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Enumeration of thraustochytrids in decomposing leaves of mangroves as influenced by physicochemical and microbial factors

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Abstract

This study enumerated thraustochytrids in the decomposing leaves of mangroves in relation to physico-chemical and microbial aspects. Senescent leaves of four mangrove species viz., *Rhizophora apiculata*, *R. mucronata*, *Avicennia marina* and *A. officinalis*, were allowed to decompose in litter bag along the intertidal zone of the Vellar estuary in south-east coast of India. The leaf samples were drawn at different days of decomposition (0, 7, 14, 21, 28, 35, and 42) for four seasons: pre-monsoon (July-September, 2017), monsoon (October-December, 2017), post-monsoon (January-March, 2018), and summer (April-June, 2018). The samples were studied for thraustochytrids and eight other microbial groups as well analysed for physicochemical parameters (temperature, pH, salinity, redox potential, N, P, and K of leaf samples). Thraustochytrids count was higher in *Avicennia marina* (0.16×10^3 CFU.g^{-1}) than that in *Rhizophora apiculata* (0.12×10^3 CFU.g^{-1}). The count was maximum (0.21×10^3 CFU.g^{-1}) on day 21 of decomposition. The count varied with seasons, and it was higher (0.15×10^3 CFU.g^{-1}) in monsoon than that (0.13×10^3 CFU.g^{-1}) in post-monsoon and pre-monsoon. Thraustochytrids count exhibited a high positive correlation ($p<0.01$) with other microbial groups such as total heterotrophic bacteria, lactobacilli, yeasts, actinobacteria, azotobacter, fungi and *Trichoderma*, but a negative correlation with cyanobacteria. The count also showed positive correlations with pH and redox potential of decomposing water, leaf N and P. Thus, the thraustochytrids in association with other saprophytic microbes are involved in the decomposition of leaf litter, making the mangrove habitat productive.

Key Words – *Avicennia* – Decomposing leaf litter – Mangroves – *Rhizophora* – Thraustochytrids

Introduction

Mangrove forests are among the most productive detritus-based ecosystems (Kathiresan & Bingham 2001, Kathiresan et al. 2011). In the mangrove biotope, fallen leaves are colonized and decomposed by microorganisms. During this process the leaves become nutritious due to the microbial enrichment (Odum 1971, Ashton et al. 1999, Kathiresan & Bingham 2001, Dewiyanti 2010, Hossain et al. 2014). The major role of microbes in detrital processes is the conversion of less palatable organic matter to a more protein-rich food for the detritivorous fishes in the mangrove

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systems (Raghukumar et al. 1994, Rajendran & Kathiresan 1999a, b, 2007). The litter decomposition is also vital for the recycling of nutrients. However, the level of decomposition depends on physico-chemical factors and saprophytic microbes (Wedderburn & Carter 1999, Kathiresan et al. 2014, Loria-Naranjo et al. 2019). Though the role of bacteria and fungi are well known in the detrital food web of mangroves, little is known about the role played by thraustochytrids (Sharma et al. 1994, Raghukumar et al. 1994, Bremer & Talbot 1995, Bremer 1995, Raghukumar 2002, Mfilinge et al. 2003, Kalidasan 2015, Taketani et al. 2018).

Thraustochytrids are a diverse group of marine osmoheterotrophic, fungi-like protists. They belong to the eukaryotic kingdom Straminipila, which includes oomycetes and diatoms (Marchan et al. 2018, Tsui et al. 2009). They are isolated using direct plating and pollen baiting methods from various habitats such as coastal waters, sediments, algae and decaying mangrove leaves (Gupta et al. 2013, Caamano et al. 2017, Unagul et al. 2017, Boro et al. 2018). Yet, most of the thraustochytrids are isolated from only a limited number of coastal regions. For example, most of the thraustochytrids have been reported only in the region of west coast of India (Raghukumar et al. 2001, Raghukumar 2002, 2008, Jaseera et al. 2018) and very limited in east coast (Gomathi 2011, Kalidasan 2015, Kabilan et al. 2018). Consequently, studies of thraustochytrids for many coastal and mangrove habitats around the world remain poorly described, which limits our understanding of their true culturable diversity, and their relationship with physiochemical parameters, nutrients and microbial group.

