

Thraustochytrid and fungal component of marine detritus II. Laboratory studies on decomposition of the brown alga *Sargassum cinereum* J. Ag.

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Abstract

The potential role of thraustochytrids and fungi in detrital dynamics of the brown alga *Sargassum cinereum* J.Ag. was investigated in the laboratory. Field detritus of 0, 7, 14, 21 and 28 days obtained from litter-bag experiments were inoculated individually with three fungi, namely *Acremonium* sp., *Pythium* sp. and *Lindra thalassiae* and two thraustochytrids, *Ulkenia visurgensis* and *Labyrinthuloides minuta*. With the exception of *L. minuta*, the others grew best on 7- and 14-day-old detritus, suggesting that microbial biomass buildup in the field would be encouraged only in later stages of decomposition, after phenolic contents are reduced. The biochemical status of the five ages of detritus were examined after growth of the test microorganisms for 1 wk. Growth often, but not always, resulted in an increase of protein values in detritus, whereas amino acid contents always decreased substantially. Total carbohydrates (TCH), reducing sugars and often the C:N ratios were enhanced in the presence of the thraustochytrid *U. visurgensis*. TCH increased also in the presence of *L. minuta*. Growth of *Acremonium* sp. resulted in reduction of TCH and increase in reducing sugars levels, while *Lindra thalassiae* caused a lowering of reducing sugars and phenolics. C:N ratios of 0–21-day detritus were consistently lowered by growth of *Acremonium* sp., *Lindra thalassiae* and *L. minuta*. All test species enhanced C:N for 28-day material. The two thraustochytrids produced only proteases in detritus out of the six enzymes tested and utilised only casein and gelatin as carbon sources. The three fungi tested elaborated all the enzymes in detritus and utilised a wide variety of carbon sources. Thraustochytrids and fungi have the potential of altering the biochemical nature of detritus in *Sargassum cinereum* by virtue of their degradative enzyme production.

Key words: Detritus; Fungi; *Sargassum cinereum*; Thraustochytrids

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1. Introduction

Decomposition and the concomitant formation of detritus from macrophytes in the sea are accompanied by significant changes in their biochemical constitution (Godschalk & Wetzel, 1978; Cundell et al., 1979; Wilson et al., 1986a,b). Initially, a large proportion of soluble organics and phenols are rapidly leached out. Structural polysaccharides and proteins subsequently decrease (Robinson et al., 1981; Rice, 1982; Bowen, 1987; Tam et al., 1990). Nitrogen content of detritus increases in the presence of microbes thereby decreasing its C:N ratio (Fell et al., 1975; Harrison & Mann, 1975; Mann, 1976; Marinucci et al., 1981). It is now widely accepted that major biochemical changes in detritus are mediated mainly by microorganisms, namely bacteria and fungi present in them, which elaborate several degradative enzymes that break down the complex organic constituents present therein (Gessner, 1980; Pomeroy, 1980; Moran & Hodson, 1989). These processes have an implication on the feeding biology of detritivorous animals (Barnes, 1980; Robinson et al., 1981). Populations and biomass of these microorganisms which are present even on the healthy marine macrophyte fluctuate as decomposition progresses (Ruble & Roman, 1982; Blum et al., 1988; Robertson, 1988).

Most of the conclusions reached regarding the microbial role in detrital transformation have been based on undefined microbial consortia. A clearer picture of their involvement in such processes might be achieved by further experimentation with physical models of coastal litter systems of known microbial construct (Stephens, 1967). The present experimental study is the sequel to our earlier field studies (Sathe-Pathak et al., 1993) in which we have shown that along with bacteria, the marine osmo-heterotrophic protists, the thraustochytrids and fungi comprise a regular component of detritus of the brown alga *Sargassum cinereum* J. Ag. in the west coast of India. The former, particularly, could attain high biomass values in certain stages of detritus. Likewise, mycelial fungal biomass in marine macrophyte detritus may often be substantial (Sathe & Raghukumar, 1991). The role of these microorganisms in detrital transformation is not sufficiently known. In order to understand this and to infer the general role of microorganisms in the process, we have carried out in vitro studies using five of the most commonly isolated species from the brown algal detritus. Results obtained on the growth and enzyme production of these organisms in detritus, the changes in organic constituents of the detritus brought about by them and their carbon nutrition are presented in this paper.

