



Litter decomposition of the invasive *Lantana camara* L. (Verbenaceae: Lamiales) in a tropical stream of South India

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Abstract

The invasive species *Lantana camara* is a common in India and this species is widespread in forests, where it is distributed along with native riparian species in streams. Decay rate and nutrient release of *L. camara* are necessary to understand the variability of aquatic communities and processes in impacted and non-impacted streams. Here, we examined native and invasive litter decomposition process and rate of sporulation by aquatic hyphomycetes on decomposition in a tropical stream. For this study, two native riparian (*Syzygium alternifolium* and *Pongamia pinnata*) and an invasive species (*L. camara*) were used at two different seasons. We found that litter decompositional rate differs with seasons, being higher in north-east monsoon. However, the invasive species decomposed faster than the native species. The species with lower sporulation rate, *S. alternifolium*, had higher sporulation rate. Our results demonstrated that abiotic process during litter decomposition in stream does not altered conversely biotic components of microbial consumers influence with the introduction of an invasive species.

Keywords: leaf litter, decomposition, invasive species, streams, India

Introduction

Stream ecosystem is often reliant on external resource inputs to endure trophic links. Entry of leaf litter into streams through direct (vertical litter fall) and laterally from the forest soil alter food webs due to litter's nutrient and chemical composition ^[1] and significant to the global carbon cycle through their subsequent decomposition ^[2]. The physical and chemical characters of toughness and secondary compounds play a key role determining litter processing and decomposer colonization ^[3, 4]. Thus, surrounding vegetation is an important source for decomposers and proficient to change trophic links in streams ^[5].

The total forest and tree cover in India is about 24.6% of the total geographical area of the country represented by Forest Survey of India (2019), of these, 3.26% of the forest covers in the province of Tamil Nadu including the Western and Eastern Ghats. In total, 5,640 species of flowering plants have been recorded in Tamil Nadu, which is one-third of the total flora in India. About 5% (279 species) of alien invasive taxa are recorded in Tamil Nadu, which influences native biodiversity and human welfare ^[6]. Plant invasions alter key ecosystem process involving water and nutrient cycles and affect the functioning of fluvial and riparian biotic communities in streams ^[7].

In spite of the variety of native plant community contributes a vital nutrient source to stream community, effect of native riparian zones with alien invasive taxa on stream community has received more attention, especially in temperate streams. For instance, exotic litter influenced the feeding pattern of aquatic communities^[8], lower colonization of aquatic fungi and bacteria^[9] and amendment of aquatic macroinvertebrates and fishes^[10]. Litter decay process of native and invasive species in tropical streams is occasional. Hence, the present study aims to inventorize the native and invasive litter decaying process and to analyze the aquatic hyphomycetes sporulation during decomposition in a tropical stream of South India.

Materials and methods

Study area

Azhagar hills (Alagarmalai as in Tamil language) lies between 10.06°N-10.17°N latitude and 78.19°E-78.28°E longitude, which cover an area of 60.83sq.km. The highest peak at Thalaianaiparai (805m) is situated in the middle of the hill. The Azhagar hill is located 20km from Madurai district, Tamil Nadu province, South India. The Eastern Ghats starting point is Azhagar hill and Sirumalai (which is parallel to Azhagar hill). Two major valleys are found in Azhagar hill: Peria-aruvi valley (290m) and Bison valley (670m). From south-west direction of the Bison valley, a Silambar valley is present. These three valleys are fed by rivulets of Peria-aruvi, Thumanimuthar and Silambar. The Azhagar hill has many intermittent and perennial streams.

In this area, a total of 25 species in herbaceous community were recorded ^[11]. In our pilot survey, we observed that the dominant riparian plant species in sampling stream site (Nooburagangai stream) are: Tree- *Syzygium alternifolium*, *Pongamia pinnata*, *Mangifera indica*, *Artocarpus hirsutus* and *Ficus callosa* and Herbs-*Mimosa pudica*, *Euphorbia hirta* and *Cyperus rotundus*. Other than these plants, some invasive plants are found nearer to streams including *Lantana camara*. *L. camara* is not observed in the survey of ^[11]. Now, this plant *L. camara* is sparsely present in Alagar hills. Therefore, we compared the native versus invasive litter decomposition and spore production patterns in this area.