Thraustochytrids may play a potential role in decomposing and enriching the mangrove litter (Bremer 1995). They have the ability to secrete extracellular hydrolytic enzymes such as cellulase, amylases, protease, phosphatase, pectinase and xylanase to decompose the litter (Raghukumar et al. 1994, Bremer & Talbot 1995, Bongiorni et al. 2005, Taoka et al. 2009, Kathiresan et al. 2011, Priyanka et al. 2017 and Taoka et al. 2017). Thraustochytrids are also an important food source for crabs, shrimps and fish that live in the mangroves due to the high amount of omega-3 fatty acids present in the thraustrochytrids (Gomathi et al. 2013, Lee Chang et al. 2014, Kalidasan 2015, Manikan et al. 2015, Yong et al. 2016, Marchan et al. 2017, 2018). Many of these ecological studies, however, have been undertaken in temperate, tropical, sub-Antarctic and Antarctic regions. Studies from the subtropical regions are few, although investigations are increasing (Raghukumar 1987a, b) especially in mangrove areas (Raghukumar 1988, Honda et al. 1998). Though, the role of bacteria and fungi are well-known in the detrital food web of mangroves, limited studies on the role of thraustochytrids in the decomposition of mangrove litter have been reported (Sharma et al. 1994, Raghukumar et al. 1994, 1995, Bremer & Talbot 1995, Bremer 1995, Raghukumar 2002, Mfilinge et al. 2003, Kalidasan 2015, Taketani et al. 2018). Interestingly, the first field litterbag study of decomposition on leaves of the *Rhizophora apiculata* was conducted for isolation thraustochytrids and fungi in west coast of India, Goa (Raghukumar et al. 1994). However, studies on thraustochytrids in decomposing leaves of mangroves in relation to duration of decomposition, other microbial groups and levels of nitrogen (N), phosphorus (P), and potassium (K) are not available, especially in restored mangrove forest. Considering the scarcity of information, the present work was undertaken in an artificially raised mangrove forest of the Vellar estuary in southeast coast of India, which is unexplored for thraustochytrids.

Materials and Methods

Collection and analysis of decomposing leaves

Fresh senescent leaves of *Rhizophora apiculata* Blume (Rhizophoraceae), *R. mucronata* Poir. (Rhizophoraceae), *Avicennia marina* (Forssk.) Vierh. (Avicenniaceae) and *A. officinalis* L. (Avicenniaceae) were collected by gentle shaking of healthy trees in the restored mangrove forest of the Vellar estuary in the southeast coast of India (11°29’25 "N; 79°45’56.2 "E).

One kilogram of the leaves collected was taken in a nylon bag (30 × 50 cm with a mesh size of 2 mm) for each species and submerged in pits with 1 m × 0.5 m x 1 m dimension, constructed along the intertidal area of the Vellar estuary. In order to allow the nylon bag to sink in water, a

stone weighing approximately 250 g was put in each bag. This experiment was conducted for four seasons: pre-monsoon (July - September), monsoon (October - December) in the year of 2017 and post-monsoon (January-March), summer (April-June) in the year 2018. Decomposing leaf samples were sampled during low tide when the mangrove substrate was fully exposed. The water from decomposing leaf litter areas was analyzed *in situ* for temperature, pH, redox potential (Eh) and salinity (‰). The temperature was measured using a thermometer with 0.5°C accuracy; pH and redox potential (Eh) by using a millivolt meter with the platinum electrode (pH 315i/ SET, Wissenschaftlich-Technische Werkstatten, Germany); and water salinity by a hand refractometer (Erma INC, Tokyo). In the decomposing leaves, levels of available N (Subbiah & Asija 1956), P (Olsen et al. 1954) and K (Guzman & Jimenez 1992) were estimated.

Isolation of microbes from decomposing leaf samples

Leaf samples were drawn randomly from any one of the six litter bags at the intervals of 0, 7, 14, 21, 28, 35 and 42 days of experiment and brought to the laboratory immediately for microbial examination. The samples were cleaned and washed with sterilized seawater to remove the debris present on the leaves. One gram of the fresh sample was aseptically ground by using a pestle and mortar, then a serial dilution was made to get different diluents (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) for the isolation of microbes in Petri dish with suitable culture media prepared in 50% seawater: glucose yeast peptone agar (GYPA) medium supplemented with antibacterial (streptomycin 100 $\mu g.l^{-1}$) and antifungal (fluconazole 100 $\mu g.l^{-1}$) agents purchased from Hi-Media (Mumbai-India) to prevent the bacterial and fungal contaminants for thraustochytrids (Kalidasan et al. 2015a,b), zobell marine agar (ZMA) for total heterotrophic bacteria (THB) (Masilamaniselvam 2003), de man, rogosa and sharpe agar (MRS) medium for lactobacillus (deMan et al. 1960), starch casein agar (SCA) medium for actinobacteria (Ravikumar et al. 2004), winogradsky agar (WA) medium for azotobacters (Buchanan & Gibbons 1974), yeast malt agar (YMA) medium for yeasts (Fell 2005), *Trichoderma* selective agar medium (TSM) for *Trichoderma* (Askew & Laing 1993), and potato dextrose agar (PDA) medium for fungi (Ravikumar et al. 2004). All analytical reagents, chemical and media components were purchased from Hi-Media (Mumbai-India).

Enumeration of the microbes

For the enumeration of microbes, 1 ml of the serially diluted samples of leaf extract was pipetted out into sterile petri dish. Then, sterile media (GYPA, ZMA, MRS, SCA, WA, YMA, TSM and PDA) was poured aseptically into dishes and swirled for a thorough mixing. After solidification the plates were incubated for 2-7 days in an inverted position at 28±2ºC. All the determinations were carried out in triplicates. Counts of different microbial groups were determined after the incubation period of 2 to 3 days for THB; 7 to 10 days for azotobacters, actinobacteria, lactobacilli and *Trichoderma;* 2 to 7 days for thraustochytrids and yeasts; and, 2 to 3 weeks for cyanobacteria. The microbial counts are expressed as the number of colony forming units (CFU) per gram of wet leaf sample.

