

Thraustochytrid and fungal component of marine detritus. III. Field studies on decomposition of leaves of the mangrove *Rhizophora apiculata*

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ABSTRACT: The sequence of colonisation, densities and biomass of fungi, thraustochytrid protists and bacteria during decomposition of leaves of the mangrove *Rhizophora apiculata* Blume, as well as the accompanying biochemical changes of the detritus, were studied in 2 litterbag experiments at Goa, India. Three phases were observed. (1) Thraustochytrids and the fungi *Cladosporium herbarum* and *Halophytophthora vesicula* colonised detritus during the first week, characterised by a rapid loss of detrital dry weight and a reduction in proteins, carbohydrates, reducing sugars, phenolics and cellulose. (2) Decline of most of the organic constituents in detritus to almost the lowest observed levels within 21 d was accompanied by an increase in fungal and bacterial biomass. Fungi and bacteria together contributed maxima of 0.052 and 0.065% to dry weight of 21 and 35 d detritus respectively. Protein values never increased with decomposition and microbial biomass buildup, although C:N values declined. (3) Total fungal and bacterial biomass decreased after 3 and 5 wk, respectively. However, thraustochytrids and marine fungal species such as *Cirrenalia basiminuta* and 'Hyphomycete XVII' attained high densities by 56 to 60 d. Most of the species present initially in the detritus were observed even in the last stages of decomposition examined (56 to 60 d). Thraustochytrids and fungi were isolated both from surface-sterilized and untreated detritus, suggesting epi- and endobiontic modes of life. Distinct sequences of fungal and thraustochytrid colonisation, presence of high fungal biomass relative to bacterial biomass, the high densities of thraustochytrids and accompanying biochemical changes indicate the importance of these organisms in the mangrove detrital processes.

KEY WORDS: Thraustochytrids · Fungi · Mangrove detritus

INTRODUCTION

Detritus derived from coastal marine macrophytes is colonised by bacteria (Pomeroy 1980, Bowen 1987, Peduzzi & Herndl 1991), whose metabolic activities are important in the breakdown of the particulate detritus, as well as release and utilisation of dissolved organic matter (Koop et al. 1982, Hodson & Moran 1989). Since bacterial biomass on detritus could serve as carbon and nitrogen sources in the nutrition of detritivores, and bacterially mediated biochemical transformations of detritus could render the refractory detritus more nutritional, these 2 aspects have been studied in great

detail (Newell & Field 1983, Mann 1988, Pomeroy & Wiebe 1988). However, the present consensus is that, except in certain cases (Kenworthy et al. 1989, Sathe & Raghukumar 1991), microorganisms generally contribute less than 1% to detritus dry weight (Findlay & Meyer 1984, Melillo et al. 1984, Bowen 1987, Blum et al. 1988). This has cast serious doubt on the importance of microbial biomass as food for metazoan detritivores (Bowen 1987, Blum et al. 1988, Kenworthy et al. 1989). In this context, a consideration of fungi and the fungi-like unicellular osmoheterotrophic protists, the thraustochytrids (Moss 1986), would give a more complete picture of the microbial role in the detrital food web.

Several mycological studies on leaf- and seedling-derived detritus in subtropical mangroves have eluci-

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dated species composition and succession (mycoseres) and abundance of thraustochytrids (Newell 1976, Fell & Master 1980, Nakagiri et al. 1989), as well as fungal colonisation and biochemical changes (Cundell et al. 1979). However, similar studies on tropical mangroves are few. In addition, information on fungal biomass and their fluctuations in mangrove detritus is meagre (Blum et al. 1988). Data on the biochemical composition of mangrove detritus accompanying mycoseres and fungal biomass which might provide clues on the role of fungi in these substrata are not available as they are for bacteria.

In this paper, we address the following problems concerning leaf detritus of the mangrove *Rhizophora apiculata* Blume: (1) the densities and biomass of thraustochytrids and fungi in different decompositional stages, with relation to bacteria; (2) the sequence of colonisation of fungal species with progress of decomposition; and (3) the broad biochemical changes which accompany the above.

MATERIALS AND METHODS

The experiments were carried out at Chorao mangrove in Goa, India (15° 27' to 15° 38' N, 73° 42' to 75° 50' E), situated about 8 km upstream from the mouth of the Mandovi river to the Arabian Sea.