The present study was carried out in a perennial and anthropogenic impacted stream of Nooburagangai stream (Fig. 1), which originates from Silambar valley and this stream is considered as sacred water and utilize for drinking and bathing purposes. The experiment was conducted during two post monsoonal periods: South-west monsoon (September 2020) and North-east monsoon (January 2021). The post monsoon period is suitable for conducting litter experiment study^[12]. From the study area, the three dominant riparian plant species were taken for the present study: two native species (*Syzygium alternifolium* and *Pongamia pinnata*) and one invasive plant species (*Lantana camara*).

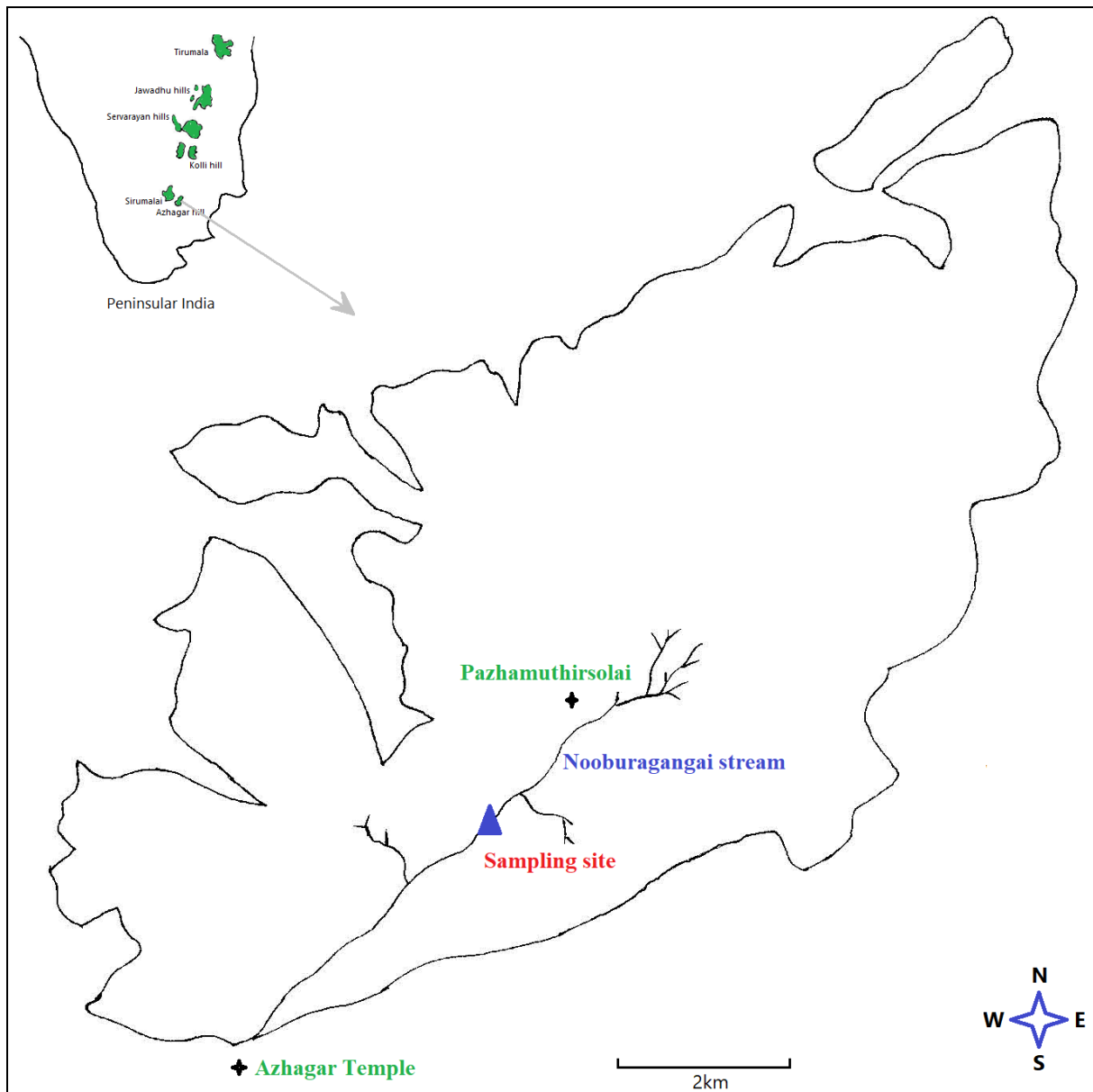


Fig. 1. Sampling site of Nooburagangai stream in Azhagar hill of South India.

