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Leaf Litter Decomposition of *Melocanna baccifera* (Roxb.) Kurz under Field and Laboratory Microcosm in Northeast India

N.S. Singh, K.K. Upadhyay and S.K. Tripathi[1*]

Department of Forestry, [1]School of Earth Science and Natural Resources Management
Mizoram University, Tanhril, Aizawl-796004, India
**E-mail: sk_tripathi@rediffmail.com*

***Abstract*:** *Melocanna baccifera* (Roxb.) Kurz is one of the most abundant bamboo species of Mizoram, Northeast India contributing about 95% of the total bamboo resources. This study was aimed to assess the rate of leaf litter decomposition of *M. baccifera* leaves in natural forest (FS) and laboratory microcosm (MC). A total of 36 litter bags measuring (15 x 15 cm, 2 mm mesh) containing 10g of air-dried litter material were placed in FS, and 36 litter bags of 10 x 10 cm filled with ~5 g of litter material were placed in the MC. MC was made from bottom sealed PVC pipe of 12 cm diameter and 16 cm height and filled with layers of 2/3 mineral soil and 1/3 with organic soil from the forest. A total of 6 litter bags were retrieved from FS and MC at monthly intervals for 6 months. Litter decay rate was higher in FS as compared to MC and litter mass remaining at the end of the study was 7 % and 13 % in FS and MC, respectively. Consequently, carbon (C) and nitrogen (N) mass remaining were 11.2% and 11.8% in MC and 7.3% and 5.6% in FS. Annual decay constant (k) was 2.2 and 1.4, respectively in FS and MC. Overall, mass loss rate and C and N release from *M. baccifera* litter was significantly higher in FS compared to MC indicating more complex synergistic effects of abiotic and biotic factors in decomposition at the FS site compared to MC site.

***Keywords*:** Bamboo leaf litter, Decay rate constant (k), Carbon and Nitrogen release, Northeast India

Bamboo is one of the fastest growing plants belonging to the family Poaceae. Various species of bamboo is widely distributed throughout the northeastern region. Bamboo forest represents ~57% of the total forest area in Mizoram. *Melocanna baccifera* is one of the most abundant bamboo species accountings for about 95% of the total bamboo resources of the state of Mizoram (Anon 2017). The species of bamboo are occurring naturally mixed with other forest species as well as planted by the villagers for its multiple uses at altitudes ranging from as low as 100 m amsl to 1500 m amsl depending on the types of bamboo (Singh et al 2015, Singha and Tripathi 2019). The studies on the litter decomposition of bamboo in the region are highly scarce. While the photosynthesis represents one of the important processes for building up of organic matter into the forest, the process of litter decomposition is equally important to recycle the organic matter in the forested ecosystems (Novara et al 2015, Lalnunzira and Tripathi 2018). Litter decomposition helps to restore organic matter and nutrients in the forest soils (Pandey et al 2007, Wapongnungsang et al 2017, Singh and Tripathi 2020 a, b). But the process of litter decomposition on the forest floor is influenced by number of factors such as initial litter quality (i.e. C, N, lignin, C/N), soil microbes and various abiotic variables (Tripathi et al 2012, Gautam et al 2019, Lalnunzira and Tripathi 2018, Singh et al 2021). Concentration of Carbon (C), nitrogen (N) and lignin in the initial litter has been reported to control the decomposition of litter in many forest sites (Gartner and Cardon 2004, Tripathi et al 2006). Further, it has been reported that the early phase of litter decomposition process is influenced by the concentration of N in the initial litter (Lalnunzira and Tripathi 2018), and in the later phase C becomes the limiting factor for the decomposition (Hauchhum and Tripathi 2019, Singh et al 2021). Rapid mass loss of litter has been reported in tropical forests as compared to the temperate forests (Thongkantha et al 2008, Nonghuloo et al 2020). This study was designed to assess the magnitude of litter decomposition and C and N release pattern in the natural forest and control laboratory condition.

MATERIAL AND METHODS

Experimental sites: The natural forest (FS) site was located at Sairang (23°49`18``N 92°39`33``E 91 m amsl) area of the Aizawl districts of Mizoram. The microcosm (MC) experiment was set up inside the mist chamber at the Department of Forestry, Mizoram University (23°44`14``N 92°39`39``E 763 m amsl). The MC is made of PVC pipe of 16 cm height sealed at the bottom with 12 cm wide opening on the top. The lower part MC was filled with 2/3 of mineral soil and the remaining 1/3 top portion was covered with organic soil from the forest (Fig. 1).

