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Seasonal Dynamics of Leaf Litter Decomposition and Fungal Population in an Undisturbed *Dipterocarpus* Forest of North East India

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ABSTRACT

The impact of climatic factors such as rainfall, temperature and seasonal variations on the rate of leaf litter decomposition and on the occurrence and abundance of microfungi were studied in an undisturbed *Dipterocarpus* forest in Manipur, North East India. The decomposition of leaf litter was determined by the litter bag method and culturable microfungal quantities were determined using serial dilution and plate count methods. The results of the study revealed a decline in the microfungal population and decomposition in environments with scanty rainfall. Significant positive correlations were observed between weight loss, rainfall, microfungal quantities and temperature. Significant variation in fungal colonies was observed in different seasons. Depletion in rainfall may be one of the important causes for the decrease in decomposition rate, which may have affected the nutrient dynamics and occurrence of microfungi. It may also affect overall forest productivity if similar adverse climatic conditions continued in the region in the future.

Keywords: climate change, litter decomposition, Manipur, microfungi
Abbreviations: cfu, colony forming unit; **MRBA**, Martin's rose Bengal agar; **PDA**, potato dextrose agar

INTRODUCTION

Litter decomposition is one of the key biogeochemical processes in forest ecosystems (Swift *et al.* 1979). Litter is an essential part of nutrient cycling and also acts as a protective layer by buffering changes in soil water content (Ginter *et al.* 1979), temperature (MacKinney 1929), soil compaction (Benkobi *et al.* 1993) and hinders erosion (Lowdermilk 1930) and leaching (Lunt 1951; Mo *et al.* 2003). Litter provides habitats and a substrate for microorganisms (Jordan *et al.* 2003). Soil physical and chemical properties, the diversity of microfungi and other soil organisms are directly or indirectly influenced by the existing litter layer. The quantity of litter fall varies greatly over a range of spatial and temporal scales and is determined mainly by climate, seasonality, topography, soil parent materials, and species distribution. Climate change may affect litter fall because changes in rainfall patterns and mean annual temperatures can affect tree phenology and species distribution (Condit *et al.* 1996). An increase in productivity and litter fall has been observed as a consequence of elevated atmospheric CO_2 levels (Allen *et al.* 2000). In tropical forest ecosystems with nutrient-poor soils, litterfall and decomposition processes are particularly important for the nutrient budget (Sundarapandian and Swamy 1999), affecting relationships between biodiversity and ecosystem properties and functions (Swift *et al.* 1979; Wardle and Lavelle 1997; Hoorens *et al.* 2003). Xiong *et al*. (2008) reported that significant soil pH increase with litter removal was a positive factor for acid soil in the studied site. Except for soil moisture, significant effects, caused by removal of litter or/and understory, on measured soil chemical characteristics were only observed in the top 10 cm soil layer, but not in the 10–20 cm layer. Soil available P and exchangeable K contents were significantly higher in the rainy season than in the dry season; however, the other soil properties were not substantially affected by season.