2. Materials and methods

Detritus of 0, 7, 14, 21 and 28 days of *Sargassum cinereum* were collected from litter-bag experiments carried out at Dona Paula Jetty, Goa, India: Lat. 15° 27'–15° 38' N; Long. 73° 42'–73° 50' E (Sathe-Pathak et al., 1993). These were done by shredding live algal material into 15-cm bits and placing them in 1 mm mesh nylon bags, which were then tied to a raft at 1 m depth below water level. After collection, the detrital material was air dried for 3 days followed by drying at 50 °C for 1 day and

milled and sieved through a 200- μ m sieve. Samples of these were used for all further experiments. The five most frequently isolated species comprising two thraustochytrids, namely *Labyrinthuloides minuta* (Watson and Raper) Perkins and *Ulkenia visurgensis* (Ulken) Gaertner and three fungi, *Acremonium* sp., *Pythium* sp. and *Lindra thalassiae* Orpurt et al., were used as test organisms for these studies. The thraustochytrids were maintained in seawater/pine pollen cultures (Gaertner, 1968) and the fungi on corn meal agar medium with 50% seawater.

2.1. Experimental design

The five species were grown on detrital substrates of five ages, except *Lindra thalassiae* which was tested only on 0, 7, 14 and 21-day material. Detritus of 250 mg dry wt was autoclaved in 100 ml seawater in 250 ml Erlenmeyer flasks and inoculated with a loopful of the thraustochytrid culture or a 5-mm disc of a 5-day-old agar culture of the fungi. Flasks inoculated with the test organisms were grown in shake cultures and the two thraustochytrids under stationary conditions. Five replicates were maintained for each treatment. Uninoculated control flasks containing the same amount of autoclaved detritus in sterile seawater were maintained. After incubation for 1 wk at room temperature in the dark, the contents of three flasks were filtered separately over sterilised Whatman GF/C glassfibre filters. The culture filtrates were frozen immediately for subsequent enzyme analyses. Detritus with microbial growth from these flasks retained on the filter, were used for biochemical analyses. Those from the other two flasks were used to estimate growth of the test organisms on detritus.

Growth on detritus was estimated by analysing the ATP content (Parsons et al., 1984) using a Turner TD-20e luminometer. The amount of growth is represented as ng ATP per 250 mg of detritus, equivalent to 100 ml of culture fluid.

2.2. Biochemical changes

Control and inoculated detritus were analysed for biochemical constituents. These were air dried for 3 days, followed by drying at 50 °C for 1 day and milled. Aliquots of these were analysed as follows.

Total carbohydrates (TCH) were estimated by the phenol-sulphuric acid method (Hellebust and Craigie, 1978) and reducing sugars by using dinitrosalicylic acid (Miller, 1960). Proteins were determined by the modified Lowry's method of Peterson (1977). Amino acids were estimated spectrophotometrically using ninhydrin reagent (Mahadevan, 1975). Total phenols were extracted in cold methanol at 4 °C for 24 h and after centrifugation, the clear supernatant analysed using Folin-Denis reagent (Swain & Hillis, 1959). Catechol (Sigma, USA) was used as a reference standard. C:N total carbon to nitrogen ratios were estimated using a Heraeus CHN. O Rapid Analyzer.

2.3. Enzyme estimations in culture filtrates

Cellulases, xylanase, laminarinase, polygalacturonase, amylase and alginate hydrolase in culture filtrates were estimated by measuring reducing sugars released from

powdered microcrystalline cellulose, xylan, laminarin, sodium polypectate, soluble starch and sodium alginate as substrates, respectively, using standard methods (Gessner, 1980; Suberkropp et al., 1983; Dewey et al., 1984; Suberkropp & Arsuffi, 1984; Schaumann & Weide, 1990). Protease was estimated by measuring release of amino acids from casein (Mahadevan, 1975). Four replicates were maintained for all estimations. One unit of proteases is defined as 1 μg of tyrosine released per ml of culture fluid per h. For all other enzymes, 1 unit corresponds to 1 μg glucose liberated per ml culture fluid per h.

2.4. Carbon nutrition

The test organisms were grown on 13 different substrates as sole carbon sources (see Table 3) at 0.25% w/v concentrations in a basal salts medium (Fell & Master, 1975). Growth in the presence of tannic acid was examined in the basal medium supplemented with 0.25% glucose (Gessner, 1980).

