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Protozoa associated with leaf litter degradation in Coringa mangrove forest, Kakinada Bay, east coast of India

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Observations (1995-'96) on mangrove leaf litter revealed a variety of microorganisms dominated by bacteria (5 types), 12 species of flagellates, 2 sarcodines, 17 ciliates, 2 suctorids and 2 sessile ciliates besides several diatoms, nematodes and nauplii. Overall, bacteria outnumbered (4.59×10^5 no. g^{-1} dry weight) all others constituting 80-90% of the population followed by flagellates (4.8%), ciliates (4.4%) and, sessile ciliates (0.2%). *Chromulina* sp., *Spumella socialis* and *Euglena acus* (flagellates), *Cyclidium* sp., *Prorodon* sp., *Euplotoides aediculatus* and *Zoothamnium* sp. (ciliates) were relatively dominant (mean density 4,331 individuals l^{-1}) in the litter collected from *Avicennia* plot. Flagellates, *Astasia* sp., *Heteronema* sp. and *Paranema* sp. and, ciliates, *Prorodon* sp., *Holosticha* sp. and *E. aediculatus* were, however, more common in *Excoecaria* (mean density 3719 individuals l^{-1}). *In situ* experiments on leaf decay showed that the entire process lasted 12-18 days in summer and 26-32 days during monsoon. Bacteria were the first to settle, followed by nanoflagellates (2-20 μm), microciliates (20-100 μm), macrociliates (100-200 μm) and sessile ciliates. Nematodes indicated culmination. Bacterial (mean) biomass registered highest value (6.43×10^{-3} mgC g^{-1}) within 24 hours but decreased (3.1×10^{-6} mgC g^{-1}) by day-3 to 5. Mean flagellate biomass peaked (32.6 mgC g^{-1}) by day-2 and microciliates (92 mgC g^{-1}) by day-5 in summer and (47mgC g^{-1}) by day-24 during monsoon. Macrociliates registered highest biomass (168.4mgC g^{-1}) by day-6 in summer but lagged behind until day-26 to day-30 (154mgC g^{-1}) during monsoon. A distinct prey predator relationship, direct dependence of ciliate species on nanoflagellate and bacterial populations as well as, a well marked microbial community succession were evident.

[**Key words**: Mangrove leaf litter, microorganisms, Protozoa, degradation, east coast of India]

In tropical and sub–tropical mangrove forests, leaf litter deposited as ungrazed material is largely processed through detritus-based food chains and accounts for considerable near shore secondary production[1]. On the forest floor, mangrove leaves and other debris accumulate constituting the bulk of organic detritus that supports a rich and heterogeneous variety of microbial communities. Absolute decay parameters for mangrove litter are site and species dependent and the rate of infestation and degradation varies considerably with individual species[2]. Leaves decompose faster in subtidal regions of mangrove systems than in the intertidal and presumably leaching and saprophytic decay are more effective when leaves are not subjected to drying[2]. The alternating inundation-exposure events in these areas seem to aid this process greatly. Bacteria decompose the leaf litter primarily and they also serve as food for Protozoa, albeit the latter known for their special role in nutrient regenerative processes otherwise bound in bacterial biomass[3]. In the classical paradigm of marine detrital-based food chain, protozoans occupy a key position as a vital link between bacterial cells and the larger zooplankton. Studies on bacteria and fungi as microbial communities of a wide variety of seagrass and mangrove ecosystems are reported[4-6] although there is little or no information on protozoans associated with mangrove litter decomposition vis-à-vis the ecological energetic[7]. Year round experiments during 1995-'96 on litter decomposition inside Coringa dominated by *Avicennia officinalis* and *Excoecaria agallocha* revealed that C: N ratio in senescent leaves changed appreciably (78-24) in 33-45 days of decay depending on whether it is dry or wet season[8] though no attempt was made to identify the causative organisms. The present study was therefore aimed at investigating the microbial communities, in particular the ciliated Protozoa and flagellates, concerned with mangrove litter degradation process in this area. The essential tasks included taxonomic listing of Protozoa, estimation of standing crop

(abundance/biomass) and microbial succession patterns, if any, through conventional techniques.