Statistical analysis

All data were analyzed statistically using SPSS 16.0 (IBM-Windows) software to find the significant differences of parameters between mangrove species or seasons or days of decomposition. Univariate 3-way ANOVA was done according to Duncan multiple range test. Pearson's correction was also calculated at $p<0.01$ between thraustochytrid counts and leaf nutrients or water parameters or other microbes.

Results

Thraustochytrids count in relation to mangrove species, days of leaf decomposition and season of analysis

Thraustochytrid counts as influenced by four seasons are shown in Table 1. Thraustochytrid

count was higher in *Avicennia marina* (1.6 $\times 10^2$ CFU.g^{-1}) than that of *Rhizophora apiculata* (1.2$\times 10^2$ CFU.g^{-1}) (Table 1). The count ranged between 6$\times 10^1$ and 2.1$\times 10^2$ CFU.g^{-1} during litter decomposition (Fig. 1a), being the maximum on day 21 of decomposition (Fig. 1b). The count was the highest (1.6$\times 10^2$ CFU.g^{-1}) in post-monsoon, and lowest (1.3$\times 10^2$ CFU.g^{-1}) in pre-monsoon (Fig. 1c).

Thraustochytrids count in relation to other microbial counts during litter decomposition

Thraustochytrids count exhibited high positive correlations (p<0.01) with other microbial groups viz., total heterotrophic bacteria, lactobacilli, yeasts, actinobacteria, azotobacter, fungi and *Trichoderma* but a negative correlation with cyanobacteria (Table 1, Fig. 2a-h).

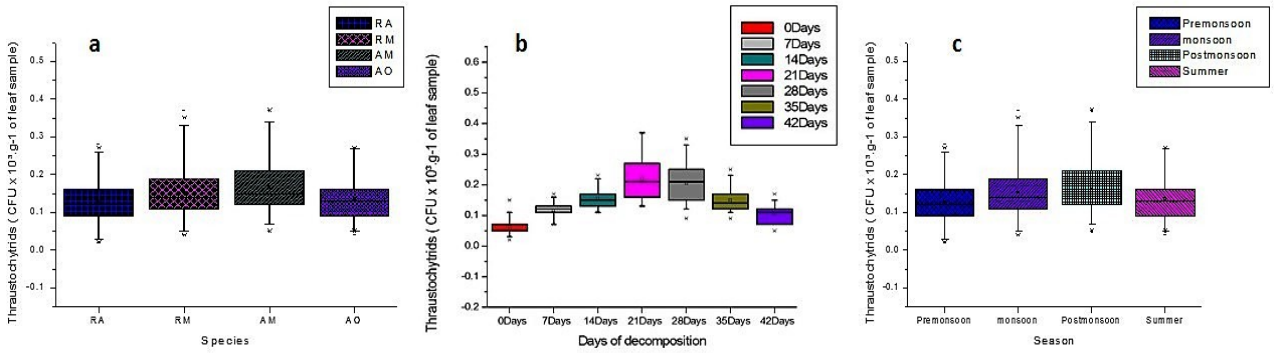

Fig. 1 – Thraustochytrids counts as influenced by (a) mangroves species (b) days of mangrove litter decomposition and (c) months/seasons (AM – *A. marina*, AO – *A. officinalis*, RA – *R. apiculata* and RM – *R. mucronata*) (bars indicate standard error)

Thraustochytrids count in relation to physicochemical changes during litter decomposition

The counts exhibited positive correlations with pH, redox potential of mangrove water, leaf N and P (p<0.01) (Fig. 3a-g) while other parameters such as temperature, salinity and potassium did not show any positive correlation.

Thraustochytrids count in relation to water temperature

Thraustochytrids count showed a negative correlation with water temperature (p<0.01). The temperature was higher in the decomposing *A. marina* (24.23±1.67ºC) than that of *R. apiculata* (23.23±1.74ºC) (Table 2). The temperature varied with days of litter decomposition ranging from 22.55±1.86ºC in 14 days to 24.66±1.62ºC in 21 days (Table 2). The temperature varied with months: maximum (25.46±0.88ºC) in summer (May) and minimum (22.55±1.18ºC) in post monsoon (Feb) (Table 2).

Thraustochytrids count in relation to water pH

Thraustochytrids count showed a significant positive correlation with water pH (p<0.01). The pH was higher in the decomposing *R. apiculata* (8.09±0.39) than that of *A. marina* (8.37±0.32) (Table 2). The pH varied with days of litter decomposition ranging from 7.81±0.44 in 28 days to 8.38±0.29 in 42 days (Table 2). The pH varied with months: maximum (8.37±0.29) in summer (May) and minimum (7.96±0.31) in pre-monsoon (Aug) (Table 2).

Thraustochytrids count in relation to water salinity

Thraustochytrids count showed a negative correlation with water salinity (p<0.01). The salinity was higher in the decomposing leaf litter of *A. marina* (27.29±1.96 ppt) than that of *A. officinalis* (26.50±1.76 ppt) (Table 2). The salinity varied with days of litter decomposition ranging from 26.69±1.88 ppt in 35 days to 27.10±1.75 ppt in 07 days (Table 2). The salinity varied with months: high (29.30±1.34 ppt) in summer (May) and low (25.13±0.66 ppt) in monsoon (Nov) (Table 2).