3. Results

3.1. Growth on detritus

Growth of all the test organisms varied significantly in different ages of detritus (Table 1). Four of the species showed maximum growth on 7- or 14-day-old detritus, although pronounced growth was produced on 0-day detritus as well (Table 1). Only the thraustochytrid *L. minuta* grew best on 0-day material. Biomass buildup was least in 28-day-old detritus. Of all the test species, the fungus *Lindra thalassiae* grew best on detritus.

3.2. Biochemical changes

The following biochemical changes were produced in detritus upon growth of fungi and thraustochytrids for 1 wk:

Table 1
Biomass (ng ATP) production by test fungi and thraustochytrids in detritus of various ages from *Sargassum cinereum* (250 mg detritus/100 ml seawater)

Species	Age of detritus (days)				
	0	7	14	21	28
<i>Acremonium</i> sp.	340	470	590	80	80
<i>Pythium</i> sp.	440	230	490	50	20
<i>Lindra thalassiae</i>	5300	7100	4400	6600	–
<i>U. visurgensis</i>	200	170	430	220	60
<i>L. minuta</i>	240	170	220	120	65

Two-way Analysis of Variance: Difference between fungi (df 4; *F* 3.01) not significant. Difference between ages of detritus (df 4; *F* 4.1) significant at 5%.

(1) All the test species enhanced protein levels in certain ages of detritus (Fig. 1). The greatest increase was caused by *Acremonium* sp. and *U. visurgensis*. Growth also resulted in a reduction of proteins in some cases. No definite pattern of changes with regard to species or ages of detritus was observed.

(2) All the test species caused a marked decrease in amino acid contents as compared to uninoculated controls (Fig. 2).

(3) Growth of the thraustochytrids *U. visurgensis* and *L. minuta* resulted in an increase of TCH of all ages of detritus (Fig. 3). Growth of *Lindra thalassiae* on 14- and 21-day detritus yielded similar results. On the contrary, *Acremonium* sp. caused a distinct lowering of TCH levels.

(4) Detritus inoculated with *Acremonium* sp. and *U. visurgensis* yielded higher levels of reducing sugars compared to the controls (Fig. 4). Growth of *Lindra thalassiae*, on the other hand, resulted in almost a total removal of these.

(5) The fungus *Lindra thalassiae* caused a distinct decline of total phenols, compared to the uninoculated control values. Phenolic values in detritus inoculated with the other three species did not differ significantly from the controls (Fig. 5).

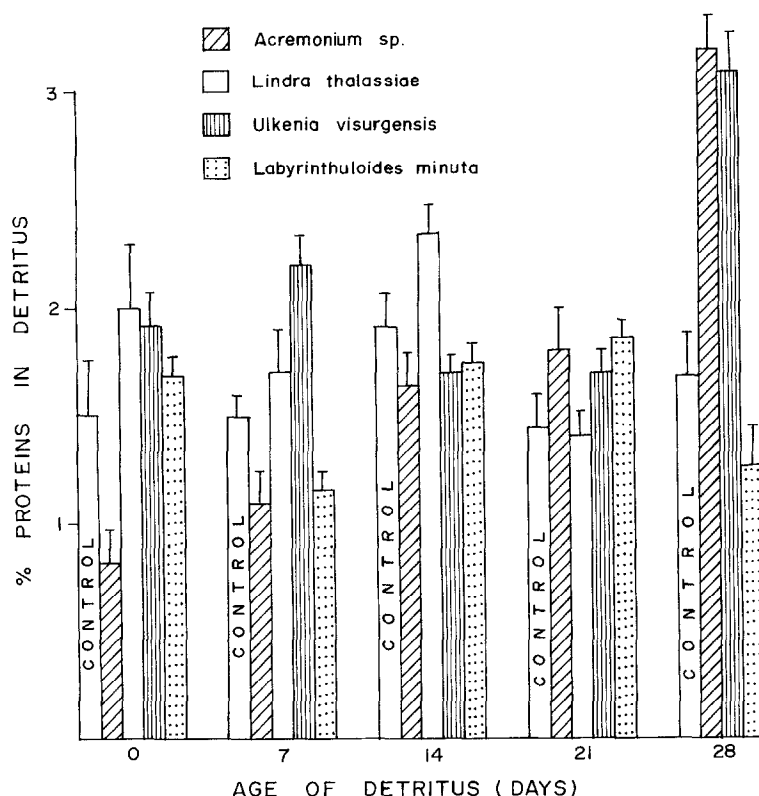


Fig. 1. Changes in protein contents of various ages of *Sargassum cinereum* detritus after growth of thraustochytrids and fungi for 1 wk.