Materials and Methods

River Godavari bifurcates towards its lower reaches, and one of the branches, the Gautami Godavari, joins the Bay of Bengal at Bhairavapalem (Fig. 1). Kakinada bay is situated north of Gautami Godavari and is connected to it by distributaries mainly Gaderu and Coringa. Bhairavapalem is also the southern limit of the Bay-Mangrove complex that covers a total area of ~350 km^2. An important feature of this region is the location of Coringa Wildlife Sanctuary (250 km^2) with high grown mangroves (*Avicennia marina, A. officinalis, A. alba, Excoecaria agallocha, Sonneratia apetala, Rhizophora apiculata, R. mucronata, Lumnitzera racemosa* and *Aegiceras corniculatum*). The long and often branching mangrove creeks, where considerable lateral trapping of water occurs, serve as an excellent habitat and nursery for a wide range of invertebrate species and fish. During the last 5-8 years, following an upsurge in shrimp farming activity much of the mangrove area has been denuded.

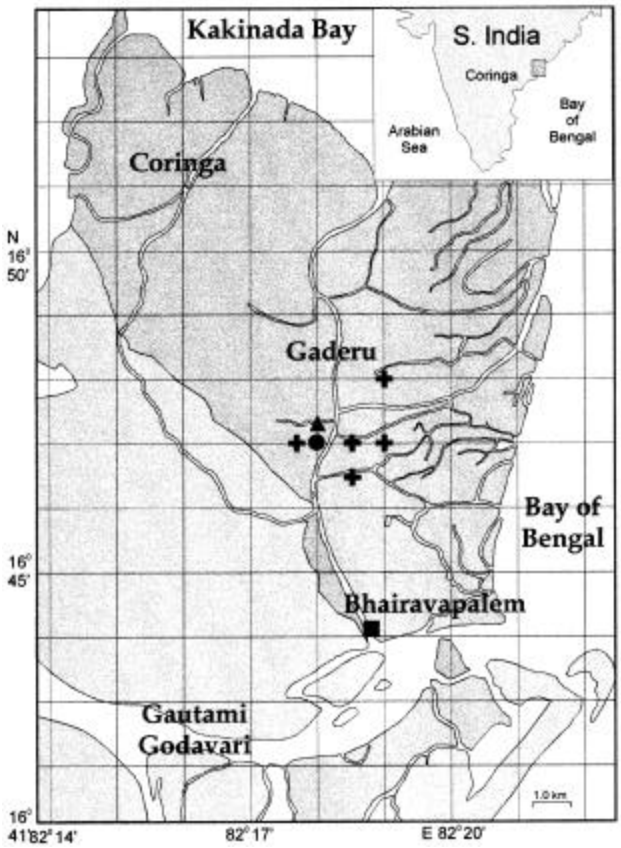

Fig. 1—Field plots of (?)*Avicennia officinalis* (?) *Excoecaria agallocha*. [**+** indicates collection sites for mangrove detritus, dark shaded area refers to mangroves].

However, within the Coringa Wildlife Sanctuary and interior areas, there is still some luxuriant vegetation. During the present study, two field-plots one dominated by *Avicennia officinalis* Linn (Family: Avicenniaceae**)** and the other by *Excoecaria agallocha* Linn (Family: Euphorbiaceae) with an inundation frequency of 17-44 times a month were chosen.