Sampling methods

The fresh fallen leaves were collected under the respective plant species in study area. The collected leaves were taken in a polythene bag and brought it to the laboratory where leaves were sorted out and dried at room temperature for 3 to 4 days. Then, leaves weighed into portions of 1g of each litter species (*S. alternifolium*, *P. pinnata* and *L. camara*). The weighed litter were packed in fine mesh bags (pore size: 1mm; bag size: 10x10cm) to prevent macroinvertebrates entry. The mesh bags were prepared as following: 1g of each species *S. alternifolium* *P. pinnata*, *L. camara* and three leaves species mixture -48 bags (3 bags x 4 types x 4 sampling

dates) for estimating to change air dry mass to oven dry mass; 48 bags (3 bags x 4 types 4 sampling dates) for estimating handling loss and 192 bags (4 litter bags x 3 replicates x 4 types x 4 sampling dates) for litter processing.

In the field, three pools across the stream were selected and this pool interval was 50m distance. The pool depth and diameter were 30-50cm and 0.5 to 1m depth. The three replicates of litter bags of each pool were placed in stream pool for studying litter decay and spore production. In total, 192 bags were placed three streams (48bags of each type x4 sampling dates). The processed litter bags were collected at 7th, 14th, 21st and 28th day's intervals. With specified intervals, litter bags in each type were carefully collected from the site and brought in to the laboratory. The decayed litters were carefully removed from the mesh bags in the laboratory and relic litter was dried at room temperature for 3 days. The physico-chemical parameters of water temperature, pH, total dissolved solids, and conductivity were analyzed in the field by PCS Testr 35 (Eutech instruments, India) and the stream flow, width and depth were measured.

From the initial stage to final period, litter from each type (*S. alternifolium*, *P. pinnata*, *L. camara* and three leaves mixture) were placed separately in muffle furnace for 1h at 500°C to estimate ash free dry weight (AFDW) for each collection date. The first set of air dry mass of each litter bags kept at 55 °C for 2 days and weighed for humidity removal. The average correction factor (D) was calculated by $D = \text{oven-dry mass} / \text{air-dry mass}$. The initial oven dry mass of litter bags brought to the streams is measured by multiplying the air-dry mass by the average correction factor (D). The second set of litter bags was recovered immediately upon exposure in the stream, which allows an estimate of losses due to initial handling.

The sporulation by aquatic hyphomycetes was estimated according to Barlocher ^[13]: the recovered leaves from each type of litter bags were taken and removed silt and sand, leaf materials (size ranged from 5 to 10 cm) were placed in Erlenmeyer flask with stream water, it was placed in shaker at 150 rpm for 2 days at room temperature for inducing spores, incubated leaf materials removed and filtered the water by membrane filter, and added a few drops of Trypan Blue for staining, and they were randomly counted with the help of microscope. Biomass, total volume and density of spores were estimated ^[14, 15].

Data analysis

All data are processed in MS Excel, where data are sorted out and assigned for different analysis. The arithmetic mean, percentage of value and standard deviation were calculated. The percentage of mass loss was expressed after correction for handling and humidity (100% = mass after corrections). The decay co-efficient (-k) was calculated by regression analysis: a linear regression for original data (independent variable= Time; dependent variable= % Mass remaining), a nonlinear curve fitting program (exponential decay; provide an estimate of a & k) and transform (% Mass remaining) to ln (% mass remaining) and run a linear regression (independent variable= Time, dependent variable= ln (% mass remaining)). To find the relationship between sporulation rate of litter type and environmental variables, Principal Component Analysis (PCA) was used by using statistical software (PAST, version 3.15).