Soil sampling and analysis: Soil samples were collected

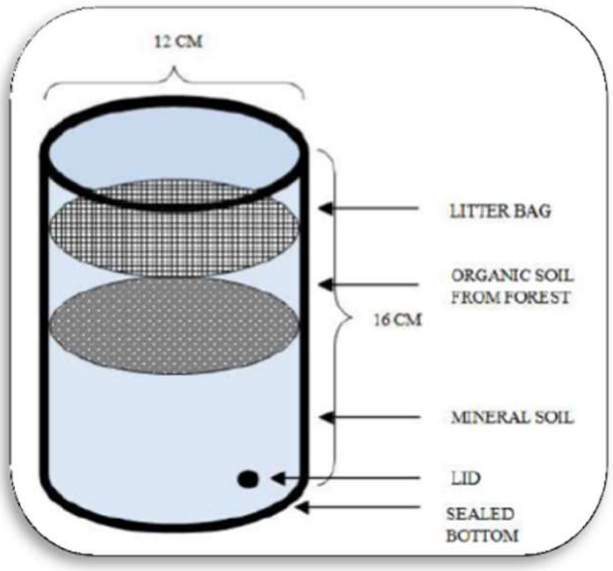

Fig. 1. Design of microcosm used for leaf litter decomposition

from 3 random locations approximately 25 m away from each other from upper soil layer (0 - 10 cm) using soil corer (4.2 cm wide). Bulk density (g cm^{-3}) was calculated using soil corer (4.2 cm wide and 10 cm high) and expressed on dry soil weight basis. Soil pH was determined using a standard pH meter (Mettler Toledo, Switzerland) at 1: 2.5 soil $water^{-1}$ suspensions. Hydrometer method was used for the determination of soil texture. The moisture content of the soil was determined by drying soil samples at 105°C for 48 h in hot air oven to constant weight (Hauchhum and Tripathi 2017). The available P was determined by the Bray-I-P method. Soil microbial biomass carbon (MBC) was estimated by chloroform fumigation extraction method (Brookes et al 1985) followed by the liquid oxidation. The difference in C content between fumigated and non-fumigated soil samples was multiplied by constant (K_{EC}) = 0.38 fixed (Vance et al 1987) and expressed in mg kg^{-1} (DW) soil. Air dried soil sample was ground and sieved at 1mm mesh, then analyst C and N using CHNS/O Elemental Analyzer with auto-sampler and TCD detector–Euro Vector Model: Euro EA 3000 at Central Instrumental Laboratory, Mizoram University.

Determining soil microbial population: Serial dilution technique was used to prepare 1 gm freshly collected soil solutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) as described in Ghosh and Tripathi 2021. Colony building units (CFUs) are calculated according to the Dilution Plate Method. Different agar media were prepared separately; fungi, potato dextrose agar (PTA) supplemented with an antibiotic 0.08% penicillin, chloramphenicol and rose Bengal; actinomycetes, starch casein agar (SCA) mixed with nystatin (0.08%) and bacteria, nutrient agar (NA) added about 0.08% nystatin and actidione. Dilutions of 10^{-3} - 10^{-5}, 10^{-4} - 10^{-6} and 10^{-5} - 10^{-7} are used to isolate fungi, actinomycetes and bacteria, respectively. Dilution of 0.5 ml is placed on petri-plates containing solid media in triplicates. Media plates were incubated at 28 ± 1°C for fungal and 25 ± 1°C for both actinomycetes and bacterial growth. Colony count for actinomycetes and bacterial started after 24 h of incubation and fungal after 72 h of incubation. The microbial population was expressed in CFUs/g of soil.