(6) Inoculation of 0–21-day-old detritus with the test species resulted in a decrease in C:N values compared to uninoculated controls, except in the case of *U. visurgensis*, where higher C:N was observed. The C:N values of inoculated 28-day detritus were higher in all the cases (Fig. 6).

3.3. Degradative enzymes

Proteases were elaborated commonly by all five test species (Table 2). Higher amounts were produced by the fungus *Lindra thalassiae* and the two thraustochytrids compared to *Acremonium* sp. and *Pythium* sp. Only proteases out of the six enzymes tested were detected in the thraustochytrids. Alginate hydrolase was present only in the two fungi *Acremonium* sp. and *Pythium* sp., laminarinase in *Pythium* sp. and *Lindra thalassiae* and amylase in *Acremonium* sp. and *Lindra thalassiae*. *Acremonium* sp. and *Lindra thalassiae* produced cellulases. Polygalacturonase was elaborated by all three fungi and xylanase only by *Pythium* sp. No discernible pattern was observed between the amounts of individual enzymes produced and different ages of detritus.

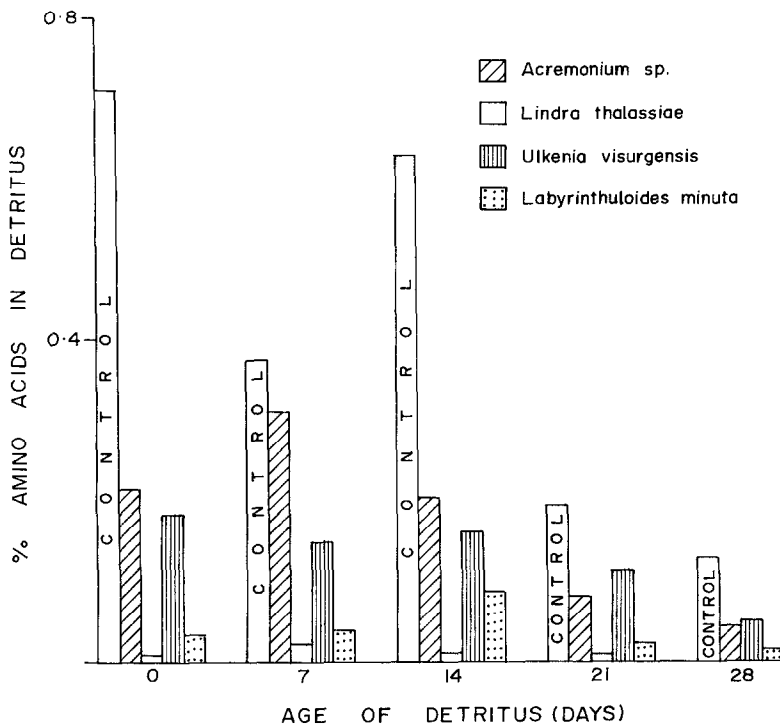


Fig. 2. Changes in amino acid contents of various ages of *Sargassum cinereum* detritus after growth of thraustochytrids and fungi for 1 wk.