Results

The physical and chemical characters of sampling site in Nooburagangai stream of Azhagar hill, South India are given in Table 1. The latitude, longitude and elevation of the sampling site are: 10°09'12N, 78°22'13E and 360m, respectively. The average water temperature during experimental period was 21.4 °C in south-west monsoon and 24.6 °C in north-east monsoon. The values of pH, conductivity, total dissolved solids and salinity were unchanged during the first experimental period (south-west monsoon) whereas these parameters were increased from initial to final stage of the second experiment during north-east monsoon. The stream water flow was high during the first experiment and low the second experiment. The width and depth of pool were less during the second experiment than the first experimental period (South-west monsoon).

The partial leaf mass loss in first week was observed in invasive species of *L. camara* (60%) and less than 50% leaf mass loss observed in other three litter bags for two seasons (Table 2). During the experimental periods, leaf mass loss in all type of litter bags showed the steady forfeit. In the end of the experiment or 28th day of South-west monsoon, 85% mass loss found in *L. camara* followed 38% in *S. alternifolium*, 39% in mixture of leaves and 35% in *P. pinnata*. The leaf dry mass loss 81% in *L. camara* followed by 50% in *S. alternifolium*, 40% in mixture of leaves and 37% in *P. pinnata* were observed at 28th day of experiment during north-east monsoon. Overall, the invasive species of *L. camara* leaf mass loss was greater than native species in both seasons (Fig. 2). The low percentage of dry mass loss found in leaf mixture bags when compared to individual bags.

The decay coefficient for four types of litter bags was estimated (Table 3). The rate of decomposition was rapid in the invasive species of *L. camara* litter bag for both seasons and these leaves would be taken 28-30 days for 95% loss. The litter decay in *S. alternifolium* was slower than invasive species and their decomposition period was about 70 days at south-west monsoon and 52 days at north-east monsoon. In *P. pinnata* leaves decomposition rate was steady and their decomposition period was more or less similar (75 and 71 days) for two

seasons. The decomposition of mixture of three leaves species bags were gradual and constant and 95% loss would be taken for 66 days at north-east monsoon and 71 days for south-west monsoon.

The sporulation rate by aquatic hyphomycetes of four types of litter bags (*S. alternifolium*, *P. pinnata*, *L. camara* and mixture of three leaf species) was calculated for two experimental periods (south-west and north-east monsoons) (Fig. 3). Comparatively, two experimental periods were relatively same and these data were pooled and analyzed for estimating sporulation rate. The sporulation rate for all type of litter bags was increased up to length of experimental period. The high rate of sporulation was observed in *S. alternifolium* (31%) followed by *L. camara* (25%), *P. pinnata* (22%) and mixture of three leaf species (22%). The average sporulation rate for the length of 28th days was 822 (no. day⁻¹ mg⁻¹ of leaf dry mass) in *S. alternifolium*, 671 in *L. camara*, 597 in leaf mixture and 589 in *P. pinnata*. The mean sporulation rate ranged from 150 to 200 for *S. alternifolium* and *L. camara* and 100 to 180 for leaf mixture and *P. pinnata*.

The relationship between sporulation rate by aquatic hyphomycetes and environmental variables recorded during experimental periods was analyzed through Principal component analysis. The two experimental period's environmental variables and sproulation rate were pooled due to similar results. The eigen value of PC1 and PC2 are 0.0297 and 0.005. The result of PCA was given as biplot (Fig. 4). The high score was found at 28th day experiment (0.161 of PC1). The high PCA loading value was observed in salinity (0.162) and total dissolved solids (0.111). Overall result of PCA revealed that four types of litter bags were related with salinity and total dissolved solids rather than other environmental variables tested.

Table 1: Physico-chemical parameters recorded in Nooburagangai stream during experimental periods.