Mangrove leaves-freshly fallen and in various stages of decomposition were hand picked from the forest floor during low tide and examined microscopically as detailed below. For experimental studies, a trap (made of a film canister, on which 2 windows were cut and fitted with plankton net of 120×120 µm mesh size), designed for studying benthic protozoans[9] was employed. Freshly sloughed senescent leaves (~10×20 mm; surface area 200 mm^2) were used as experimental bait. The canisters containing the leaf were laid on the forest floor inside field plots, well secured, ensuring periodic inundation. The traps were recovered at 24 hr intervals during the first week and at weekly intervals thereafter. Dummy traps with a piece of plastic sheet of a similar size were used as controls. After each retrieval, the traps were carefully transported to the laboratory in cold condition for further analysis. Since it was not possible to measure the surface area of the leaf owing to its bad state during decomposition the contents were washed with filtered 50% seawater and made up to 250 ml quantity. After examining under fresh conditions the samples were fixed in Lugol's iodine for further study. The experiments were carried out twice during 1995 and 1996, in summer (April-May, salinity 14-27‰; mean temp. 33.1°C) and monsoon (September-October, salinity 2-11‰; mean temp. 32.7°C). Duration of experiment varied between 20 and 32 days or until the leaf is completely decomposed. Samples for bacterial isolation were initially inoculated in Peptone Water and Zobell's Marine Agar and later in selective media such as MacConkey Agar and Thiosulphate Citrate Bile salts Sucrose Agar (TCBS). For bacterial enumeration, samples were fixed immediately after collection with filtered 40% formaldehyde and refrigerated. Bacterial densities were determined by direct counting[10] employing Acridine Orange (AO) and by epifluorescency[11]. In addition, spread plate was used to determine number of Colony Forming Units/ml, 37°C/48 hrs, in ZMA. For computation, average number per plate was divided by sample volume. About 100 µl of sample was pipeted out on to the surface of the medium (ZMA) and evenly spread out with a

sterile glass spreader. Specific single colonies were sub-cultured and the resulting pure cultures were used for biochemical tests. Biomass was calculated as carbon using a conversion factor[12] of 20 femtogram (fg) C $cell^{-1}$. For taxonomic identification of protozoans, fresh observations were made using vital stains such as 1% methyl green in acetic acid, 1% aqueous eosin or 1% neutral red. Quantitative enumeration (based on 3-6 replicates) was carried out on Lugol's iodine preserved samples (250 ml) concentrated through gravity sedimentation technique[13]. Aliquots of 1 ml were drawn into a Sedgwick- Rafter counting chamber and protozoan abundance was estimated per 250 ml. These numbers were converted into numbers per gram dry weight of leaf. For this purpose, leaves of the same surface area (200 mm^2) were dried in the oven at 60°C till a constant weight was obtained. The mean dry weight of 10 leaves for both *Avicennia officinalis* and *Excoecaria agallocha* was taken as the standard dry weight and the abundance was expressed as numbers per gm dry weight. Species biomass was estimated after measuring cell dimensions ($n>20$) and calculation of cell volume through suitable geometric equations based on cell shape. A cell volume to cell carbon conversion factor of 220fg C μm^{-3} for flagellates and 190 fg C μm^{-3} for ciliates and dinoflagellates was used to calculate their biomass[14]. Individual species biomass was obtained by multiplying it with numerical abundance. Bacterial and protozoan identification was made following Barrow & Feltham[15], Lee *et al.*[16] and Corliss[17].

Results and Discussion

Field observations

Microscopic examination of mangrove litter collected from the forest floor revealed a heterogeneous variety of microorganisms consisting of 40 species dominated by bacteria (5 types), 12 species of flagellates, 2 sarcodines, 17 ciliates, 2 suctorids and 2 sessile ciliates in addition to diatoms, nematodes and nauplii. Overall, bacteria (4.59×10^5 no.g^{-1} dry weight) outnumbered all other groups constituting 80-90% of the population. Next in order were the flagellates (4.8%) followed by vagile (4.4%) and sessile ciliates (0.2%). Heterogeneity and density of constituent groups increased as the process of decomposition advanced. At the onset of decomposition (indicated by leaching), bacteria alone were found on senescent leaves. Under partly decomposing conditions, heterotrophic nannoflagellates were the predominant organisms. At the end, when only leaf debris and humus were left, mostly grazing ciliates represented the community. While flagellates such as *Chromulina* sp., *Spumella socialis* and *Euglena acus* were dominant in the litter collected from *Avicennia* plot, there were *Astasia* sp., *Heteronema* sp. and *Pseudoparanema* sp. in the *Excoecaria* plot. Among ciliates, *Cyclidium* sp., *Prorodon* sp., *Euplotoides aediculatus* and *Zoothamnium* sp., were predominant in *Avicennia* plot and from *Excoecaria* the important forms were *Prorodon* sp., *Holosticha* sp. and *Holophrya* sp.. Mean faunal density was 4,331 no l^{-1} in *Avicennia* plot and 3,719 no l^{-1} in *Excoecaria* plot.