Measuring litter decomposition: Freshly fallen (senescence) leaves of *Melocanna baccifera* was used for decomposition experiment using nylon net bags technique. A total of 72 litter bags were used for the two set of litter decomposition experiments. Litter bag measuring 15 x 15 cm (2 mm mesh) filled with ~10 g of air-dried litter was used for FS. Whereas, in MC litter bag measuring 10 x 10 cm filled with ~5 g of litter material was used. In FS, litter bags were prepared and placed randomly at six locations approximately ~25 m distances from each other. Six litter bags were recovered at monthly interval from both FS and MC. Litter bags were brought to the laboratory and cleaned to remove adhering soil particles. Litter was dried separately in hot air oven at 70°C for 24 h to constant weight. Weight of litter was recorded and litter samples were ground and sieved in 1.5 mm mesh for further analysis.

Analysis of litter material: Air dried litter samples were ground and sieved through 1mm mesh screen and analysed for C and N concentrations using CHNS/O Elemental Analyzer with auto-sampler and TCD detector–Euro Vector, Model. Euro EA 3000 at Central Instrumental Laboratory, Mizoram University. Litter lignin was determined using Fibrotron Automatic Fiber Analysis, Model FRB 6, Version 1, Tulin Equipments, Chennai, India.

Computations: The annual decay constant (k) was calculated using negative exponential decay model of Olson (1963). $W_t/W_0 = \exp^{(-kt)}$, where W_0 = initial weight and W_t = weight remaining after time t. As suggested by Olson (1963), the time required for 50% and 95% weight loss was calculated as $t_{50} = 0.693/k$ and $t_{95} = 3/k$.

Data were compared and analyzed statistically using SPSS ver-18, Pearson correlation (r) was performed to assess significant (p <0.01) correlation between litter mass remaining of FS and MC with time elapse and mass remaining with C/N ratio in both FS and MC.

RESULTS AND DISCUSSION

Soil physico-chemical analysis: Concentrations of C, N and C/N ratio in the soil were 2.4%, 0.21% and 11.2, respectively. P_{avail} and MBC were 4.26 mg kg^{-1} and 525 mg kg^{-1}. Soil pH was strongly acidic (4.8). Bulk density was 0.93 whereas percent sand, silt and clay content were: 69.3, 16.6

and 14 (Fig. 2). Soil physico-chemical properties such as bulk density and pH affects soil permeability and microbial activities during decomposition (Krishna and Mohan 2017).

Colony forming unit of soil microbes were 9 x 10^{-3} (fungi), 49 x 10^{-6} (actinomycetes) and 174 x 10^{-5} (bacterial), respectively (Table 1). Soil microbes play an important role in breaking down of litter, example in initial stage of decomposition, bacteria help in breaking labile substances followed by fungi in decaying complex substances in later stages (Singh and Tripathi 2020 b). However, activities of soil microbes depend on various abiotic factors like temperature, moisture and litter substrate quality (Sun et al 2019).

Initial litter chemical composition and litter mass loss through time: In *M. baccifera* litter, per cent concentration of lignin, C and N were 15, 37 and 2.2, respectively, whereas C/N ratio was 16.7 (Fig. 3). Temporal changes in mass loss as per cent ash-free mass remaining (natural logarithm) against time elapsed showed significant negative correlation in litter materials at both studies (Table 3). Correlation coefficient (r) value was 0.93 in FS and 0.99 in MC. Slope was higher (0.43) in FS compared to QF (0.34) (Fig. 4).

Mass loss in initial two months of decomposition was significantly higher (62%) in FS compared to MC (43%). At the end of the study (180 days) mass remaining was 7.3% at FS and 12.9% at MC (Fig. 4). Rapid decomposition in initial stage of decomposition was also reported by the other from different ecosystems (Pandey et al 2007, Bohara et al 2019). In *Tephrosia candida* litter decomposition similar trend was reported in shifting cultivation of Mizoram (Wapongnungsang and Tripathi 2017, Ghosh and Tripathi 2021). Such rapid degradation of litter material in initial stage of litter decomposition is mainly due to loss of easily decomposable labile substances like sugar, starch (Aerts and Chapin 2000).