Litterbag experiments. Two litterbag experiments were carried out: 5 December 1988 to 3 February 1989 (Expt 1) and 8 November 1989 to 3 January 1990 (Expt 2). The water temperature during these periods varied from 25 to 30°C and salinity from 25 to 30‰. Nylon litter bags of 1 mm mesh, measuring 1 × 0.25 m and divided into 4 pouches, were used. Two yellow, senescent leaves of *Rhizophora apiculata* plucked from the trees were placed in each pouch of the bag (8 leaves per bag). Fifteen such bags, with a total of 120 leaves, were attached to a plastic frame and placed in a creek in the mangrove below low tide level. One or two bags were removed at intervals of 4 to 7 d, for a total period of 60 and 56 d in Expts 1 and 2 respectively, and brought to the laboratory for analysis.

Isolation and enumeration of fungi and thraustochytrids. Discs of 5 mm diameter from the detritus were used directly or following surface-sterilization with a 0.5% sodium hypochlorite solution (Newell & Fell 1982). Twenty discs were homogenised in 15 ml of sterile seawater. Aliquots of 0.1 ml were plated onto 5 Petri plates with Corn Meal Agar made up of 50% seawater or a 'thraustochytrid medium' [glucose 0.01%, peptone 0.01%, yeast extract 0.05%, vitamin mix according to Vishniac (see Jones 1971), agar 1.2%, seawater 100 ml]. The media contained 0.05 g streptomycin and 10 000 units penicillin per 100 ml. After

incubation for 1 wk at room temperature, mean numbers of colonies of thraustochytrids and tentatively identified individual species of fungi for the different media and treatments were recorded. These values were converted to mean numbers per g dry wt detritus based on that of a leaf disc. From among the fungal species growing out of non-surface-sterilized material, only those which were also observed on surface-sterilized ones were taken into account, since the others might have arisen from spore contaminants. From among the 2 media and treatments, the highest mean numbers of such individual species were pooled and expressed as total derived numbers.

Thraustochytrids were also enumerated by using the pine pollen baiting/MPN method of Gaertner (1968), in which 1.0, 0.5, 0.25, 0.1 and 0.05 ml of the homogenates of surface-sterilized and non-surface-sterilized detritus were added to 5 ml of sterile seawater in 20 ml flat-bottomed screw-cap tubes and baited with sterilised pine pollen. Numbers per ml of homogenate were estimated using Thomas' formula (American Public Health Association 1981) and converted to numbers per g detritus based on the dry weight material present in 1 ml suspension.

Fungi were also isolated by moist-chamber (Kohlmeyer & Kohlmeyer 1979) and sterile-seawater (Newell & Fell 1980) incubations.

Biomass estimations. All estimations were carried out with material fixed in 3% formalin. Biomass of mycelial fungi was estimated after clearing the detrital material with KOH and hydrogen peroxide and staining for hyphae with lactophenol-cotton blue (Koske & Gemma 1989). Total length and average widths of hyphae were measured in 5 sets of 10 random microscopic fields (total of 50) for each detrital sample. The mean of the 5 sets was converted to biovolume, considering the hyphae as cylinders. Biomass was derived from biovolumes using a conversion factor of 200 mg dry wt cm⁻³ fungus (Newell et al. 1986).

Mean numbers of bacteria were estimated from 5 sets of 10 microscope fields as above using the Acridine Orange Direct Count (AODC) method (Parsons et al. 1984). The values were converted to bacterial carbon using a factor of 20 fg per bacterial cell (Peduzzi & Herndl 1991), and a factor of 2 was used to convert bacterial carbon to dry wt (Blum et al. 1988).

Biochemical analyses. Proteins were estimated using the modification of Lowry's method (Peterson 1977), reducing sugars by the DNS method (Miller 1960) and carbohydrates as described by Dawes (1981). Total phenols were extracted in methanol overnight and spectrophotometrically estimated using Folin Denis reagent (Swain & Hillis 1959). Lignin and cellulose were estimated gravimetrically following Allen (1974). Ash content was determined as loss of

weight after heating at 450 to 500°C for 5 h. Total C and N were analysed using a Heraeus CHN.O Rapid Analyser at the Indian Institute of Technology, Madras.