Leaf litter decomposition experiments

Leaf decay experiments showed that the entire process lasted 14-18 days during summer and 28-32 days during monsoon (Table 1). Bacteria were the first to colonize followed by nanoflagellates (2-20 µm), microciliates (20-100 µm), macrociliates (100-200 µm), sessile ciliates (e.g. *Vorticella* sp., *Zoothamnium* sp.) and nematodes indicative of an intricate prey–predator relationship. Fungal spores did not appear in any appreciable numbers and the few found occasionally were not taken into consideration since they do not form food for protozoans and therefore not contribute to the microbial succession in the present context. Bacterial isolation and identification, although sketchy, from the observations, bacteria consisted of mostly *Micrococcus* sp., *Pseudomonas* sp. and *Spirosoma* sp. On the contrary, protozoans were very diverse and numerically rich. As many as 54 species represented by 3 major functional groups namely, heterotrophic flagellates, vagile (both micro and macrociliates) and, sessile ciliates were encountered. Leaves retrieved after a 24-hr period showed sufficient leaching indicated by a layer of bacteria on their surface. On day-1 the autochthonous cellulolytic *Micrococcus varians* registered its highest density (3.23×10^8no. g^{-1}) and biomass (6.5×10^{-5} mg C g^{-1}). The presence of other bacteria namely *Pseudomonas* sp. (3.34×10^7 no. g^{-1}; 6.7×10^{-4} mg C g^{-1}) and *Spirosoma* sp. (3.0×10^7 no.l^{-1}; 6.0×10^{-4}mg C g^{-1}) in *Avicennia* and, the blue green alga, *Merismopaedia* sp. in large numbers (1.8×10^6 no. g^{-1}) in *Excoecaria*, was suggestive of the differences between the two examples and the biochemical composition of their leaves. Bacterial counts, however, dwindled after 48 hr in *Excoecaria* and 60-72 hr later in *Avicennia*. Heterotrophic nanoflagellates peaked (1.4×10^6 no. g^{-1} in *Avicennia;* 4.6×10^6 no. g^{-1} in *Excoecaria*) by day-2.

Table 1 — Leaf litter decomposition experiments in Coringa mangroves: Summary of experimental data