Litter decomposition is influenced by environmental factors and physiochemical properties of plant parts such as stem wood, leaves, roots, etc. and decomposer organisms present in the soil (Vesterdal 1999). Three important key factors responsible for the control of litter decomposition processes include environmental conditions (particularly climate), litter quality and the soil biota (Swift *et al.* 1979). Climate is known to be the dominant factor influencing litter decomposition on a large geographic scale under unfavorable conditions (Meentemeyer 1978; Dyer *et al.* 1990; Austin and Vitousek 2000). In contrast, under favorable climatic conditions, litter quality becomes a significant factor in determining litter decomposition processes (Bargali *et al.* 1993; Austin and Vitousek 2000). Therefore, litter decomposition in wet tropical regions is considered to be more rapid than in temperate regions, although decomposition rates vary widely in both climatic regions (Anderson and Swift 1983; Aerts 1997; Takeda 1998). Litterfall inputs and litter decomposition represent important components of the carbon (C) and nitrogen (N) cycles in forest ecosystems because the turnover of litter is a major pathway by which C and N enter forest soil (Berg and Laskowski 2006; Kamei *et al.* 2009). Forest type can have a significant influence on litterfall C and N because of the difference in input of leaf litter or non-leaf litter produced by different species (Kamei *et al.* 2009). Decomposition is the breakdown of dead organic matter through the action of leaching, fragmentation and chemical reduction into CO_2 and inorganic nutrients (Chapin *et al.* 2002). Leaching removes soluble materials from decomposing organic matter, fragmentation occured by large soil fauna and/or abiotic agents produces a fresh substrate for microbial colonization and a food source for smaller soil animals (Gavazov 2011). Chemical alteration of dead organic matter is primarily due to the catabolic activity of bacteria and fungi (Gavazov 2011). The population of microfungi varies in different seasons of the year (Behera 1985). The decomposing ability of species within fungal

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Research Note

assemblages was greater in warmer than in cooler climates and in broad-leaved than in coniferous tree species. In particular, the greatest abilities to cause mass loss were found among fungi with ligninolytic activity in broad-leaved tree species in warmer climates (Osono 2011). Uselman *et al.* (2011) reported that Greater UVB exposure resulted in decreased rates of decomposition and P release for the lower quality litter and no change in rates of decomposition and nutrient release for the two higher quality litter types, possibly due to a negative effect of UVB on soil microbes. Salah and Scholes (2011) reported the effect of temperature and litter quality on decomposition rate of *Pinus patula* needle litter. Litter quality was not a strong predictor of decomposition rates implying temperature is the major factor influencing the decomposition rate of *Pinus patula* needle litter. Results of this study are consistent with the hypothesis that the rate of nutrient cycling in non-limiting environments will increase, due primarily to an increase in litter decomposition as a result of increased temperatures. He *et al.* (2010) reported that elevation of temperature caused higher mass loss of *Altingia obovata* leaf litter. However the results were not similar in the two forest stands where the studies were conducted. This showed that spatial variation is one important factor of decomposition pattern. He *et al*. (2010) also reported that response of decomposition of leaf litter to forest type and temperature was positively related to the difference in microbial activities between both montane rainforests. Lecerf and Richardson (2010) studied the effects of high and moderate levels of forest disturbance on stream condition and reported that across all forest treatments fungal biomass or diversity remained fairly similar. They have suggest that stream ecosystems are extremely sensitive to small changes in riparian and upland forest cover and propose that litter decomposition as a key ecosystem function in streams could be incorporated into further efforts to evaluate and improve forestry best management practices. Prevost-Boure *et al.* (2010) reported that fresh litter quantity directly affects soil and litter bacterial community structure and litter and abiotic factors interact in modifying soil/litter bacterial communities. Hättenschwiler *et al.* (2011) reported that plant litter and microbial diversity matters for decomposition, but that considering numbers of taxonomic units appears overall as little relevant and less useful than functional diversity. However, despite easily available functional litter traits and the well-established theoretical framework for functional litter diversity, the impact of functional litter diversity on decomposition is not yet well enough explored. Defining functional diversity of microorganisms remains one of the biggest challenges for functional approaches to microbial diversity. The North-East part of India received considerably scanty rainfall in 2009. Changes in abiotic variables such as temperature and rainfall affect various processes in different ecosystems, including litter decomposition. The study was conducted in a tropical deciduous forest (Champion and Seth 1968) located near Moreh town, Chandel District in Manipur (India). In this study, the variation in leaf litter decomposition of *Dipterocarpus tuberculatus* in different seasons were evaluated and correlated with the decomposer fungal populations.