We have expressed the amounts of various organic constituents in detritus as (1) percentages of ash-free dry weight (AFDW) and (2) percentages of dry weight detritus, as has been conventionally done in literature. However, fresh detritus gradually loses weight through leaching of soluble organics and a given area of detritus weighs much less during later stages of decomposition than at the beginning. Therefore, the changes in detritus cannot be related to the original material in terms of weight, but only as values normalised to a parameter which remains constant. Assuming that no substantial additions or removal of minerals from detrital material occurs, we have presented values of the detrital organic constituents (*a*) as those normalised to the original ash content using the following formula:

$$\text{Normalised value of } a \text{ in detritus} = \frac{\% \text{ ash in detritus on Day 0}}{\% \text{ ash in detritus on Day } x} \times \% a \text{ in detritus on Day } x \quad (1)$$

RESULTS

Sequences of colonisation

Three distinct sequences were observed.

(1) Senescent, attached leaves (0 d) harboured yeasts, and terrestrial species of fungi such as *Fusarium moniliforme* Sheld., and non-sporulating species in Expt 1 (Table 1) and species of *Acremonium* and *Aspergillus*, *Cladosporium herbarum* (Pers.) Link ex Gray, *F. moniliforme* and non-sporulating species in Expt 2 (Table 2). All these species persisted in detritus up to the last stage of decomposition examined (56 and 60 d) or re-appeared then.

(2) The first samplings carried out after submergence of leaves (4 and 7 d) yielded *Cladosporium herbarum* in Expt 1 (Table 1), the oomycete *Halophytophthora vesicula* (Anast. et Church) Ho et Jong in Expt 2 (Table 2) and thraustochytrid protists in both experiments. *Labyrinthula* sp. was recorded after 53 and 14 d respectively (Tables 1 & 2). These species also persisted until the end of the study period. *H. vesicula* and thraustochytrids attained peak densities by 56 to 60 d.

Table 1. *Rhizophora apiculata*. Densities of fungi in decomposing mangrove leaves (no. g⁻¹ dry wt). Expt 1

Species	Age of detritus (d)								
	0	4	7	14	21	28	46	53	60
<i>Acremonium</i> sp.	0	0	3 500	0	0	0	5 500	0	0
<i>Aspergillus</i> spp.	0	0	1 200	0	0	0	0	0	24 000
<i>Cladosporium herbarum</i>	0	63 300	44 600	158 000	16 600	12 900	7 370	3 690	475 000
<i>Cirrenalia basiminuta</i>	0	0	0	0	0	0	16 600	22 100	0
<i>Fusarium moniliforme</i>	173 000	0	0	0	0	0	0	0	5 530
'Hyphomycete XVII'	0	0	0	0	11 100	10 600	81 100	103 000	0
<i>Labyrinthula</i> spp.	0	0	0	0	0	0	0	5 530	0
Non-sporulating forms	12 900	15 200	11 700	31 300	18 400	20 300	47 900	88 500	12 900
<i>Penicillium</i> sp.	759	4 690	1 170	0	0	0	3 690	0	14 700
Yeasts	7 590	7 040	7 040	1 633 000	12 900	5 530	0	1 840	5 530

Table 2. *Rhizophora apiculata*. Densities of fungi in decomposing mangrove leaves (no. g⁻¹ dry wt). Expt 2

Species	Age of detritus (d)								
	0	7	14	21	28	35	42	49	56
<i>Acremonium</i> sp.	2 610	0 ^a	2 860	6 350 ^a	25 400 ^a	28 600	19 000	2 860 ^a	2 860
<i>Aspergillus</i> spp.	2 610	0	0	0	2 860 ^a	19 000	0	0	19 000
<i>Cladosporium herbarum</i>	2 610	303 000	44 400	2 860	12 700	12 700	19 000	25 400	31 700
<i>Cirrenalia basiminuta</i>	0	0 ^a	0 ^a	0 ^a	6 350	5 720 ^a	4 290 ^a	12 700	25 400
<i>Fusarium moniliforme</i>	2 610	4 040	0	12 700	0	0	0	0	0
<i>Halophytophthora vesicula</i>	0	7 830 ^a	0 ^a	0 ^a	0	6 350 ^a	5 720 ^a	7 150	7 150
'Hyphomycete XVII'	0	0	0 ^a	6 350 ^a	19 000 ^a	8 670	7 150 ^a	12 700 ^a	19 000 ^a
<i>Labyrinthula</i> spp.	0	0	2 860	635 000	0	0	1 430	793 000	0
<i>Lulworthia</i> sp.	0	0	0 ^a	0	0 ^a	0 ^a	0	0	0
Non-sporulating forms	22 300	23 300	12 700	5 720	19 000	12 700	19 000	25 400	44 400
Yeasts	0	0	0	6 350 ^a	0	6 350	0	6 350	0