Characteristics	*Excoecaria agallocha*		*Avicennia officinalis*	
	Dry season (April-May)	Wet season (Sep-Oct)	Dry season (April-May)	Wet season (April-May)
Duration of experiment (days)	12 – 14	26 –28	14 – 18	28 – 32
Species number	29	22	40	26
Bacteria				
No of predominant types	1	3	5	1
Highest abundance ($\times 10^8$no. g^{-1})	8.31	0.5	3.9	0.2
Day on which found.	Day 1	Day 1	Day 1	Day 1
Dominant species	*Micrococcus varians* (99.0%)	*M.varians* (96.5%)	*M. varians* (78.1%)	*M. varians* (98.8%)
Biomass (mg C. g^{-1})	0.017	0.0009	0.0078	0.0004
Flagellates				
No. Species	12	5	10	1
Highest abundance ($\times 10^6$no. g^{-1})	8.8	0.46	2.6	0.1
Day on which found.	Day 3	Day 3	Day 4	Day 3
Dominant species	*Astasia klebsii* (20.3%) *Heteronema acus* (19.3%) *Pseudoparanema fusi-forme* (18.5%)	*Spumella socialis* (32.9%) *Petalomonas tricarinata* (28.8%) *Heteronema acus* (25.6%)	*Chromulina pascheri* (45.7%) *S. socialis* (27.8%) *Euglena acus* (15.0%)	*S. socialis* (100 %)
Biomass (mg C. g^{-1})	16.1	72.5	42.0	0.0009
Microciliates				
No. Species	9	9	11	8
Highest abundance ($\times 10^6$no.g^{-1})	2.0	1.4	1.1	0.74
Day on which found.	Day 5	Day 24	Day 5	Day 28
Dominant species	*Nassula citrea* (34.8%) *Lacrymaria sapropelica* (29.3%)	*Holosticha warreni* (37%) *Lagynophrya salina* (14.2%)	*N. citrea* (28.1%) *C. citrillus* (23.4%)	*C. citrillus* (43..1%) *H. warreni* (28.4%)
Biomass (mg C.g^{-1})	114.7	90.9	69.4	3.0
Macrociliates				
No. Species	4	4	8	9
Highest abundance ($\times 10^6$no. g^{-1})	1.7	0.17 & 0.5	0.57	1.0
Day on which found.	Day 5	Day 7 & 26	Day 6	Day 30
Dominant species	*Prorodon discolor* (78.1%) *Chilodinella cucullulus* (18.3%)	*Holophrya nigricans* (39.7%) *Spathidiodes armata* (26.8%)	*E. aediculatus* (33.3%) *Prorodon discolor* (17.5%)	*P. discolor* (35.8%) *H .nigricans* (14.7%)
Biomass (mg C.g^{-1})	145.7	161.0	191.1	147.0
Sessile ciliates				
No. Species	2	1	6	7
Highest abundance (x10^4no. g^{-1})	8.7	8.7	77.2	50.0
Day on which found	Day 8	Day 14	Day 8	Day 30-32
Dominant species	*Lagotia* sp. (66.7%)	*Vorticella microstoma* (100.0%)	*Zoothamnium simplex* (82.4%)	*V.microstoma* (65.4%) *Carchesium polypinum* (14.4%)
Biomass (mg C. g^{-1})	0.34	0.15	286.0	10.3