MATERIALS AND METHODS

Study site

The study was conducted in a tropical deciduous forest located near Moreh town, Chandel District, between 23°49`–24°28`N lat. and 94° 09′ – 94° 31′ E long. in Manipur along the Indo-Myanmar border of Northeast India at 300–360 msl. The study area was dominated by *Dipterocarpus tuberculatus* Roxb. and other associated species, including *Ardisia peniculata*, *Wendlandia wallichii*, *Ficus cunia* among others. The site experiences three seasons: spring (March to May), rainy (June to October) and winter (November to February).

Collection of samples

Freshly fallen leaves of *D. tuberculatus* were collected using the litter trap method (Bréda 2003) using large nylon nets hanged 2 feet above ground to prevent leaves from falling on forest floor. The nets were placed in three locations where the density of *Dipterocarpus* trees are maximum and trees are adjacent, ensuring maximum *Dipterocarpus* leaf litter collection for microbial and decomposition studies.

Estimation of litter decomposition

Leaf litter decomposition was measured following the litterbag method (Bocock and Gilbert 1957). The leaves were air dried to constant weight and 10 g of samples were placed in autoclaved nylon net bags stitched in the local market (20 × 20 cm; 2 mm mesh size) and were pinned to the forest floor, ensuring a fixed position for each bag. To determine the leaf litter weight loss per season one set of three bags was picked up in every season. The recovered litter bags were brushed and washed using sterile distilled water with gentle agitation on a 1 mm mesh screen and oven dried at 80°C for 24 h until constant weight. The fresh and dry weight was recorded.

Enumeration of decomposer microbes

The dilution plate method was followed for isolation of decomposer fungi (Parkinson *et al.* 1971). One gram of leaf litter pieces (5 mm in diameter) was suspended in 100 ml of distilled water and shaken vigorously to detach the fungal propagules and serially diluted before inoculation. PDA (potato dextrose agar) and MRBA (Martin's rose Bengal agar) media were used for routine culturing and isolation. The experiment was replicated three times and colony forming units (cfu/g) of dried litter was calculated by the following formula:

$$\text{CFU} = \frac{\text{Mean number of fungal colonies}}{\text{Weight of dry leaf litter}} \times \text{Dilution factor}$$

Meteorological data

Meteorological data on rainfall, temperature and humidity for the study area was collected from the Regional Meteorological Centre, Guwahati, Assam, India.

Statistical analysis

Statistical analysis was performed following completely randomized design (CRBD) with three replicates in each treatment. The data was subjected to Analysis of Variance (ANOVA) using statistical software MINITAB 11.2 (http://www.minitab.com) and significance of various treatments was evaluated by *F*-tests ($P < 0.05$) by calculating CD values. The data recorded were subjected to ANOVA to see the significant variations between the sampling seasons. Pearson's correlation coefficient was used for Correlation analysis to find out the relationship between percent weight loss of leaf litter, number of fungal colonies with abiotic variables.

RESULTS

Meteorological data

The meteorological data on rainfall and temperature of the study area for 2008 and 2009 revealed that the mean monthly average temperature was 23.1 and 23.2°C in spring, 25.8 and 26.1°C in the rainy season and 17.0 and 16.8°C in winter, respectively. The area received 1146.4 mm of annual rainfall in 2008 and 1124.1 mm in 2009; maximum rainfall occurred during the rainy season. A decrease in average rainfall was recorded in the rainy season during 2009 while a slight hike in the average temperature during 2009 was observed compared to 2008 (**Table 1**). There were no significant differences in the temperature and rainfall of both years ($P < 0.05$) although there were significant differences between seasons within the same year ($P < 0.05$).

Table 1 Total rainfall and average temperature.

Season	Total rainfall (mm)	Average temperature (°C)
Spring08	230.8	23.1
Rainy 08	849.0	25.8
Winter 08-09	24.1	17.0
Spring09	313.2	23.2
Rainy 09	776.6	26.1
Winter 09-10	26.7	16.8
	F = 13193.54	F = 5156.4
	SE ± m = 29.83	SE ± m = 0.01
	CD = 103.37	CD = 0.03
	p = 0.05	p = 0.05

Table 2 Litter decomposition and microbial population.