^aAlso recorded by 'seawater incubation' method

(3) The obligate marine species *Cirrenalia basiminuta* Raghukumar et Zainal and an unidentified hyphomycete (termed 'Hyphomycete XVII' in the text) were recorded only after 21 d using the plating technique, attaining peak densities in the last stages of decomposition (Tables 1 & 2). However, moist-chamber and seawater incubation yielded these 2 species, as well as the marine ascomycete *Lulworthia* sp., even after 7 d (Table 2).

Densities of thraustochytrids and biomass of fungi and bacteria

Thraustochytrids were recovered from non-surface-sterilized as well as surface-sterilized detritus using plating and baiting techniques (Figs. 1 & 2). Numbers of up to 25×10^3 and $28 \times 10^3 \text{ g}^{-1}$ dry wt detritus were detected in the 2 experiments using the former. However, the baiting technique yielded thraustochytrids more consistently (Fig. 1). Fungi contributed 0.002 to 0.014% to the detrital dry wt in Expt 1 (Fig. 3). Their

biomass was higher in Expt 2 (0.017 to 0.052% of the detrital dry wt). Maximum biomass in both the experiments occurred in 21 d detritus. There was an almost linear increase of fungal biomass in Expt 2, starting from 0 d (Fig. 3). A second, smaller biomass peak was observed in 42 or 46 d detritus. Bacteria were not detected on attached, senescent leaves (0 d detritus). Bacterial biomass in Expt 2 was less than that of the fungi up to 21 d, but increased later (see Fig. 6). The maximum bacterial biomass of 0.0624% was attained at 35 d (Fig. 4).

Biochemical changes

Decaying leaves showed a rapid loss of dry wt, attaining 66% of original values in 7 d and 50% by 14 d. No marked change was observed after this (Fig. 5). Substantial decline in reducing sugars, carbohydrates, total phenols and proteins occurred within 21 d of submergence (Figs. 5 & 6). The former was lost more rapidly, reaching minimal values in 14 d. In con-

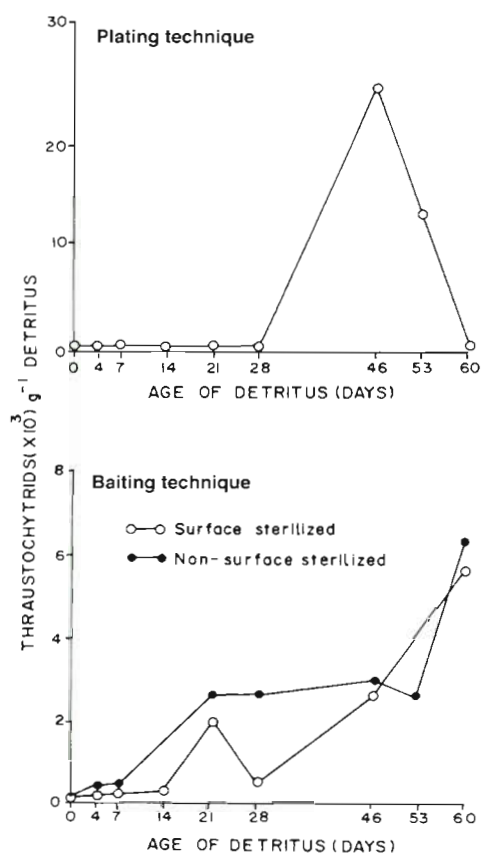


Fig. 1. *Rhizophora apiculata*. Densities of thraustochytrids in decomposing mangrove leaves, estimated by culture techniques. Expt 1