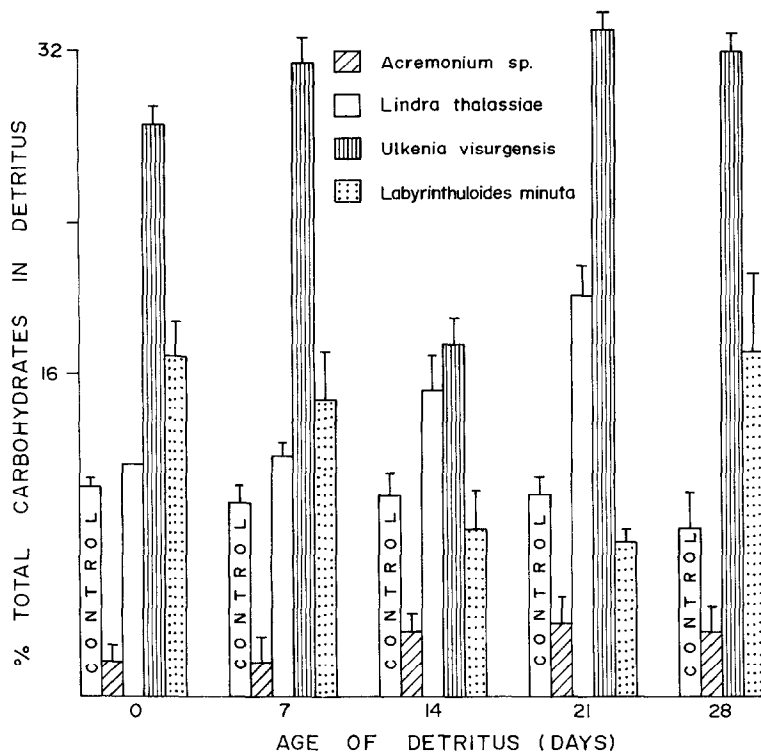


Fig. 3. Changes in total carbohydrate contents (TCH) of various ages of *Sargassum cinereum* detritus after growth of thraustochytrids and fungi for 1 wk.

3.4. Growth on various carbon sources

The three fungi, *Acremonium* sp., *Pythium* sp. and *Lindra thalassiae* grew on a wide variety of carbon sources. *Pythium* sp. and *Lindra thalassiae* produced maximum growth on laminarin. Growth on the lipids tripalmitin and tributyrin was poor in the case of *Acremonium* sp. and *Pythium* sp. and absent in others. The thraustochytrids showed measurable growth only on casein and gelatin. *Ulkenia visurgensis* showed a slight growth on mannitol (Table 3).

4. Discussion

4.1. Biomass buildup in detritus

The fact that 4 of the 5 species grew better on aged detritus than on 0-day material (Table 1) indicates that biomass buildup may be encouraged only after a certain stage of decomposition. Field studies on detritus of *Sargassum cinereum* showed that although healthy material of *Sargassum cinereum* (0-day detritus) contained higher

Table 2

Production of degradative enzymes (U/ml) culture filtrate by test fungi and thraustochytrids grown on different ages of detritus from *Sargassum cinereum*

Species	Age of detritus (days)				
	0	7	14	21	28
Proteases					
<i>Acremonium</i> sp.	2 (1.0)	17 (0.81)	10 (0.81)	8 (2.09)	5 (0.92)
<i>Pythium</i> sp.	10 (1.5)	3 (0.66)	12 (0.24)	8 (0.12)	5 (0.28)
<i>Lindra thalassiae</i>	8 (7.2)	46 (12.0)	46 (14.2)	9 (1.1)	–
<i>U. visurgensis</i>	16 (1.7)	14 (1.9)	17 (2.1)	17 (0.6)	6 (2.4)
<i>L. minuta</i>	35 (0.3)	22 (3.0)	14 (1.1)	13 (4.0)	11 (1.2)
Alginate hydrolase					
<i>Acremonium</i> sp.	23 (2.6)	4 (0.4)	27 (0.5)	26 (1.9)	2 (0.5)
<i>Pythium</i> sp.	104 (1.0)	104 (0.7)	106 (0.9)	94 (0.7)	70 (0.7)
<i>Lindra thalassiae</i>	0	0	0	0	0
<i>U. visurgensis</i>	0	0	0	0	0
<i>L. minuta</i>	0	0	0	0	0
Laminarinase					
<i>Acremonium</i> sp.	0	0	0	0	0
<i>Pythium</i> sp.	66 (0.4)	45 (0.6)	86 (0.7)	65 (0.5)	44 (1.6)
<i>Lindra thalassiae</i>	450 (25.0)	490 (70.0)	530 (90.0)	295 (55.0)	–
<i>U. visurgensis</i>	0	0	0	0	0
<i>L. minuta</i>	0	0	0	0	0
Amylase					
<i>Acremonium</i> sp.	0	22	15	24	21
<i>Pythium</i> sp.	0	0	0	0	0
<i>Lindra thalassiae</i>	29 (7.4)	30 (3.5)	23 (3.0)	22 (2.0)	–
<i>U. visurgensis</i>	0	0	0	0	0
<i>L. minuta</i>	0	0	0	0	0
Polygalacturonases					
<i>Acremonium</i> sp.	87 (2.9)	122 (2.3)	103 (0.3)	72 (0.3)	82 (1.4)
<i>Pythium</i> sp.	54 (2.5)	139 (4.9)	167 (3.3)	121 (2.8)	143 (4.8)
<i>Lindra thalassiae</i>	91 (6.4)	87 (8.9)	112 (10.0)	105 (19.0)	–
<i>U. visurgensis</i>	0	0	0	0	0
<i>L. minuta</i>	0	0	0	0	0

Table 2
(Continued)