Annual decayed constant k was higher in FS (2.16) compared to MC (1.42). Using the Olson model (1963) time (days) required (117 days) for 50% mass loss was under

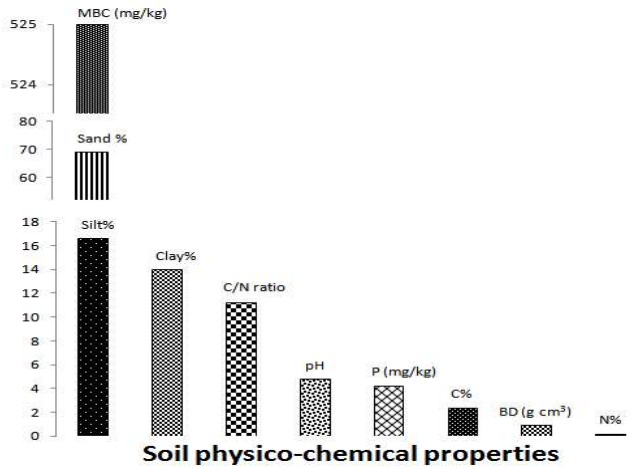

Fig. 2. Initial soil physico-chemical properties from forest site at 0-10cm depths

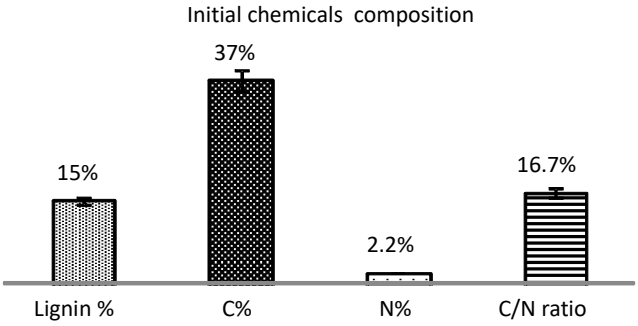

Fig. 3. Original chemical composition of *Melocanna baccifera* leaf litter

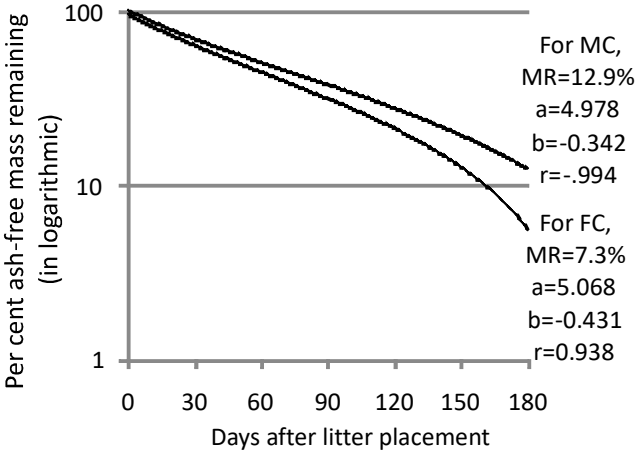

Fig. 4. Mass remaining (MR) at the end of experiment in FS and MC. Relationship between *ln* of per cent ash-free mass remaining (y) with time (days, x) with correlation coefficients (r), intercept (a) and slope (b) of the linear regression equation y = a + bx

Table 1. Colony forming units (CFUs) of various soil microbes viz. fungi, actinomycetes and bacteria from initial collected soil

Types of soil microbes	Dilution factor	Microbial count	Obtained soil microbes	CFU^{-g}
Fungi	10^3	(CFU x 10^3) colonies g^{-1}	9	9 x 10^{-3}
Actinomycetes	10^6	(CFU x 10^6) colonies g^{-1}	49	49 x 10^{-6}
Bacteria	10^5	(CFU x 10^5) colonies g^{-1}	174	174 x 10^{-5}

predicted and (505 days) for 90% mass loss was over-predicted for FS and was almost same for 178 days for 50% decomposition and 769 days for 90% decomposition in MC (Table 2). The present k value was considerably higher compared to understory dwarf bamboo (*Sasa kurilensis*) in young secondary forest of northern Japan (Tripathi et al 2006). However, k value was lower compared to recent finding in four agroforestry tree species in western Himalaya reported for *C. australis* (2.3) but comparable to *G. optiva* (2.12) with FS (Singhal et al 2019). Variations in litter mass loss in two litter decomposition set ups were likely affected by abiotic factors (i.e. temperature, precipitation etc.) along with soil microbial activities (Powers et al 2009).