Table 1 Changes of microbial counts as influenced by four mangrove species (*Avicennia officinalis*, *A. marina*, *Rhizophora apiculata* and *R. mucronata*), days of decomposition and four seasons of sampling.

Source	Microbial counts (CFUx $10^3.g^{-1}$ of leaf tissue)								
	Cyanobacteria	**THB**	**Lactobacillus**	**Azotobacter**	**Actinobacteria**	**Fungi**	**Yeasts**	***Trichoderma***	**Thraustochytrids**
Species									
Rhizophora apiculata	0.07 ± 0.08^{ab}	2.68 ± 0.69^{a}	0.10 ± 0.06^{a}	0.05 ± 0.02^{a}	0.17 ± 0.10^{a}	0.26 ± 0.08^{a}	0.17 ± 0.10^{a}	0.21 ± 0.0^{a}	0.12 ± 0.06^{a}
Rhizophora mucronata	0.07 ± 0.04^{ab}	2.91 ± 0.80^{a}	0.12 ± 0.06^{bc}	0.06 ± 0.03^{b}	0.20 ± 0.11^{b}	0.30 ± 0.09^{b}	0.20 ± 0.10^{a}	0.24 ± 0.08^{b}	0.15 ± 0.07^{bc}
Avicennia marina	0.08 ± 0.03^{b}	2.99 ± 0.84^{a}	0.13 ± 0.07^{c}	0.06 ± 0.03^{b}	0.21 ± 0.11^{b}	0.30 ± 0.09^{b}	0.20 ± 0.11^{a}	0.25 ± 0.08^{b}	0.16 ± 0.07^{c}
Avicennia officinalis	0.06 ± 0.03^{a}	2.77 ± 0.72^{a}	0.10 ± 0.05^{a}	0.04 ± 0.02^{a}	0.18 ± 0.09^{a}	0.26 ± 0.09^{a}	0.17 ± 0.10^{a}	0.21 ± 0.0^{a}	0.13 ± 0.05^{ab}
Days of decomposition									
0	0.10 ± 0.02^{c}	0.28 ± 0.04^{a}	0.05 ± 0.0^{a}	0.01 ± 0.00^{a}	0.06 ± 0.01^{a}	0.10 ± 0.03^{a}	0.05 ± 0.01^{a}	0.11 ± 0.03^{a}	0.06 ± 0.02^{a}
7	0.11 ± 0.01^{c}	0.23 ± 0.03^{a}	0.10 ± 0.0^{c}	0.04 ± 0.01^{b}	0.05 ± 0.01^{a}	0.21 ± 0.04^{b}	0.04 ± 0.01^{a}	0.15 ± 0.03^{b}	0.11 ± 0.02^{b}
14	0.09 ± 0.03^{c}	3.07 ± 0.14^{b}	0.15 ± 0.0^{e}	0.08 ± 0.01^{d}	0.16 ± 0.03^{b}	0.30 ± 0.03^{c}	0.16 ± 0.03^{b}	0.27 ± 0.03^{d}	0.15 ± 0.03^{c}
21	0.07 ± 0.01^{b}	4.04 ± 0.22^{c}	0.24 ± 0.0^{f}	0.09 ± 0.02^{e}	0.21 ± 0.03^{c}	0.36 ± 0.03^{e}	0.19 ± 0.03^{c}	0.27 ± 0.03^{d}	0.21 ± 0.07^{d}
28	0.05 ± 0.01^{b}	5.08 ± 0.33^{d}	0.11 ± 0.0^{d}	0.06 ± 0.01^{c}	0.23 ± 0.03^{d}	0.35 ± 0.03^{e}	0.22 ± 0.02^{d}	0.30 ± 0.04^{e}	0.20 ± 0.06^{d}
35	0.05 ± 0.02^{b}	4.08 ± 0.27^{c}	0.07 ± 0.0^{b}	0.05 ± 0.01^{b}	0.27 ± 0.03^{e}	0.32 ± 0.03^{d}	0.31 ± 0.03^{e}	0.31 ± 0.03^{e}	0.14 ± 0.03^{c}
42	0.03 ± 0.01^{a}	3.10 ± 0.24^{b}	0.05 ± 0.0^{a}	0.04 ± 0.01^{b}	0.35 ± 0.03^{f}	0.32 ± 0.03^{d}	0.34 ± 0.03^{f}	0.19 ± 0.04^{c}	0.10 ± 0.03^{b}
Season									
Premonsoon	0.06 ± 0.03^{a}	2.91 ± 0.80^{a}	0.11 ± 0.06^{ab}	0.05 ± 0.02^{ab}	0.18 ± 0.10^{ab}	0.28 ± 0.09^{a}	0.19 ± 0.10^{a}	0.22 ± 0.07^{a}	0.13 ± 0.07^{a}
Monsoon	0.09 ± 0.08^{b}	2.94 ± 0.81^{a}	0.12 ± 0.06^{b}	0.06 ± 0.02^{b}	0.20 ± 0.10^{ab}	0.29 ± 0.09^{a}	0.19 ± 0.11^{a}	0.25 ± 0.07^{b}	0.15 ± 0.06^{a}
Postmonsoon	0.07 ± 0.03^{ab}	2.79 ± 0.76^{a}	0.10 ± 0.06^{ab}	0.06 ± 0.03^{b}	0.21 ± 0.11^{b}	0.29 ± 0.08^{a}	0.19 ± 0.11^{a}	0.23 ± 0.08^{ab}	0.16 ± 0.06^{a}
Summer	0.06 ± 0.03^{a}	2.73 ± 0.70^{a}	0.10 ± 0.06^{a}	0.04 ± 0.02^{a}	0.17 ± 0.10^{a}	0.27 ± 0.09^{a}	0.18 ± 0.11^{a}	0.22 ± 0.08^{b}	0.14 ± 0.06^{a}
ANOVA									
Species	*	**	**	**	**	**	**	**	**
Days of decomposition	**	**	**	**	**	**	**	**	**
Seasons	**	**	**	**	**	**	**	**	NS
Species X Days of decomposition	NS	**	NS	NS	NS	NS	NS	NS	NS
Species X Seasons	NS	**	NS	NS	NS	NS	NS	NS	NS
Days of decomposition X Seasons	NS	**	NS	NS	*	NS	NS	NS	NS
Species X Days of decomposition X Seasons	NS	**	NS	NS	NS	NS	NS	NS	NS

