza*				0.61	1.82	1.82		4.25
Fusarium sp.	*A. marina*	1.67	1.67	1.67	2.78	2.22	3.89	3.89	17.79
(F 1)	*B. gymnorrhiza*	1.82	1.82	1.21	2.42	3.64	7.88	4.85	23.64
Fusarium sp.	*A. marina*					0.56	0.56	1.67	2.79
(F 2)	*B. gymnorrhiza*						1.21	0.61	1.82
Penicillium sp.	*A. marina*		0.56	0.56		1.11	1.11	1.67	5.01
(P)	*B. gymnorrhiza*				0.61	0.61	0.61	1.21	3.04
Trichoderma reesii	*A. marina*			0.56	2.22	2.78	3.89	5.56	15.01
(T 1)	*B. gymnorrhiza*				2.42	1.82	4.24	6.06	14.54
Trichoderma sp.	*A. marina*						0.56	0.56	1.12
(T 2)	*B. gymnorrhiza*								–
Ascomycotina									
Yeast 1 (orange)	*A. marina*					0.56	0.56		1.12
(Y 1)	*B. gymnorrhiza*						1.21		1.21
Rhodotorula sp.	*A. marina*					1.67	1.67	0.56	3.90
(Y 2)	*B. gymnorrhiza*				3.03	1.21	1.21	2.42	7.87
Yeast 3 (white)	*A. marina*					1.11			1.11
(Y 3)	*B. gymnorrhiza*				1.82	1.21			3.03
Neurospora sp.	*A. marina*								–
(N)	*B. gymnorrhiza*				1.21	0.61			1.82
Zygomycotina									
Mucor sp.	*A. marina*				1.11	1.67	0.56	0.56	3.90
(M 1)	*B. gymnorrhiza*					1.21	1.82	1.82	4.85
Mucor sp.	*A. marina*					1.12	0.56	2.23	3.91
(M 2)	*B. gymnorrhiza*						0.61	0.61	1.22

known as an enzyme inhibitor and antimicrobial agent, has been shown to inhibit bacterial and fungal growth (Lewis & Papavizas 1967; Benoit & Starkey 1968). Anatomical sections from this study confirmed that *A. marina* has low or different tannin (polyphenolic) concentrations, especially relative to *B. gymnorrhiza* (Smith 1987; Robertson 1988). It is probably reasonable to assume that the slower rate of decomposition of leaves of the latter species is due at least in part to the higher tannin (polyphenolic) content.

Earlier colonization of abaxial, as compared to adaxial, leaf surfaces was observed. The adaxial surfaces of both species are relatively smooth: the hairs on the abaxial surface of *A. marina* have already been noted; while on *B. gymnorrhiza* leaves, spores were frequently seen lodged within the abaxial stomatal pits. The anatomical sections also revealed smaller amounts of tannin on the abaxial side of the leaves of the latter species.

Micro-organisms can readily digest leaves and convert substantial fractions into their own biomass — fungi with an efficiency of 40–70%, bacteria with an efficiency of approximately 50% (Brock 1966). The growing hyphae of many mangrove fungi produce cellulolytic and pectolytic enzymes which are responsible for degradation of plant tissue (Rai & Chowdhery 1976). Two of the more common genera, namely *Trichoderma* and *Fusarium*, are strongly cellulolytic (Rai & Chowdhery 1976; Araujo *et*

al. 1981) and it is clear that they must have played a significant role in the breakdown of the leaf tissue.

The increase in numbers of both bacteria and fungi during the period of this study suggests that both groups were important in decomposition. In decomposing mangrove leaves there is a decrease in carbon (Cundell *et al.* 1979; Fell & Master 1980; Robertson 1988) which is attributable to the fragmentation of the cell walls (Torzilli 1982). In the estuarine food web there is evidence that micro-organisms degrading the structural material of the mangrove leaves support a population of detritus consumers including invertebrate species and fish (Odum & Heald 1975).

There has been little research on the role of micro-organisms in the decomposition of litter in the west Indian Ocean mangroves, but these preliminary results have confirmed the importance of microbial activity in this process and therefore probably in the food web of our estuaries. Further research in this field is in progress.

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References

ARAUJO, A.D., D'SOUZA, J. & KARANDE, A. 1981. Studies on fungi and yeasts from the west coast of India. *Ind. J. Mar. Sci.* 10: 341–345.

BENOIT, R.D. & STARKEY, R.L. 1968. Enzyme inactivation as a factor in the inhibition of decomposition of organic matter by tannins. *Soil Sci.* 105: 203–209.

BOONRUANG, P. 1978. The degradation rates of mangrove leaves of *Rhizophora apiculata* (Bl.) and *Avicennia marina* (Forsk.) Vierh. at Phuket Island, Thailand. *Phuket Mar. Biol. Centre, Res. Bull.* 26: 1–7.

BROCK, T.D. 1966. Principles of microbial ecology. Prentice Hall, New Jersey.