Changes in C, N and C/N ratio during litter decomposition: Carbon and N release in both FS and MC decreased consistently throughout the decomposition period. C and N release almost followed the pattern similar to that of mass loss showing higher mass loss in first two months. C and N release in FS (i.e. 69.8% & 73.6%) was faster as compared to MC (i.e. 53.7% & 53.1%) (Fig. 5). C remains at the end of experiment were 7.3% in FS and 11.3% in MC, whereas N remains were 5.6% in FS and 11.8% in MC, respectively (Fig. 5). The C and N mass remaining at the end of experiment (180 days) was considerably lower compared to reported C and N value in *Tephrosia candida* (22 – 24% for C, 6 – 13 % for N) in Mizoram (Wapongnungsang and Tripathi 2017).

Increase in litter C/N ratio up to 120 days was observed in FS followed by continuous decrease in the later period of the decomposition. However, it was almost stable in MC (Fig. 6). Lower C/N ratio in initial litter has been reported to speed up the process of litter decomposition (Krishna and Mohan 2017, Ghosh and Tripathi 2021) and similar value (16.6) was observed in present finding which explain quicker decomposition. Litter ash-free mass remaining in both FS and MC was significantly negatively correlated with time duration. Significantly positive correlation was observed between mass remaining of both sites (Table 3).

Table 2. Annual decay rate constant (k), time (days) required to achieve 50% (t_{50}) and 95% (t_{95}) decompose of bamboo litter mass in FS and MC

Study types	k	t_{50}	t_{95}
FS	2.16	117	505
MC	1.42	178	769

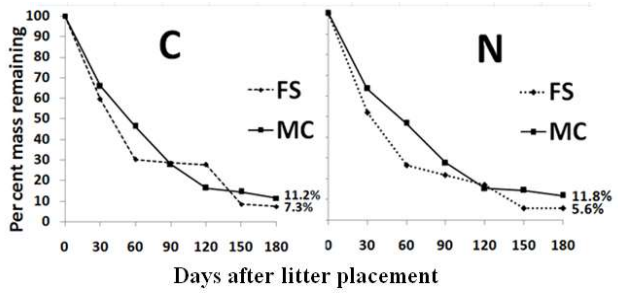

Fig. 5. Per cent mass remaining of C and N during the course of litter decomposition of bamboo in FS and MC

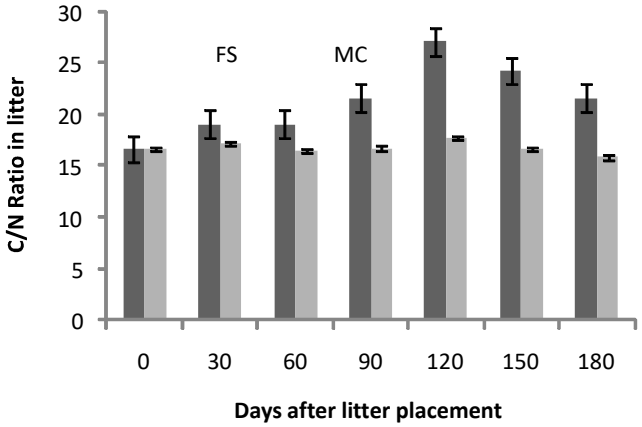

Fig. 6. Temporal changes in litter C/N ratio during litter decomposition

Table 3. Correlation coefficient (r) between leaf litter mass remaining of FS and MC, mass remaining and C/N ratio in FS, mass remaining and C/N ratio in MC

Interaction	Correlation	*p*-value
Decomposition days Vs Mass remaining of FS	-0.939**	0.002
Decomposition days Vs Mass remaining of MC	-0.969**	0.00
Mass remaining of FS Vs Mass remaining of MC	0.97**	0.00
Mass remaining of FS Vs C/N ratio in FS litters	-0.726	0.065
Mass remaining of MC Vs C/N ratio in MC litters	0.137	0.796

CONCLUSIONS

The study demonstrates that the pattern of litter mass loss, and carbon and nitrogen release varied during decomposition from same species in natural and control environment under laboratory condition. Further, study suggests that the same litter material when decomposing under different environments (i.e. variations in temperature, moisture, humidity etc.) and soil microbial composition

(changes in bacteria, fungi and actinomycetes populations) in natural and under laboratory conditions shows varied rates of organic matter decomposition and nutrient release as these are the triggering factors for the decomposition kinetics in two experimental set ups.

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