Species	Age of detritus (days)				
	0	7	14	21	28
Cellulases					
<i>Acremonium</i> sp.	130 (6.1)	145 (1.4)	155 (2.7)	155 (4.8)	155 (3.1)
<i>Pythium</i> sp.	0	0	0	0	0
<i>Lindra thalassiae</i>	74 (19.0)	52 (8.4)	79 (11.0)	46 (13.2)	– –
<i>U. visurgensis</i>	0	0	0	0	0
<i>L. minuta</i>	0	0	0	0	0
Xylanase					
<i>Acremonium</i> sp.	0	0	0	0	0
<i>Pythium</i> sp.	390 (2.5)	360 (29.0)	395 (3.5)	260 (44.0)	240 (7.1)
<i>Lindra thalassiae</i>	0	0	0	0	0
<i>U. visurgensis</i>	0	0	0	0	0
<i>L. minuta</i>	0	0	0	0	0

SD in parentheses.

amounts of all nutrients tested, namely, protein, TCH, reducing sugars and alginates, biomass increase followed a spurt only after 7 or 14 days (Sathe-Pathak et al., 1993), reaching a peak by 21 days. It is likely that the high amount of phenolics in 0-day detritus (see Sathe-Pathak et al., 1993) retards biomass buildup. These are significantly reduced by 14 days. The antimicrobial nature of various compounds in healthy algae is well documented (Jones, 1988). Microbial decomposition rates of terrestrial as well as marine litter are known to be affected by their phenolic contents (Berg & Agren, 1984; Gonzalez-Farias & Mee, 1988). The buildup of bacterial biomass in seagrass and mangrove detritus with progress of decomposition (Rublee & Roman, 1982; Blum et al., 1988; Robertson, 1988; Blum & Mills, 1991) may also be related to this fact. The substantial growth of the thraustochytrid *L. minuta* in 0-day detritus, in contrast to the others, correlates with its presence in healthy *Sargassum cinereum* (Sathe-Pathak et al., 1993) and indicates a tolerance to phenolics in the brown alga.

The poor growth of the test species in late stages of detritus may reflect the recalcitrant nature of detritus at this stage. However, the production of various enzymes by the test species in such advanced decompositional stages of detritus was not diminished in proportion to the decrease in growth (Table 2). This suggests that microorganisms might maintain a high metabolic activity during the recalcitrant phase of detrital decay, even if the conversion efficiency is low.

4.2. Biochemical changes in detritus

Field studies on *Sargassum cinereum* revealed that all the biochemical parameters examined, namely, reducing sugars, TCH, phenols, proteins, mannitol and alginate

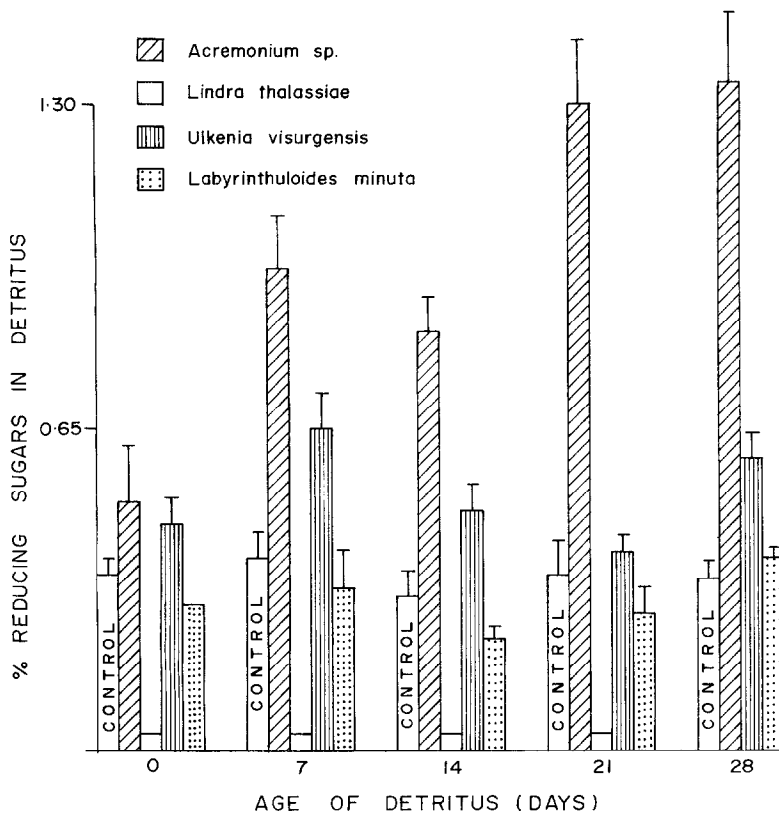


Fig. 4. Changes in reducing sugar contents of various ages of *Sargassum cinereum* detritus after growth of thraustochytrids and fungi for 1 wk.