CUNDELL, A.M., BROWN, M.S., STANDFORD, R. & MITCHELL, R. 1979. Microbial degradation of *Rhizophora mangle* leaves immersed in the sea. *Est. Coast. Mar. Sci.* 9: 281–286.

FAHN, A. & SHIMONY, C. 1977. Development of the glandular and nonglandular leaf hairs of *Avicennia marina* (Forsskal) Vierh. *Bot. J. Linn. Soc.* 74: 37–46.

FELL, J.W., CEFALU, R.C, MASTER, I.M. & TALLMAN, A.S. 1975. Microbial activities in the mangrove (*Rhizophora mangle*) leaf detrital system. In: Proc. Internat. Symp. Biol. & Mgmt Mangroves, Hawaii, Vol. 2., eds Walsh, G.E., Snedaker, S.C. & Teas, H.J., Univ. of Florida, Gainesville.

FELL, J.W. & MASTER, I.M. 1973. Fungi associated with the degradation of mangrove (*Rhizophora mangle* L.) leaves in South Florida. In: Estuarine microbial ecology, eds Stevenson, L.H. & Colwell, R.R., Univ. S. Carolina Press, Columbia.

FELL, J.W. & MASTER, I.M. 1980. The association and potential role of fungi in mangrove detrital systems. *Bot. Mar.* 23: 257–263.

GOLLEY, F., ODUM, H.T. & WILSON, R.F. 1962. The structure and metabolism of a Puerto Rican red mangrove forest in May. *Ecology* 43: 9–19.

GREGORY, P.H. 1971. The leaf as a spore trap. In: Ecology of leaf surface micro-organisms. eds Preece, T.F. & Dickinson, C.H., Academic Press, New York.

KOHLMEYER, J. 1969. Ecological notes on fungi in mangrove forests. *Trans. Brit. Mycol. Soc.* 53: 237–250.

KOHLMEYER, J. & KOHLMEYER, E. 1979. Marine mycology. Academic Press, New York.

LEH, C.M.U. & SASEKUMAR, A. 1985. The food of sesarmid crabs in Malaysian mangrove forests. *Malay. Nat. J.* 39: 135–145.

LEWIS, J.A. & PAPAVIZAS, G.C. 1967. Effects of tannins on spore germination and growth of *Fusarium solani f. phaseoli* and *Verticillium albo-atrum*. *Can. J. Microbiol.* 13: 1655–1661.

MALLEY, D.F. 1978. Degradation of mangrove litter by the tropical sesarmid crab *Chiromanthes onychophorum*. *Mar. Biol.* 49: 377–386.

NEWELL, R.C. 1982. The energetics of detritus utilization in coastal lagoons and nearshore waters. *Oceanologica Acta* 30: 347–355.

NEWELL, S.Y. 1976. Mangrove fungi: the succession in the mycoflora of red mangrove (*Rhizophora mangle* L.) seedlings. In: Recent advances in aquatic mycology, ed. Jones, E.B.G., Wiley, New York.

ODUM, W.E. & HEALD, E.J. 1972. Trophic analyses of an estuarine mangrove community. *Bull. Mar. Sci.* 22: 671–738.

ODUM, W.E. & HEALD, E.J. 1975. The detritus-based food web of an estuarine mangrove community. In: Estuarine research, ed. Cronin, L.E., Academic Press, New York.

PADIA, R. 1980. A vegetation map of the Mgeni/Beachwood Nature Reserve, Durban, from aerial photographs. B.Sc. Hons. report, Univ. of Durban-Westville.

RAI, J.N. & CHOWDHERY, H.J., 1976. Cellulolytic activity and salinity relationship of some mangrove swamp fungi. *Nova Hedwigia* 27: 609–617.

ROBERTSON, A.I. 1986. Leaf-burying crabs: their influence on energy flow and export from mixed mangrove forests (*Rhizophora* spp.) in north-eastern Australia. *J. Exp. Mar. Biol. Ecol.* 102: 237–248.

ROBERTSON, A.I. 1988. Decomposition of mangrove leaf litter in tropical Australia. *J. Exp. Mar. Biol. Ecol.* 116: 235–247.

SMITH, T.J. 1987. Seed predation in relation to tree dominance and distribution in mangrove forests. *Ecology* 68: 266–273.

SNEDAKER, S.C. 1978. Mangroves: their value and perpetuation. *Nat. Resour.* 14: 6–13.

STEINKE, T.D. & WARD, 1987. Degradation of mangrove leaf litter in the St Lucia Estuary as influenced by season and exposure. *S. Afr. J. Bot.* 53: 323–328.

TEAS, H.J. 1976. Productivity of Biscayne Bay mangroves. Univ. Miami Sea Grant Special Report No. 5: 103–112.

TORZILLI, A.P. 1982. Polysaccharide production and cell wall degradation by several salt marsh fungi. *Mycologia* 74: 297–302.