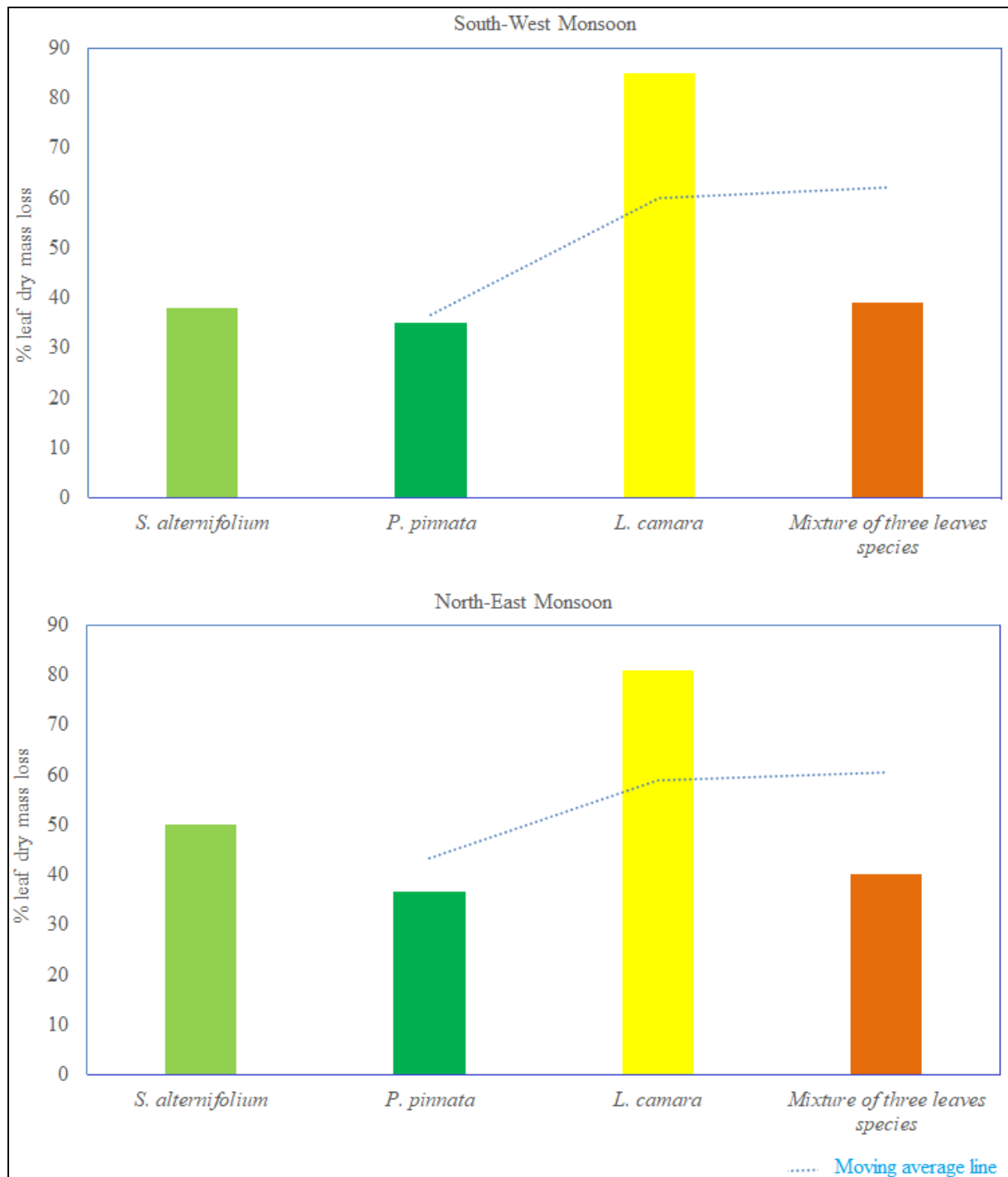
	Initial	7 th day	14 th day	21 st day	28 th day	Mean	Standard Deviation
South-west monsoon							
Water temperature (°C)	22.0	21.5	21.0	21.0	21.5	21.4	0.42
pH	7.4	7.3	7.4	7.2	7.3	7.32	0.08
Conductivity (µs)	350	355	362	382	365	363	12.2
Total dissolved solids (ppm)	254	242	284	295	254	266	22.5
Salinity (ppt)	175	168	185	190	195	183	11
Stream flow (sec/m)	3.8	3.6	2.5	2.9	3.1	3.2	0.53
Stream pool depth (cm)	45	40	40	40	40	41	2.24
Stream width (m)	1.5	1.5	1.8	1.7	1.6	1.62	0.13
North-east monsoon							
Water temperature (°C)	23.5	24.3	24.5	25.2	25.3	24.6	0.73
pH	7.9	7.8	7.7	7.7	7.7	7.76	0.09
Conductivity (µs)	401	431	463	498	512	461	46
Total dissolved solids (ppm)	285	306	329	354	363	327	32.5
Salinity (ppt)	190	208	224	237	247	221	22.8
Stream flow (sec/m)	4.2	5.6	7.9	8.3	12	7.6	2.98
Stream pool depth (cm)	30	24	16	12	10	18.4	8.41
Stream width (m)	0.9	0.8	0.75	0.7	0.65	0.76	0.1

Table 2: Leaf dry mass loss (in g) during experimental periods in Nooburagangai stream of Azhagar hill, South India.