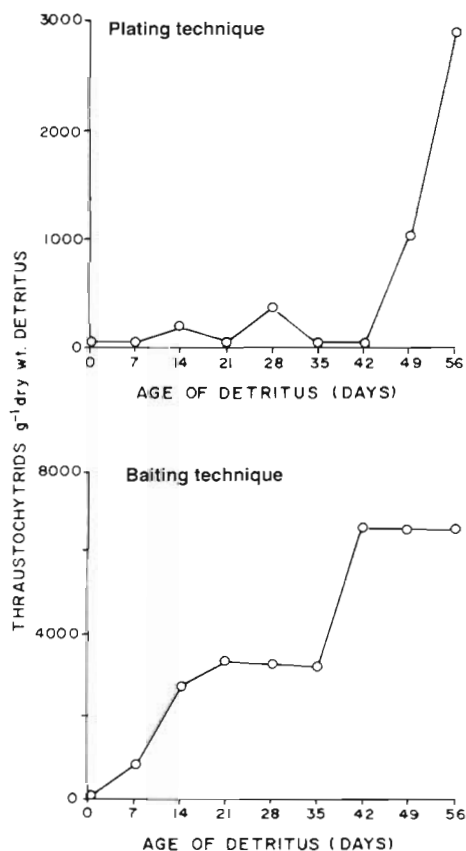


Fig. 2. *Rhizophora apiculata*. Densities of thraustochytrids in decomposing mangrove leaves, estimated by culture techniques. Expt 2

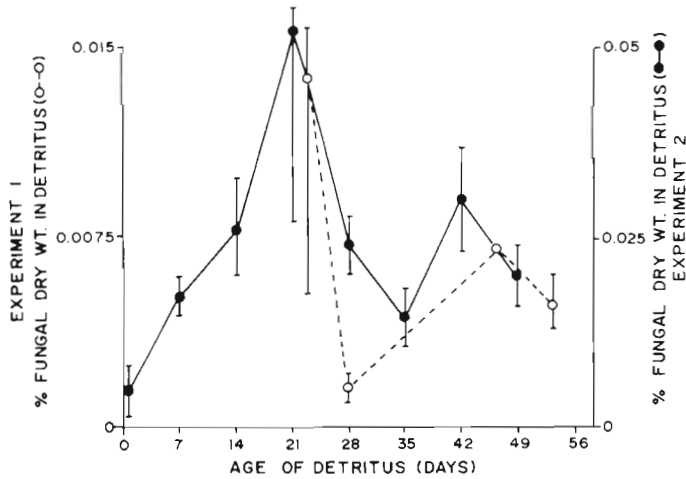


Fig. 3. *Rhizophora apiculata*. Fungal biomass in decomposing mangrove leaves

trast, loss of carbohydrates was more gradual up to 60 d (Fig. 5). Proteins decreased further after 21 d, reaching minimal values within 60 d (Fig. 5). Cellulose and lignin contents showed a steady decline with age in terms of normalised values, but not as percentages of AFDW or dry weight (Fig. 6). C/N ratios of senescent leaves decreased from 31 in 0 d detritus to 12 in 60 d detritus (Fig. 6).

DISCUSSION

The sequence of colonisation of thraustochytrids and fungi, biomass buildup of fungi and densities of thraus-

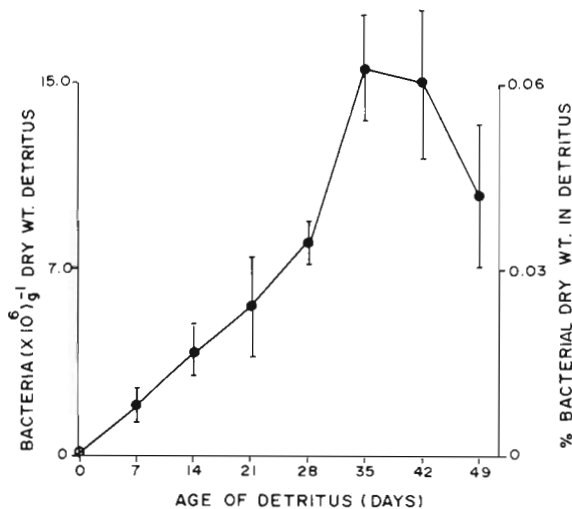


Fig. 4. *Rhizophora apiculata*. Bacterial densities and biomass in decomposing mangrove leaves. Expt 2

tochytrids on submerged mangrove leaves appears to be related to the 3 well-recognized phases of macrophytic decomposition (Godschalk & Wetzel 1978, Valiela et al. 1984) (Table 3).