decreased significantly over 28 days of decomposition (Sathe-Pathak et al., 1993), presumably by abiotic leaching (Godschalk & Wetzel, 1978; Cundell et al., 1979; Valiela et al., 1984; Wilson et al., 1986a,b) as well as microbial breakdown (Marinucci et al., 1984; Robertson, 1988). The precise role of microorganisms in this process of organic matter removal is still an incompletely understood phenomenon.

This study indicates that fungi and thraustochytrids have the following potentials in bringing about biochemical changes in detritus. However, these cannot be quantitatively compared with those in the field owing to our use of autoclaved, and, therefore, somewhat modified detritus, necessitated by axenic experiments.

(1) They may contribute to increases in detrital protein by immobilisation of inorganic nitrogen from ambient water in the form of cell biomass (Fig. 1). This, in turn, could result in the lowering of C:N values (Fig. 6) (Fenchel & Jorgensen, 1976; Fell & Master, 1980; Rublee & Roman, 1982; Melillo et al., 1984; Rice & Hanson, 1984; Sathe-Pathak et al., 1993). Such a reduction in C:N of inoculated detritus might also result from production of microbial exoenzymes, their condensation with reactive phenolics and

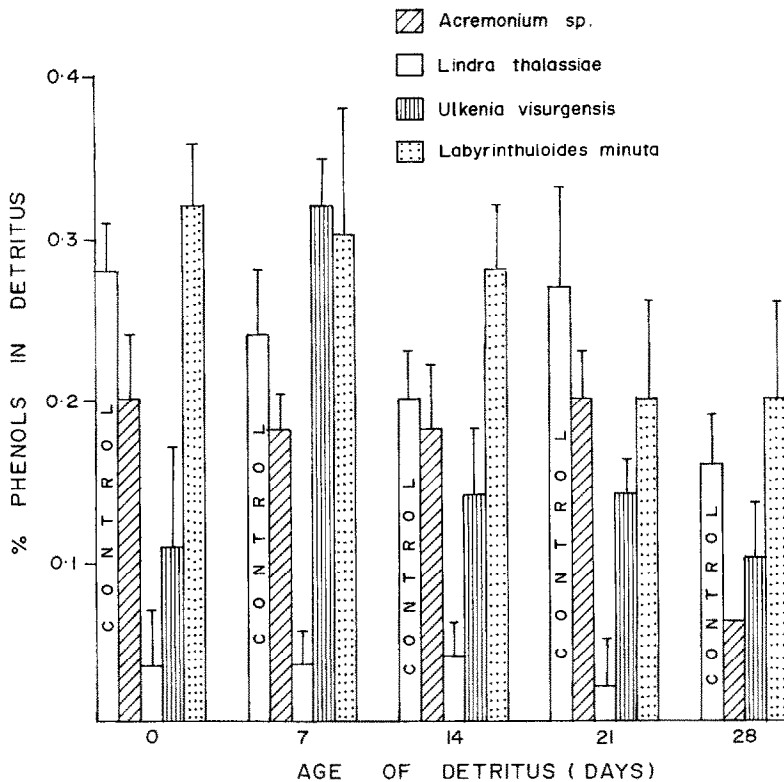


Fig. 5. Changes in total phenolic contents of various ages of *Sargassum cinereum* detritus after growth of thraustochytrids and fungi for 1 wk.

carbohydrates and formation of non-protein nitrogen (Melillo et al., 1984; Rice & Hanson, 1984; Wilson et al., 1986b).

The steady decline of protein levels in the field (Sathe-Pathak et al., 1993) in spite of the potentials of microbes to increase protein levels, could be explained by the fact that such gains are offset by: (1) the low microbial biomass in natural detritus; (2) the continued abiotic leaching that might be taking place in nature and (3) the degradation of proteins by microorganisms themselves, as evidenced by the lowering of protein levels by the test species in this study, in certain ages of detritus (Fig. 1).