Values not sharing a common alphabet superscript differ significantly at $p > 0.05$; ** = $p < 0.01$;* = $p < 0.05$; NS= Not Significant

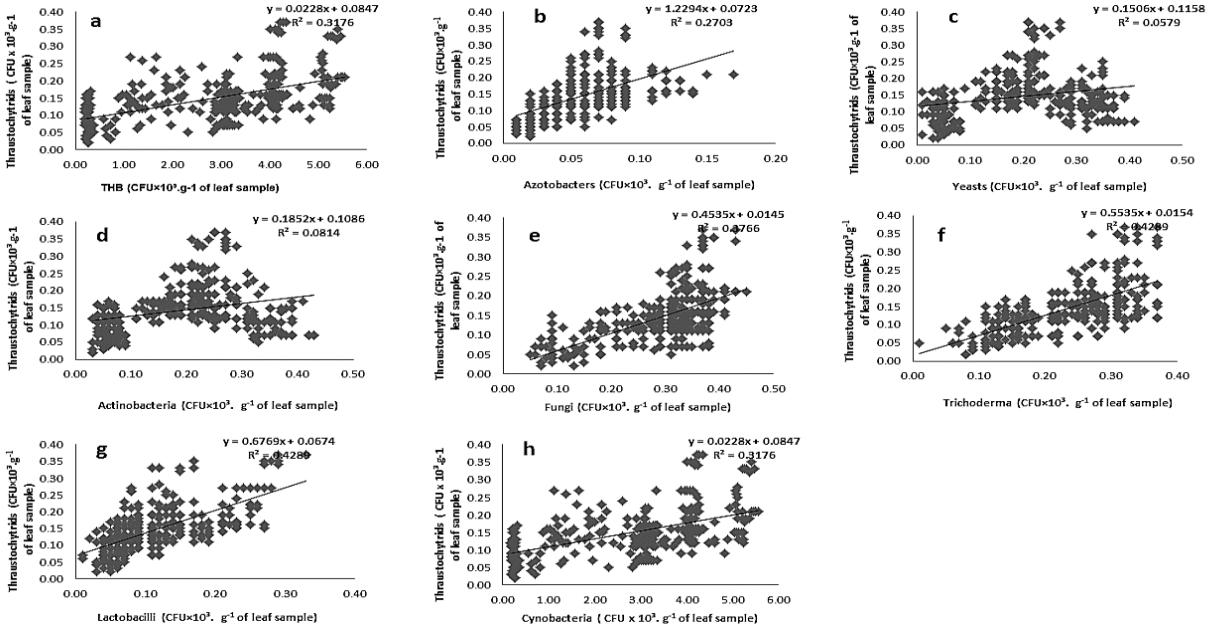

Fig. 2a–h – Relationships between *Thraustochytrids* counts and other microbial groups during decomposition of mangrove leaf litter

Thraustochytrids count in relation to water redox potential

Thraustochytrids count showed a significant positive correlation with water redox potential (p<0.01). The redox potential was higher in the decomposing leaf litter of *A. marina* (124.45±17.45 mV) than that of *R. apiculata* (112.00±14.59 mV) (Table 2). The redox potential of water varied with days of litter decomposition ranging from 91.82±8.95 mV in 14 days to 135.85±13.97 mV in 21 days (Table 2). The redox potential varied with months: high (118.91±16.51 mV) in summer (May) and low (116.00±16.04 mV) in monsoon (Nov) (Table 2).

Thraustochytrids count in relation to leaf nitrogen

Thraustochytrids count showed a significant positive correlation with leaf N (p<0.05). Nitrogen content was higher in the leaves of *A. marina* (1.98±0.67%) than that of *R. apiculata* (1.77±0.61%) (Table 2). The leaf N varied with days of litter decomposition ranging from 0.64±0.10% to 2.51±0.20 % in 0 and 42 days of decomposition, respectively (Table 2). The leaf nitrogen varied with months: high (1.93±0.65 %) in monsoon (Nov) and low (1.82±0.65) in summer (April) (Table 2).