Experimental period	<i>S. alternifolium</i>	<i>P. pinnata</i>	<i>L. camara</i>	Mixture of three leaves species
South-west monsoon				
Initial	1.0	1.0	1.0	1.0
7 th day	0.82	0.85	0.42	0.71
14 th day	0.75	0.75	0.34	0.68
21 st day	0.68	0.70	0.25	0.65
28 th day	0.62	0.65	0.15	0.61
% of loss	38.0	35.0	85.0	39.0
North-east monsoon				
Initial	1.2	1.0	1.2	1.0
7 th day	0.83	0.83	0.47	0.77
14 th day	0.77	0.77	0.43	0.73
21 st day	0.67	0.67	0.33	0.63
28 th day	0.60	0.63	0.23	0.60
% of loss	50.0	36.7	80.8	40.0

Table 3: Decay coefficients (-k SD) and days to 95% AFDW loss (SD) for leaf litter, Coefficients of determination (r²) are presented

Leaf type	γ^2	-k 95% CI \pm SD	Days to 95% loss \pm SD
South-west monsoon			
<i>S. alternifolium</i>	0.968	-0.0128 \pm 0.0001	70 \pm 0.5
<i>P. pinnata</i>	0.968	-0.0121 \pm 0.0001	75 \pm 0.1
<i>L. camara</i>	0.887	-0.0267 \pm 0.0009	28 \pm 0.4
Mixture of leaves	0.854	-0.0120 \pm 0.0002	71 \pm 1
North-east monsoon			
<i>S. alternifolium</i>	0.923	-0.0195 \pm 0.0001	52 \pm 2
<i>P. pinnata</i>	0.971	-0.0128 \pm 0.0001	71 \pm 1.5
<i>L. camara</i>	0.854	-0.0297 \pm 0.0007	30 \pm 1
Mixture of leaves	0.937	-0.0133 \pm 0.0002	66 \pm 0.5