The dominant species were *Chromulina pascheri*, *Spumella socialis*, *Heteronema acus*, *Astasia klebsii* and *Euglena acus*, all of which were bacterivorous. Microciliates represented by *Nassula citrea*, *Holosticha warreni*, *Cyclidium citrullus* and *Lacrymaria sapropelica*, also primarily bacterivorous, were abundant by day-5 and 7. Maximum abundance varied between 1.3×10^6 no. g^{-1} (*Excoecaria*) and 5.5×10^5 no. g^{-1} (*Avicennia*). Macrociliates such as *Prorodon discolor* and *Euplotoides aediculatus*, grazing on heterotrophic flagellates and microciliates, were abundant by day-6 (maximum abundance 9.2×10^5 no g^{-1} in *Excoecaria* and 3.2×10^5 no. g^{-1} *Avicennia*). Appearance of nematodes on day-9 indicated culmination of the decay process. At that stage, sessile ciliates such as *Vorticella microstoma* and *Zoothamnium simplex* colonized the fibrous remnants of *Avicennia* leaves in large numbers (6.3×10^5 no. g^{-1}; 148 mg C g^{-1}). In *Excoecaria*, there was no such strong colonization by sessile forms since there was no leftover material. Seasonally, the process of decay was faster during summer (14-18 days in *Avicennia*; 12-14 days in *Excoecaria*) (Fig. 2A, C) than in monsoon (28-32 days in *Avicennia*; 26-28 days in *Excoecaria*). (Fig. 2B, D) Such differences were also reflected in species composition and nature of their succession (Table 1). For example, among bacteria while *Micrococcus varians* was predominant during monsoon (salinity 2-11‰), there was a mixed population during summer (salinity 14-27‰) mainly *Micrococcus varians*, *Bacillus* sp., *Spirosoma* sp. and *Pseudomonas syringe*. In addition *Merismopedia* sp., a 'facultative autotroph' common in organically enriched water was also noticed. Among protozoans, low saline species such as *Spumella socialis*, *Cyclidium citrullus*, *Prorodon discolor*, *Holosticha warreni*, *Paramoecium bursaria* and *Vorticella microstoma*, were predominant during monsoon evidently due to their tolerance to low ambient salinity (mean 6‰). This period was also characterized by bimodal abundance of macrociliates (0.5×10^6 no. g^1 in *Excoecaria* and 1.0×10^6 no. g^{-1} in *Avicennia)* coinciding with the culmination of decomposition process after 26-30 days. In contrast, during summer (salinity 14-27‰), the predominant species were *Chromulina pascheri*, *Astasia klebsii*, *Heteronema acus*, *Nassula citrea* and *Zoothamnium simplex* all of which are mesohaline in nature. It is noteworthy that succession and species composition patterns remained same even when the order is changed i.e. traps with *Avicennia* leaf placed in