(1) The initial leaching phase, in which soluble organics are rapidly lost and detrital dry weight decreases (up to 7 d in our experiments; Fig. 5), may be characterised by colonisation of thraustochytrids and fungi such as *Halophytophthora vesicula* and *Cladosporium herbarum* (Tables 1 & 3). These organisms may ecologically resemble bacteria at this stage by way of responding rapidly to the leaching nutrients in terms of metabolism and growth (Blum & Mills 1991, Peduzzi & Herndl 1991). They may also be more tolerant of the higher levels of phenolics in such detritus (Fig. 6), which are generally known to be inhibitory to microbial growth (Cundell et al. 1979, Gonzalez-Farias & Mee 1988). The earliest

samplings that revealed their presence were carried out only 4 to 7 d after submergence of the leaves. However, it is likely that these species colonise detritus even earlier, as is known from subtropical mangroves (Newell 1976, Fell & Master 1980, Newell et al. 1987). Indeed, *H. vesicula* may colonise fallen mangrove leaves within 24 h, attain 100% frequency of isolation in 30 h and totally pervade the detrital tissue (Newell et al. 1987). In addition to the above organisms, terrestrial species of fungi (geofungi) that were present even on senescent leaves attached to the trees and which were also isolated from submerged ones (Table 1) might have been involved in the initial phases of detrital dynamics.

(2) Attainment of peak fungal biomass and an increase in total microbial biomass characterize the period from 7 to 21 d, when a rapid decline of proteins, carbohydrates, reducing sugars and phenols, as well as cellulose, took place (Figs. 3 to 6). This corresponds to the second decompositional phase of detritus which normally lasts from a few days to a few weeks (Godschalk & Wetzel 1978, Valiela et al. 1984) and results in high bacterial assimilation efficiencies and turnover times (Blum & Mills 1991). Fungal biomass peaked at the end of this period (Fig. 3). Contrary to our results, Cundell et al. (1979) observed scant fungal hyphae in decomposing mangrove leaves up to 28 d. This difference might be attributed to the fact that these authors, using scanning electron microscopy, observed only the hyphae on detrital surface, and not the pervading ones. Our microscopic technique enabled us to look for both epi- and endobiontic hyphae (see also Sathe & Raghukumar 1990).

Increase in microbial biomass resulted in the buildup of total organic nitrogen content and a lower C:N ratio,