Thraustochytrids count in relation to leaf phosphorus

Thraustochytrids count showed a significant positive correlation with leaf P (p<0.05). Phosphorus was higher in the leaves of *A. marina* (1.58±0.22 mg.g^{-1}) than that of *R. apiculata* (1.34±0.19 mg.g^{-1}) (Table 2). Leaf P varied with days of decomposition ranging from 1.21±0.13 to 1.68±0.16 mg.g^{-1} in 7 and 35 days of decomposition, respectively (Table 2). It also varied with months: high (1.49±0.21 mg.g^{-1}) in monsoon and post monsoon (Nov and Feb) and low (1.38±0.23 mg.g^{-1}) in summer (May) (Table 2).

Thraustochytrids count in relation to leaf potassium

Thraustochytrids count showed a negative correlation with leaf K (p<0.05). P was higher in the leaves of *R. mucronata* (0.37±0.08 mg.g^{-1}) than that of *R. apiculata* (0.21±0.06 mg.g^{-1}) (Table 2). Leaf K varied with days of decomposition ranging from 0.14±0.03 to 0.41±0.04 mg.g^{-1} in 21 and 14 days of decomposition respectively (Table 2). The leaf K varied with months: high (0.34±0.09 mg.g^{-1}) in summer (May) and low (0.23±0.07 mg.g^{-1}) in pre monsoon and post monsoon (Aug and Feb) (Table 2).

Table 2 Mangrove water characteristics during leaf litter decomposition in four mangrove species (*Avicennia officinalis, A. marina, Rhizophora apiculata* and *R. mucronata*), of different durations of decomposition and four seasons of sampling.

Source	Temperature (ºC)	pH	Salinity (ppt)	Redox potential (mV)	Leaf nitrogen (%)	Leaf phosphorus (mg.g^{-1})	Leaf potassium (mg.g^{-1})
Species							
Rhizophora apiculata	23.23±1.74^{a}	8.09±0.39^{a}	26.57±1.69^{a}	112.00±14.59^{a}	1.77±0.61^{a}	1.34±0.19^{a}	0.21±0.06^{a}
Rhizophora mucronata	24.03±1.51^{b}	8.21±0.51^{b}	27.18±1.77^{b}	116.19±14.83^{ab}	1.95±0.66^{a}	1.48±0.20^{c}	0.37±0.08^{a}
Avicennia marina	24.23±1.67^{b}	8.37±0.32^{c}	27.29±1.96^{b}	124.45±17.45^{c}	1.98±0.67^{a}	1.58±0.22^{d}	0.26±0.07^{a}
Avicennia officinalis	23.73±1.57^{b}	8.12±0.23^{ab}	26.50±1.76^{a}	117.60±15.48^{b}	1.79±0.63^{a}	1.41±0.19^{b}	0.22±0.06^{a}
Days of decomposition							
0	23.79±1.16^{bc}	8.21±0.29^{b}	27.06±1.80^{a}	105.50±5.95^{b}	0.64±0.10^{a}	1.43±0.20^{cd}	0.31±0.05^{ab}
7	23.44±1.76^{b}	8.24±0.26^{bc}	27.10±1.75^{a}	118.37±8.99^{c}	1.41±0.12^{b}	1.21±0.13^{a}	0.26±0.05^{ab}
14	22.55±1.86^{a}	8.20±0.55^{b}	26.79±1.81^{a}	91.82±8.95^{a}	1.65±0.23^{c}	1.39±0.18^{bc}	0.41±0.04^{b}
21	24.66±1.62^{d}	8.29±0.21^{bc}	26.89±1.79^{a}	135.85±13.97^{f}	2.21±0.16^{d}	1.48±0.18^{d}	0.14±0.03^{a}
28	24.26±1.20^{cd}	7.81±0.44^{a}	26.69±2.08^{a}	129.03±7.35^{e}	2.28±0.21^{d}	1.63±0.16^{e}	0.23±0.04^{ab}
35	24.06±1.30^{bcd}	8.28±0.32^{bc}	26.69±1.88^{a}	123.53±7.11^{d}	2.41±0.17^{e}	1.68±0.16^{e}	0.30±0.05^{ab}
42	23.87±1.81^{bc}	8.38±0.29^{c}	26.96±1.69^{a}	118.81±5.74^{c}	2.51±0.20^{f}	1.34±0.12^{b}	0.20±0.03^{ab}
Season							
Premonsoon	23.79±1.47^{b}	7.96±0.31^{a}	25.89±0.62^{b}	116.94±16.12^{a}	1.89±0.66^{a}	1.44±0.22^{ab}	0.23±0.07^{a}
Monsoon	23.44±1.16^{a}	8.15±0.47^{b}	25.13±0.66^{a}	116.00±16.04^{a}	1.93±0.65^{a}	1.49±0.21^{b}	0.26±0.06^{a}
Postmonsoon	22.55±1.18^{c}	8.32±0.32^{c}	27.22±0.85^{c}	118.38±16.22^{a}	1.86±0.65^{a}	1.49±0.21^{b}	0.23±0.07^{a}
Summer	25.46±0.88^{d}	8.37±0.29^{c}	29.30±1.34^{d}	118.91±16.51^{a}	1.82±0.65^{a}	1.38±0.23^{a}	0.34±0.09^{a}
ANOVA							
Species	**	**	**	**	**	**	NS
Days of decomposition	**	**	NS	**	**	**	NS
Seasons	**	**	**	**	**	**	NS
Species X Days of decomposition	NS	*	NS	*	NS	NS	NS
Species X Seasons	**	NS	NS	**	NS	NS	NS
Days of decomposition X Seasons	**	NS	NS	**	NS	NS	NS
Species X Days of decomposition X Seasons	NS	NS	NS	NS	NS	NS	NS