**Fig 2:** Leaf dry mass loss (%) for four types of litter bags during experimental periods.

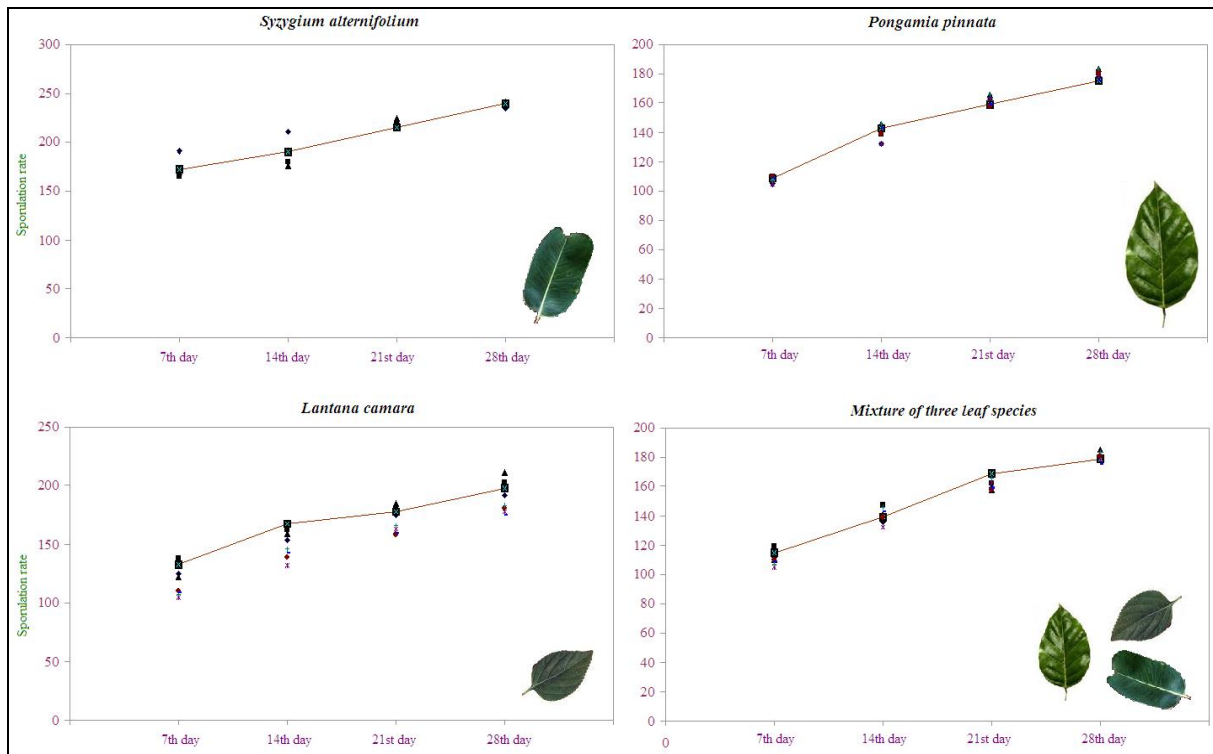


Fig 3: The spore production rate (no. day⁻¹ mg⁻¹ of leaf dry mass) observed from four types of litter bags in stream during experimental periods.

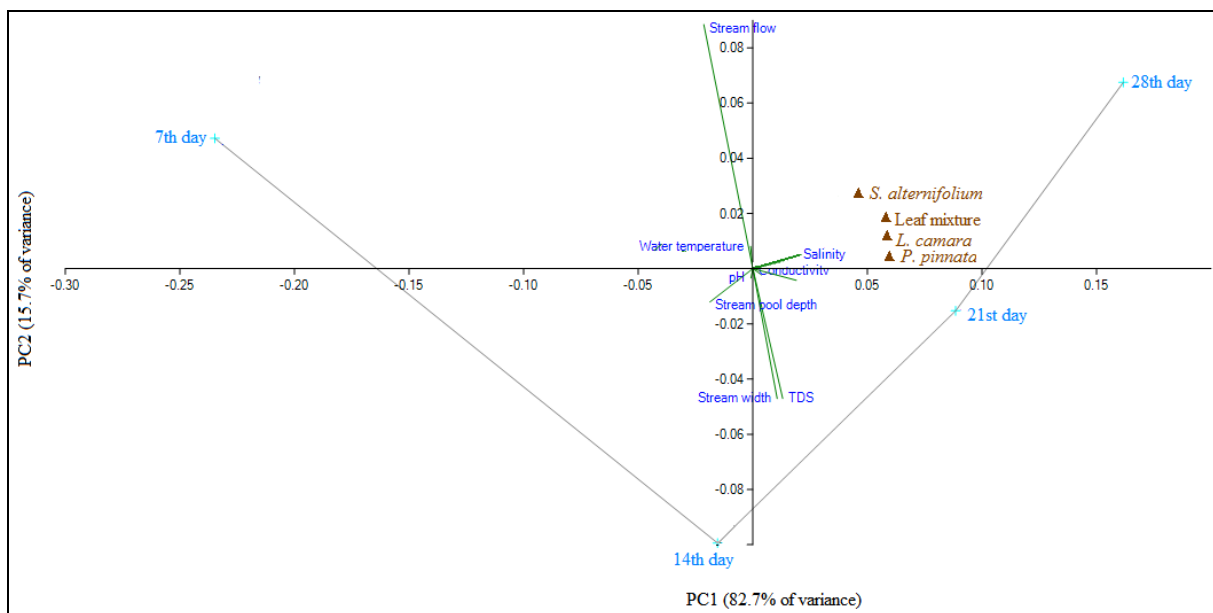


Fig 4: Biplot showing the relationship between environmental variables and sporulation rate of litter bags during experiments.