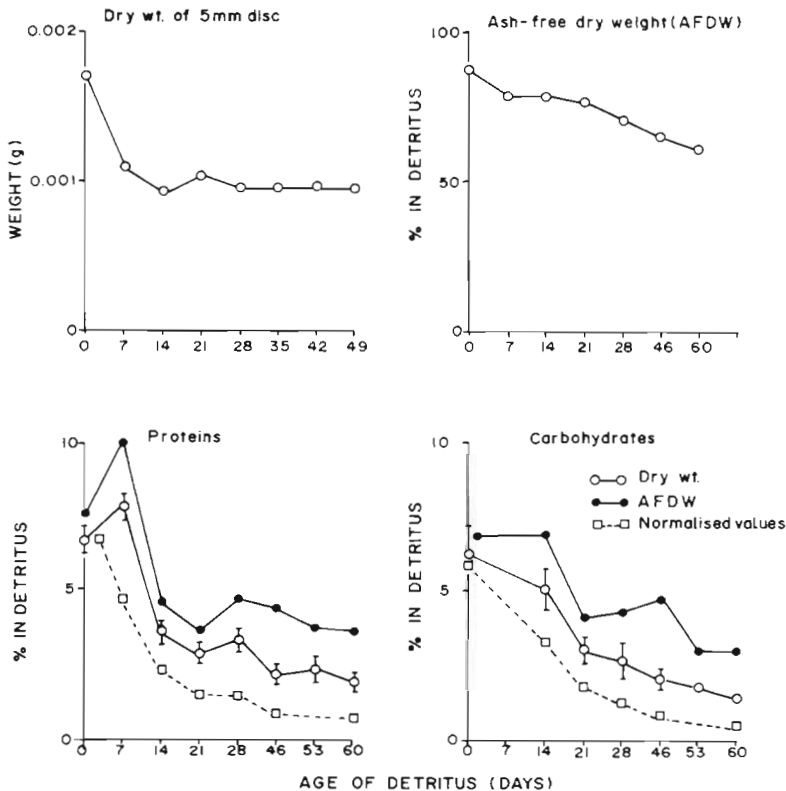


Fig. 5. *Rhizophora apiculata*. Biochemical changes during decomposition of mangrove leaves. Expt 2

but not in an enhancement of protein levels (Figs. 5 & 6). Such increases in non-protein nitrogen have been attributed to the complexing of microbial enzymes and other exudates, such as mucopolysaccharides, with carbohydrates and phenolics, resulting in recalcitrant humic nitrogen (Rice 1982, Rublee & Roman 1982, Melillo et al. 1984, Wilson et al. 1986, Sathe-Pathak et al. 1993).

(3) The third phase, following 21 d of decomposition, during which the substrate had become depleted in nutrients and phenolics, appears to be characterised by an increase in densities of marine species such as *Cirrenalia basiminuta* and 'Hyphomycete XVII'. These may, however, colonise detritus much earlier (7 d in Expt 2; Table 2). Such species may either have a slow rate of biomass buildup or an intolerance to the high levels of phenolics in early stages. The high densities attained by these species as well as thraustochytrids in late stages of this third phase of decomposition, and a further decline in cellulose and lignin during this period, indicate an active role for these organisms in the recalcitrant detritus. Although cellulases and other enzymes capable of degrading complex polysaccharides have not been demonstrated in *Halophytophthora vesicula* and thraustochytrids (Bahnweg 1978, 1979), this aspect may need further examination.

The attainment of peak bacterial biomass by 35 d in this study, much later than in the case of fungi, is similar to results obtained for seagrass and mangrove leaf detritus by Rublee & Roman (1982), Blum et al. (1988), and Robertson (1988). These authors observed maximum bacterial standing crops after 35 to 40 d of decomposition. Rublee & Roman (1982) contended that bacteria may play important roles in both the early and late stages of decomposition, while fungi may predominate in the intermediate stages. Blum et al. (1988) sug-

Table 3. *Rhizophora apiculata*. Major events during colonization of decomposing mangrove leaves by thraustochytrids and fungi

Event	Age of detritus	Thraustochytrids and fungi	Biochemical changes
1	0 d: Senescent, attached leaves	Phylloplane fungi present; few bacteria; low microbial biomass	High amounts of all organic constituents
2	0-7 d after submergence	Colonization by <i>Halophytophthora vesicula</i> and thraustochytrids; low microbial biomass	Rapid loss of dry wt; leaching of proteins, carbohydrates, reducing sugars and phenolics
3	7-21 d after submergence	Rapid increase in fungal and bacterial biomass; peak fungal biomass	Rapid decline of proteins, carbohydrates, reducing sugars, phenolics and cellulose
4	21-60 d after submergence	Peak bacterial biomass by 35 d; subsequent decline of fungal and bacterial biomass; high densities of marine fungi and thraustochytrids	Further decline of cellulose and lignin