(2) Microbial uptake and metabolism of simple organic compounds might result in their loss from detritus. Removal of detrital amino acids in the case of all test species (Fig. 2) and reducing sugars in that of *Lindra thalassiae* (Fig. 4) might be attributed to this. Hicks et al. (1991) showed that much of the free amino acid nitrogen in detrital extracts may be incorporated into cell proteins by microorganisms. In the case of thraustochytrids, such as *U. visurgensis*, amino acids may also serve as excellent carbon sources (Bahnweg, 1979), probably by virtue of deamination reactions. We suspect that increases in TCH (Fig. 3) reducing sugars (Fig. 4) and C:N ratios (Fig. 6) of detritus upon growth of this thraustochytrid resulted from this.

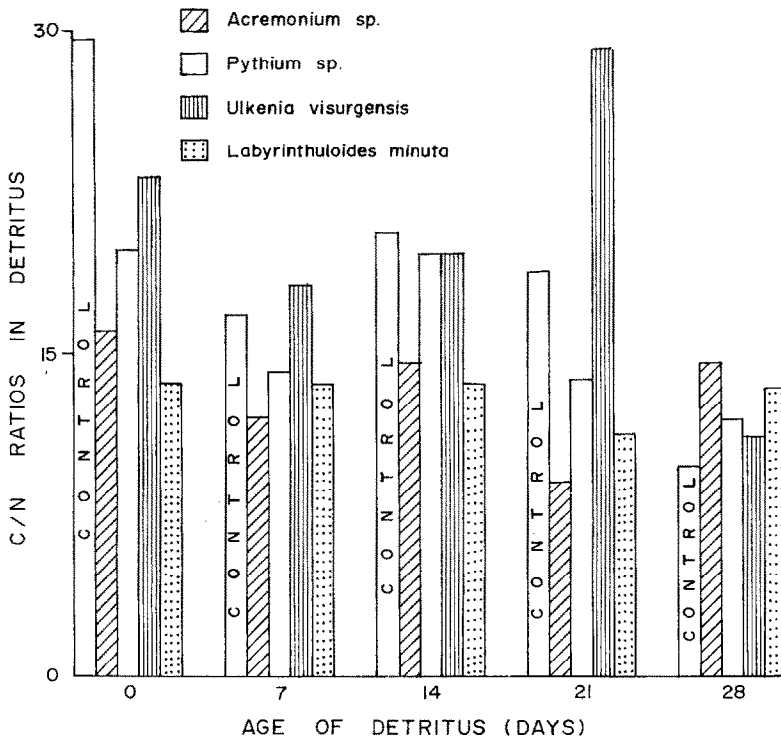


Fig. 6. Changes in C:N ratios of various ages of *Sargassum cinereum* detritus after growth of thraustochytrids and fungi for 1 wk.

(3) Thraustochytrids and fungi may be responsible for losses in plant structural and storage compounds present in detritus by means of enzymatic degradation (Gessner, 1980; Pomeroy, 1980; Suberkropp et al., 1983). Thus, *Acremonium* sp., which was capable of elaborating a wide variety of degradative enzymes (Table 2) in detritus substantially reduced the TCH contents (Fig. 3). *Lindra thalassiae* and *Pythium* sp., which revealed substantial growth on laminarin (Table 2) and production of laminarinase (Table 2), might utilise this compound in detritus. The former also grew well on sodium alginate, although no alginate hydrolase was detected when grown on detritus. In contrast to fungi, the role of thraustochytrids in the brown algal detritus might be restricted to the breakdown and utilisation of proteins, since no enzymes other than proteases were detected in them (Table 2). This is further supported by the fact that they utilised only casein and gelatin as carbon sources (Table 3).

(4) Microbial breakdown, combined with a loose uptake of the degraded products (sloppy feeding) (Moran & Hodson, 1989; Peduzzi & Herndl, 1991) might result in an accumulation of substances such as reducing sugars, as in the case of *Acremonium* sp. and *U. visurgensis* (Fig. 4).

(5) Fungi such as *Lindra thalassiae*, which could grow in the presence of tannic acid (Table 3) and reduce phenolic contents of detritus (Fig. 5), might perform the useful function of rendering the detritus more accessible to those species not capable of tol-

Microorganisms may, by various mechanisms, cause both addition and removal of different detrital organic constituents such as proteins, amino acids, TCH, reducing sugars and phenols (Fig. 7). The actual changes would depend upon the particular microbial species colonising the detritus and the age of the latter. The overall biochemical status of detritus would be a balance of the net biotic and abiotic processes.

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