Discussion

Leaf litter decomposition is an important process in freshwater environment that deeply sensitive by the allochthonous input, nutrient composition, colonization of microorganisms and macro invertebrates [12]. Stream consumers obtain most of their energy from leaf litter provided by the terrestrial vegetation and litter decomposition by consumers in streams naturally differing in riparian vegetation [16]. Thus, understanding the relationship between natural versus invasive vegetations, aquatic communities and litter decomposition may assist explaining the variability in aquatic communities and processes in streams, which result of their responses would significant for assessing anthropogenic impact in streams. Therefore, the present study focused to inventorize the litter decompositional pattern of natural vegetation and invasive *L. camara* in a tropical stream of south India.

Our results showed that the invasive *L. camara* decomposed faster for both seasons, than in the native vegetations (*S. alternifolium* and *P. Pinnata*). This result supports report that soft litter type decomposed faster than medium and hard types [17, 18]. The partial weight loss occurred in invasive *L. camara* during first week,

whereas the low percentage of weight loss found in natural vegetations and leaf mixture bags. This finding contrasts with our early study that *P. pinnata* and other two natural vegetations (*Syzygium cumini* and *Commiphora caudata*) decomposed more than 50% within first week^[12]. The lower rate dry mass loss of natural vegetation observed in both seasons in the present study area may be dependent with nature of stream health. Since, the study site Nooburagangai stream is a sacred stream and possible to more utility by pilgrims, decompositional rate is greatly influenced.

The decomposition rates of leaves in streams are influenced by external and internal factors. The external factors (temperature, physical abrasion, pH and nutrient availability) and intrinsic factors (nutrient content, hardness and presence of defensive compounds) are greatly affected the litter breakdown rates^[19]. Of these, factors, temperature and seasonality play vital factors in streams to predict litter decomposition rate^[20]. Likely, the present experimental study indicates that variation in litter decay rate occurred between seasons, and decay rate was high during north-east monsoon rather than south-west monsoon. During north-east monsoon, the study area receives high rainfall, in turn to increase stream flow, which greatly increase litter decomposition rate. The flow velocity as driving force for decomposing leaves and twigs^[21].

Microbial consumers of bacteria and fungi assemblage in leaf litter play distinctive roles in microbial decomposition and differ in their sensitivity to seasons of Mediterranean stream^[22]. In the present study, sporulation by aquatic hyphomycetes associated with litter types were slightly differed between seasons in tropical stream. Seasonal variations in rain fall in tropical and subtropical systems are probably to influence litter fall, and the reproduction of aquatic hyphomycetes and therefore the community composition of aquatic hyphomycetes throughout the year^[23]. The high rate of sporulation was associated with *S. alternifolium* litter bag, whereas invasive *L. camara* had lesser sporulation rate observed in the present study. Although invasive *L. camara* decomposition was faster, the lower rate of sporulation observed rather than native species. This finding indicates that leaf softness of *L. camara* predicts faster decomposition but fungal colonization may depend with nutrient quality.

The leaf litter chemical composition, along with nature of stream characteristics and abiotic factors of temperature, rainfall and total dissolved solids, control the process of decomposition which makes nutrient release from the leaf litter^[24]. In the present study, the environmental factors of total dissolved solids and salinity are significantly related with litter decomposition and sporulation by aquatic hyphomycetes. Since the study site is one of the famous tourist and pilgrimage place, stream water may be polluted due to human discharges, wastes, bathing activities. Consequently, non-polluted stream litter decomposition rate is faster and anthropogenic impacted site of the present study area had the slower rate of decomposition and there is no difference of litter decomposition occurred between native and invasive vegetation.

In conclusion, we found that invasive *L. camara* decomposed twice faster than native vegetation in stream. The decomposition rate was greatly influenced with seasons, especially in north-east monsoon had fast decomposition rate. The high rate of sporulation by aquatic hyphomycetes was observed in native species of *S. alternifolium* rather than invasive species *L. camara*. Further, leaf mixture bags had low decomposition rate as well as sporulation rate rather than other litter bags. These results demonstrated that introduction of an invasive species of *L. camara* into small tropical stream decomposed faster and fungal assemblage as sporulation rate was lesser than native vegetation in both seasons. Further research is needed to investigate the effects of physical leaf characteristics, litter quality and macroinvertebrate assemblages of native and invasive plant litter species on decomposition should be investigated to further understand the nutrient release process.

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