Seasons	% weight loss	cfu/g of leaf litter
Spring08	12.1	100
Rainy 08	17.4	156.4
Winter 08-09	5.7	51.8
Spring09	9.1	88.7
Rainy 09	11.9	126.6
Winter 09-10	5.8	36
	F = 2677.29	F = 331.81
	SE ± m = 0.022	SE ± m = 18.39
	CD = 0.08	CD = 63.71
	p = 0.05	p = 0.05

Table 3 Season wise occurrence of different litter decomposing microfungi.

Genera recorded	Rainy season	Spring	Winter	% Relative abundance
Alternaria	+	+	+	8.30
Aspergillus	+	+	+	27.28
Cephalosporium	+	+	-	0.55
Chaetonium	+	-	-	0.17
Cladosporium	+	+	+	8.66
Fusarium	+	+	+	12.23
Mucor	+	+	+	11.88
Penicillium	+	+	+	12.59
Pythium	+	+	-	0.21
Rhizomucor	+	-	-	0.08
Rhizopus	+	+	+	7.72
Sporotrichum	+	-	-	0.93
Trichoderma	+	+	+	8.84
Verticillium	+	-	-	0.46

Litter decomposition

Litter decomposition evaluated as percent mass loss in different seasons, it recorded significant seasonal variations. The trend of decomposition was similar in both years; it was highest in rainy season followed by spring and winter season. The weight loss was recorded a maximum of 17.4% during rainy season of 2008 in comparison to 11.9% for same season of year 2009 (**Table 2**). The tendency of weight loss was almost similar in both years; for spring it was 12.1% and 9.1 and for winters it was 5.8 and 5.7, for 2008 and 2009, respectively. Statistically the significant differences were recorded for percent weight loss for both years and among the seasons of same year ($P < 0.05$).

Diversity and population of decomposer microfungi

In agreement with the rate of decomposition, the decomposer mycoflora recorded a similar pattern of occurrence (**Table 2**). Highest fungal cfu were recorded in 2008 rainy season (1.56×10^5) followed by spring (1.0×10^5) and least in winter (5.18×10^4). Similarly, in year 2009, it was highest in rainy season (1.27×10^5) followed by spring (8.87×10^4) and least in winter (3.6×10^4). The data revealed that the total cfu of microfungi during the entire study period was recorded to be 5.6×10^5; out of which 50.5% (2.83×10^5) cfu were recorded in rainy season, 33.7% in spring (1.89×10^5) and 15.7% in winter (8.87×10^4). The population of decomposer microfungi was significantly different in both years and within seasons of same year ($P < 0.05$).

A total of 14 genera of decomposer microfungi were isolated in different seasons. All 14 genera were recorded in the rainy season, 10 genera were recorded in summer and 8 in winter (**Table 3**). The relative abundance of different genera reveled that about 97.5% of the population was dominated by only 8 genera: *Aspergillus* (27.28%), *Penicillium* (12.59%), *Fusarium* (12.23%), *Mucor* (11.88%), *Trichoderma* (8.84%), *Cladosporium* (8.66%), *Alternaria* (8.30%) and *Rhizopus* (7.72%).

The most common micro-fungi recorded in all seasons were *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Trichoderma* species while *Chaetonium*, *Verticillium*, *Sporotrichum* and *Rhizomucor* were unique to the rainy season; *Aspergillus*, *Alternaria*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Pythium*, *Rhizopus* and *Trichoderma* species were isolated in spring and *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma* were recorded in winter only. The dominant microfungi of the rainy season were *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Trichoderma* while *Mucor* and *Rhizopus* were dominant in spring and *Alternaria* and *Cladosporium* were dominant in winter. *Aspergillus* was the dominant genus across all seasons in both years. *Chaetonium*, *Rhizomucor*, *Sporotrichum*, and *Verticillium* were not encountered in spring and *Cephalosporium*, *Chaetonium*, *Pythium*, *Sporotrichum*, *Verticillium* and *Rhizomucor* did not occur in winter.