Values not sharing a common alphabet superscript differ significantly at $p > 0.05$; ** = $p < 0.01$; * = $p < 0.05$; NS= Not Significant

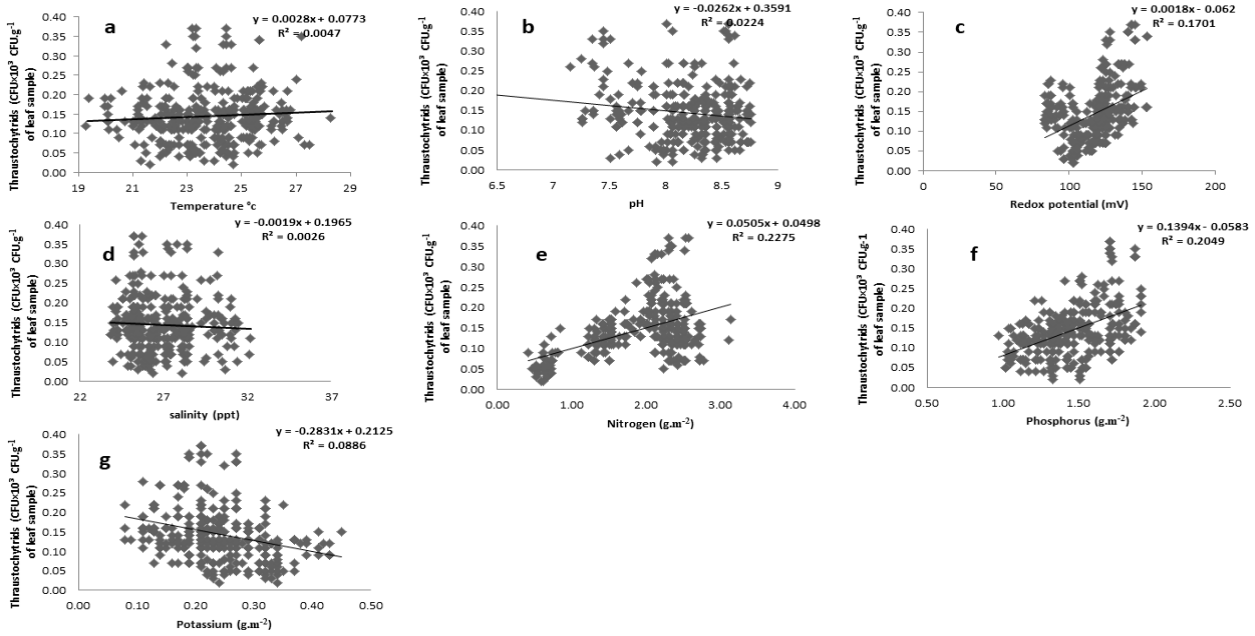

Fig. 3a–g – Relationships between *Thraustochytrids* counts and leaf nutrients and water during leaf litter decomposition

Discussion

The present study revealed that the decomposing mangrove leaf litter was colonized by different groups of microorganisms in descending order of numerical abundance: total heterotrophic bacteria, fungi, *Trichoderma*, actinobacteria, yeasts, thraustochytrids, cyanobacteria and azotobacters. These microbes involve synergistically in leaf decomposition, as evident by the positive correlation between thraustochytrids counts and other microbial groups, except cyanobacteria. Oomycetes and thraustochytrids colonize fallen mangrove leaves from early to late stages of decay (Raghukumar et al. 1994, 1995, Bremer 1995, Nakagiri et al. 1989, Leano et al. 1998). Cellulase production by thraustochytrids is particularly important in the process of degradation of refractory leaf litters and enhancement of its biomass (Raghukumar et al. 1994, Kathiresan et al. 2011, Taoka et al. 2017). This process changes the leaf debris into a nutritious detritus food for consumption of fish and crustaceans. The present study also revealed that the levels of leaf N and P increased with thraustochytrids count and other microbes. However, the increased level of N is likely to inhibit the cyanobacteria as they are autotrophic and nitrogen-fixing organisms, and hence the cyanobacterial count exhibited negative correlation with saprophytic thraustochytrids.