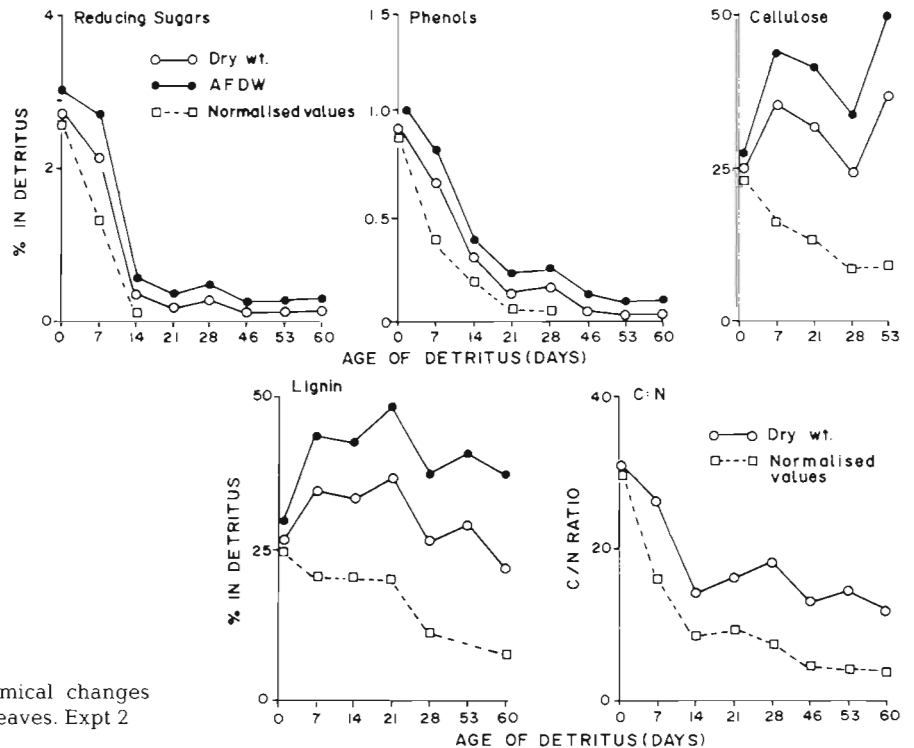


Fig. 6. *Rhizophora apiculata*. Biochemical changes during decomposition of mangrove leaves. Expt 2

gested that bacteria are likely to predominate during later stages of decomposition when the detritus is refractory.

According to this study, peak fungal biomass in mangrove leaf detritus was 80% of the highest bacterial biomass, in contrast to the observations of Blum et al. (1988), who reported the maximum fungal contribution to total microbial biomass to be only 20%. This might be attributable to the technique we employed in estimating fungal biomass, whereby we used unhomogenised detritus for staining and fungal detection in contrast to the Mollison's technique employed by Blum et al. (1988) (see Sathe & Raghukumar 1991 for discussion). Therefore, fungi may form a significant component of the total microbial biomass in mangrove detritus. However, we observed maximum biomass values of only 0.052 and 0.065% for fungi and bacteria, while Blum et al. (1988) reported 0.1 and 0.68% biomass for the 2 groups respectively in mangrove leaf detritus. The maximum combined fungal and bacterial biomass was only 0.076 to 0.077% of detrital dry wt after 21 and 35 d. Reasons for this discrepancy could be: (1) the geographical location and environmental characteristics of the 2 sampling sites; and (2) more vigorous microbial grazing by protozoans and nematodes in our samples.

Beside fungal and bacterial biomass, 3 further points will have to be considered in the future. (1) Microbial biomass may actually be greater than what is so far

recognized, if thraustochytrids and yeasts are taken into account. Thraustochytrids in brown algal detritus amounted to 0.065% C of detrital dry wt (0.13% dry wt biomass if C to dry wt is assumed to be 0.5) (Sathe-Pathak et al. 1993). Application of a newly developed epifluorescence technique for biomass estimations of thraustochytrids (Raghukumar & Schaumann 1993) might shed more light on this. Biomass studies on yeasts in marine detritus are few, essentially due to the lack of an adequate technique. These might make a significant contribution to microbial biomass as in the 14 d detritus of Expt 1, where an apparent yeast bloom occurred (Table 1). Meyers (1974) reported similar blooms in decaying *Spartina alterniflora* Loisel. (2) More important than biomass per se might be the possible supply of essential nutrients such as fatty acids, sterols, vitamins and other growth factors to detritivores (Phillips 1984). The recent finding of high contents of polyunsaturated fatty acids (PUFAs) in thraustochytrids by Findlay et al. (1986), which appear to be important in reproduction of crustaceans (Harrison 1990), lends further credence to this. (3) The actual significance of fungi and thraustochytrids in terms of degradation and flux of dissolved and particulate organic matter will have to be examined.

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