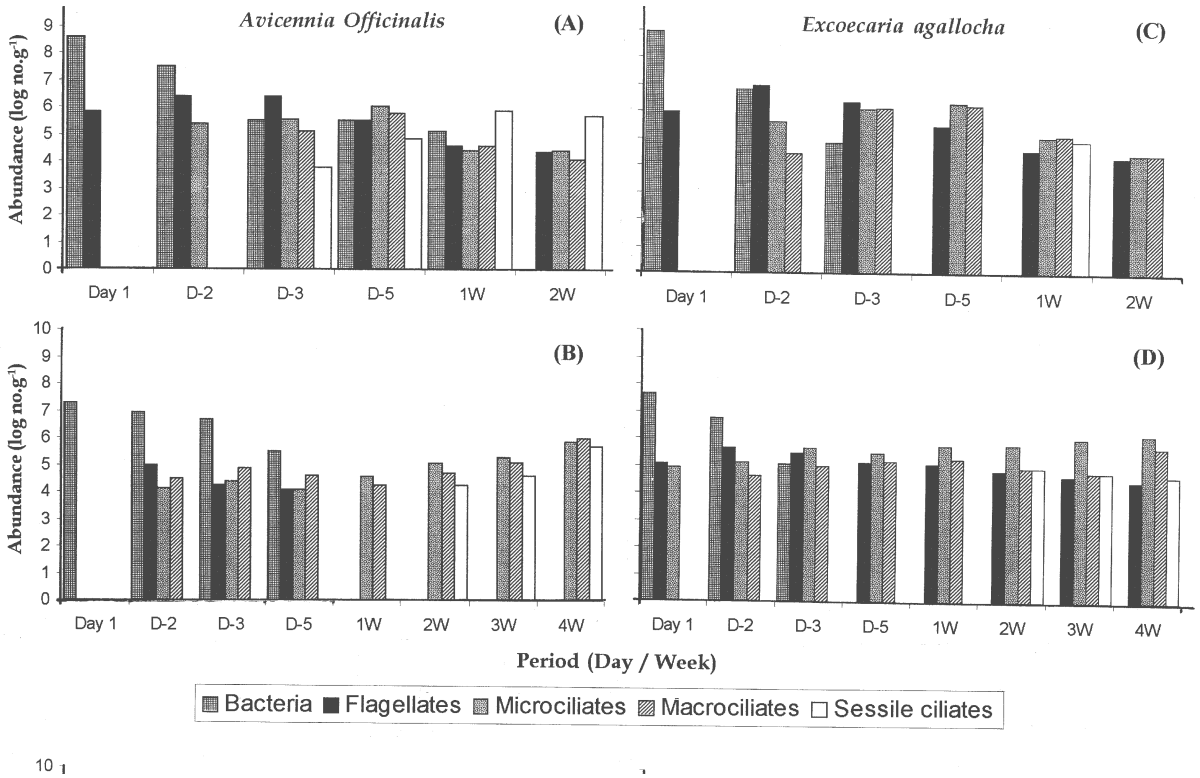

Fig. 2— Succession of microorganisms associated with *Excoecaria agallocha* and *Avicennia officinalis* litter decomposition (*in situ*) in Coringa mangroves. Abundance (log no.g^{-1}) during dry (A, C) and wet (B ,D) seasons.

Excoecaria plot or *vice versa*. At the end of the experiment, fine sediment (clayey-silt ~1-10 g dry weight) collected in blank (control) traps showed gram positive (autochthonous *Lactobacillus*, *Micrococcus* and *Bacillus*) and gram-negative (*Alcaligenes*) bacteria. Often, the variety of bacterial populations mirrored those from the forest floor but did not, however, trigger succession of Protozoa or any other organisms even after 28 days of exposure. Bacterial abundance (3×10^3 no.g^{-1} dry weight) was low.

Bacterial (mean) biomass registered its highest value (6.5×10^{-3}mg C g^{-1}) within 24 hours but decreased (3.1×10^{-6} mg C g^{-1}) by day-3 to 5. Mean flagellate biomass peaked (32.6 mg C g^{-1}) by day-2 but decreased by day-14 (6.9 mg C g^{-1}). Micro (20-100 µm) ciliate biomass varied considerably between the two seasons. It was highest (92 mg C g^{-1}) by day-5 in summer (Fig.3A, B) and (47 mg C g^{-1}) by day-24 to day-28 in monsoon (Fig.3C, D). Macro (100-200 µm) ciliate biomass was highest (mean 168.4 mg C g^{-1}) by day-6, but they lagged behind until day-26 to day-30 (154 mg C g^{-1}) during monsoon (Fig.3C, D). It is noteworthy that protozoan biomass increased by 2-3 folds relative to bacteria suggestive of a distinct prey predator relationship and direct dependence of ciliate species on nanoflagellate and bacterial populations.

Analysis of carbon isotope ratios in producers and consumers of tropical and sub-tropical mangrove systems[2] indicated the need for more detailed analysis of mangrove food chains and trophodynamics of mangrove inhabiting species[2]. Based on the earlier Florida model of food chains in mangrove forests[1], much work was carried out on the role played by detritus consumers and, lower and higher carnivores in the flow of energy through these systems[16-20]. In an experimental study on detritus consisting of vascular plant material, Fenchel & Harrison[21] observed peak bacterial abundance between 50 and 150 hr, flagellates 200 hr and ciliates after 200-300 hr. In the seagrass, *Cymodocea nodosa*, Peduzzi & Herndl[6] found development of nanoflagellates within 39 hr after incubation and densities peaked by 250 hr (density 53×10^3 cells ml^{-1}; biomass 103.1 µg C l^{-1}). Their contention was particle size is the primary criterion