Thraustochytrids count was higher in decomposing leaf litter of *Avicennia marina* than that in *Rhizophora apiculata*. This is due to the fact that *A. marina*, which has thinner leaves with higher protein and lower tannins, decomposes faster than *R. apiculata* does (Steinke et al. 1990, Kathiresan & Bingham 2001). In the present study, the maximum biomass of thraustochytrids was attained on day 21 of mangrove detritus. Microbial biomass, including that of fungi, generally peaks during intermediate stages of decomposition. Raghukumar et al. (1994) have also found the highest mycelial fungal and bacterial biomass in the mangrove leaves of *R. apiculata* after 21 days of decomposition. Similarly, Sathe-Pathak et al. (1993) have observed the maximum biomass of thraustochytrids and bacteria after 21 days of brown algal detritus. Thraustochytrid count increased with increasing days of decomposition, which is similar to the previous findings (Raghukumar et al. 1994, 1995, Kathiresan et al. 2011, Gomathi 2011). However, only low count of thraustochytrids was encountered during initial stages of decomposition. Generally, the fresh/young plant leaves contain more amount of phenolics and tannins, which are inhibitory to microbes. Latter during the decomposition process the phenolic and other second metabolites gradually decreased due to leaching, this will result in the colonization by other microbial group, bacteria and fungi,

particularly thraustochytrids (Raghukumar et al. 1994, 1995, Sharma et al. 1994). Fungal colonization is promoted by the loss of phenolics from mangrove leaves. Findlay et al. (2002) have found that bacterial biomass is higher on fine smaller particles relative to leaves or wood surfaces, fungi on the other hand, as indicated by high concentration of ergosterol, are significantly greater on leaves and wood. This confirms the predominance of fungal biomass in the larger size classes. Fungi therefore deal with biomass in senescent leaves and are most prevalent during intermediate stages of decomposition, such as yellow leaves. Meanwhile, bacteria are present in low levels in senescent leaves, becoming most abundant in black leaves and fine particulate detritus. Thraustochytrids are not present in senescent leaves but they colonize the leaves soon after they come in contact with water. Thraustochytrids increased in abundance as the leaves get decayed and become significant in black leaves.

Thraustochytrids count was under the influence of physicochemical factors. It exhibited positive correlation with pH and redox potential of mangrove water, but did not show significant correlation with temperature and salinity. Being obligate marine microbes, the thraustochytrids prefer high pH and they are also known to be affected by acidic substrates (Domsch et al. 1980). However, thraustochytrids are able to withstand the continuous fluctuations in temperature and salinity in mangrove environment. These organisms are able to grow in a wide range of temperatures (15-30ºC) and salinity (7.5-30 ppt) (Yaguchi et al. 1997, Fan et al. 2002). Interestingly, Bahnweg & Sparrow (1974) have isolated thraustochytrids from the Antarctic and Sub-Antarctic regions with a temperature ranging from 0 to 9ºC. Thraustochytrid strains used in this study showed growth responses to varying salinity and temperature levels. This indicates that the temperature and salinity of the mangrove environment have no significant effect on their vegetative cell growth.

The redox potential was higher in decomposing leaf litter of *Avicennia marina* than that of *Rhizophora apiculata*, indicating that the former was more aerated than the latter. Generally, saprotrophic microbes such as thraustochytrids are present on top layers of soils in the forests to decompose organic matter (Danielson & Davey 1973). Thraustochytrid count exhibited positive correlations with leaf N and P, but not with K. Such increase in the N and P levels are essential for the growth of mangroves associated microorganisms (THB, fungi, *Trichoderma*, actinobacteria, yeast, thraustochytrids, cyanobacteria and azotobacter) and fauna (fishes, craustaceans, molluscs, crab, benthic organism) (Sengupta & Chaudhuri 1991, Holguin et al. 1992, 1999, 2001, Alongi et al. 1993, Vazquez et al. 2000, Kathiresan et al. 2014). The K level of decomposing leaf litter varied in this study, but showed a negative correlation with thraustochytrids. The level of K content in decomposing leaf litter is lower than that of other nutrients, which indicates the reduction of thraustochytrids growth Bahnweg 1979, Garrill et al. 1992). However, seawater contains many major nutrients (Na, Ca, Mg, and K), which balance the growth of marine fungi, including thraustochytrids (Jennings 1983). The ecological niche of thraustochytrids in an aquatic biotope is dominated by heterotrophic and saprophytic microbes (Raghukumar 2002) as evident by the high positive correlation between thraustochytrids and other saprophytic microbes. The microbial role in decomposition of mangrove leaf litter is highly significant to balance the nutrient deficiency in the detritus-based mangrove ecosystem.

Conclusion

This paper has revealed that the thraustochytrids in association with other saprophytic microbes are involved in the decomposition of leaf litter, essential for ensuring soil fertility in the detritus-based mangrove system. This process changes the leaf debris into a nutritious detritus food for consumption of fish and crustaceans. The enumeration of thraustochytrids in decomposing leaves of mangroves as influenced by physicochemical and microbial factors is very much essential for studying detritus-based mangrove ecosystem. Interestingly, thraustochytrids count was significant between mangrove species and days of decomposition but not between seasons. Thraustochytrids count was high in *A. marina* at day 21 of decomposition. The thraustochytrids count exhibited a high positive correlation with THB, lactobacilli, yeasts, actinobacteria,

azotobacter, fungi, and *Trichoderma*, but negative correlation with autotrophic cyanobacteria. The counts exhibited positive correlations with pH of mangrove water, redox potential, leaf nitrogen and leaf phosphorus. This study provides baseline information on the role of thraustochytrids in decomposition process of mangrove leaves. Further studies of litter metagenomics will reveal more insights on the interactions between diverse microbial floras of the mangrove biotope.

Conflict of interest statement

We declare that we have no conflict of interest.

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