Table 4 Correlation between rate of weight loss and cfu/g of leaf litter with abiotic variables.

Parameter	r
Total rainfall (mm) and % weight loss	0.86*
Total rainfall (mm) and cfu/g of leaf litter × 10^3	0.94*
Average temperature (°C) and % weight loss	0.87*
Average temperature (°C) and cfu/g of leaf litter × 10^3	0.94*
cfu/g of leaf litter × 10^3 and % weight loss	0.96*
Average temperature (°C) and cfu/g of leaf litter × 10^3	0.94*

*Shows significant at $P < 0.05$.

Correlation analysis

The data recorded during the experiment were subjected to one-way ANOVA to see the significant variation and correlation analysis was conducted between the average temperature, rate of decomposition and fungal cfu. A significant correlation was observed between average temperature and the rate of decomposition ($P < 0.05$) and between average temperature and fungal cfu ($P < 0.05$). Total rainfall was also correlated with the occurrence of fungal cfu and the rate of decomposition. Both fungal cfu and the rate of decomposition were significantly correlated with the pattern of rainfall ($P < 0.05$) (**Table 4**).

DISCUSSION

Microfungi play an important role in organic matter decomposition in forest ecosystems. Scanty reports of decomposer microfungi from the North Eastern part of India exist. In the *Dipterocarpus* forest of North East India microfungal populations inhabiting leaf litter are reported for the very first time in this study. Leaf litter decomposition is closely associated with related phenomenon such as CO_2 emission associated with a positive feedback to global warming. Faster decomposition may leave soil unprotected for leaching and erosion. Thus, the rate of decomposition and its controlling factors are important factors to study. Favorable climatic conditions, including higher rainfall and temperature, promote microfungal growth and litter decomposition. The results clearly depicted a higher population of micro-

fungi and maximum leaf litter decomposition during rainy seasons in comparison to spring and winter. Devi and Yadava (2007) also reported maximum leaf litter decomposition in a *Dipterocarpus tuberculatus* forest during the rainy season followed by spring and minimum in winter. However, no attempt was made by them to isolate decomposer mycoflora. They reported a positive correlation between soil moisture and rainfall ($P < 0.01$) stating that soil moisture is also a regulating factor of decomposition. In the current study, a positive correlation between rainfall and temperature was observed with both litter decomposition and microfungal populations. Like all microbes, fungi also responded to abiotic variables. A positive correlation was observed between microfungal quantity and percent weight loss in different seasons. Less weight loss and fewer microfungal populations in winters may be due to the cool and dry conditions. Moorhead *et al.* (1999) also reported a higher litter mass loss in moist and warm environments in comparison to cold or dry sites. Behera and Mukerji (1985) reported higher fungal quantity in the rainy season. In some ecosystems fungi dominate other microbes in decomposing litter. Duarte *et al.* (2010) reported a greater role of fungi than bacteria in leaf-litter decomposition in streams. Variation in fungal populations is governed by changes in atmospheric temperature, relative humidity and availability of soil moisture and nutrients (Tresner *et al.* 1954; Stevenson *et al.* 1957; Wabster *et al.* 1960; Christensen *et al.* 1962). The rate of litter decomposition is largely a determining factor for productivity of forest ecosystems as plant nutrients became available for recycling within the system during this process. Variation in climatic factors alters the pattern of decomposition and occurrence of decomposer fungal populations. Compositional shifts in soil microbial communities, mediated by ecological interactions among soil saprotrophs, appear to lie at the biogeochemical heart of ecosystem response to environmental change. Further investigations are required to determine the potent microfungi responsible leaf litter decomposition in a *Dipterocarpus* forest in a particular season.

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