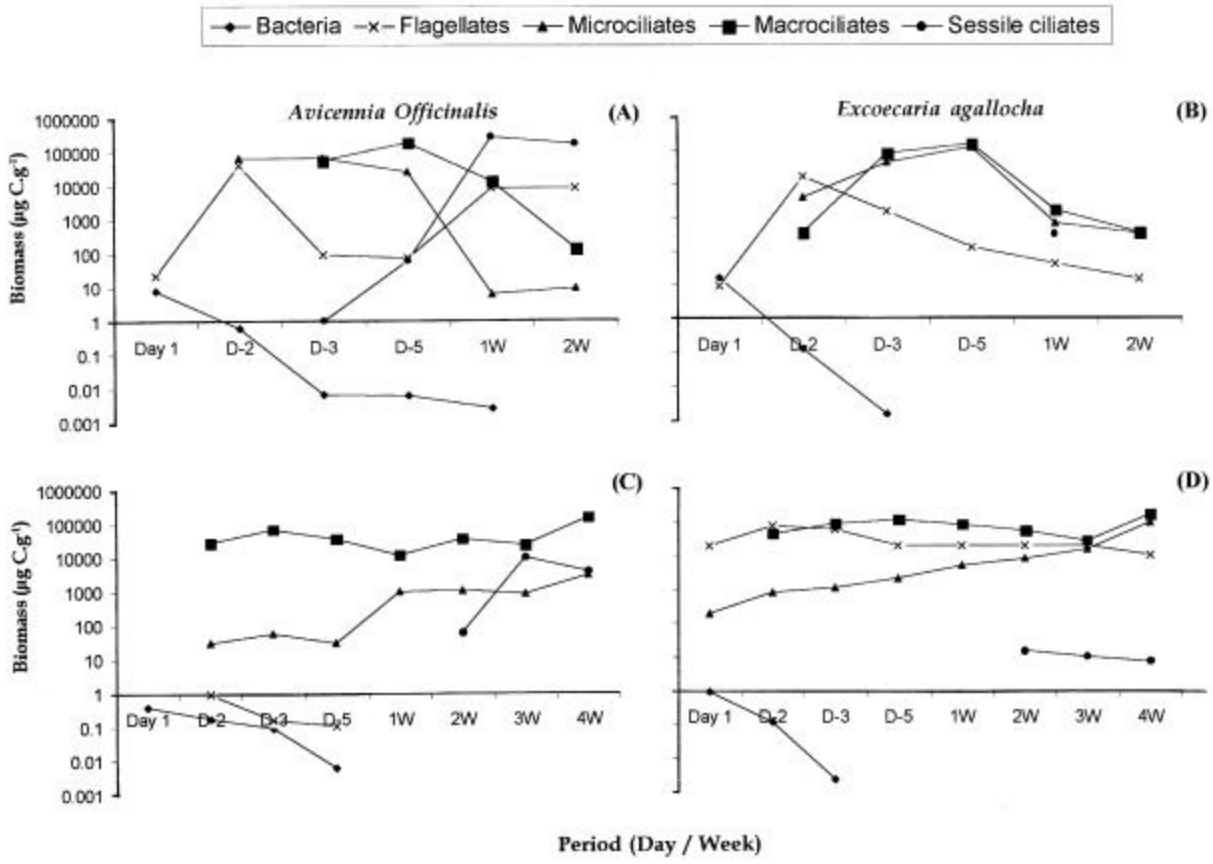

Fig. 3— Succession of microorganisms associated with *Excoecaria agallocha* and *Avicennia officinalis* litter decomposition (*in situ*) in Coringa mangroves. Biomass (µg C.g^{-1}) during dry (A, C) and wet (B, D) seasons.

since the micro-communities develop mainly on the surface of particles and protozoan grazing is a significant factor controlling bacterial numbers. The present findings have shown that within Coringa mangrove forest, litter decomposition processes are characterized by a well-marked succession of events beginning with microbes and culminating in metazoa. In both *Excoecaria* and *Avicennia*, peak bacterial, flagellate and ciliate abundance on the decomposing leaves occurred within 24, 72, 96 and 145, 150 hr respectively during summer and 24, 72 and after 25days (600 hr) during monsoon. The rapidity with which decomposition and succession events took place were suggestive of the effect of local environmental conditions such as temperature, salinity, tidal inundation etc.

In conclusion, differences between *Avicennia* and *Excoecaria*, other conditions having remained more or less same, indicated that the nature of decomposing leaf and associated leached material could play a significant role in determining the initial colonizers and later the protozoan species. This is further confirmed by the absence of flagellates or ciliates in blank traps though the sediments retained had compatible particle size (5.04 μm) normally meant to herald the whole process of mangrove leaf decay.

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