Open Journal of Soil Science, 2018, 8, 175-185
http://www.scirp.org/journal/ojss
ISSN Online: 2162-5379
ISSN Print: 2162-5360

Effect of Leaf Litter Treatment on Soil Microbial Biomass

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How to cite this paper: Sen Oli, P., Mandal, T.N. and Adhikari, U. (2018) Effect of Leaf Litter Treatment on Soil Microbial Biomass. *Open Journal of Soil Science*, **8**, 175-185.
https://doi.org/10.4236/ojss.2018.88014

Received: May 5, 2018
Accepted: August 12, 2018
Published: August 15, 2018

Abstract

Soil microbial biomass is an active fraction of soil organic matter. It shows quicker response than soil organic matter to any change in the soil environment. Being an index of soil fertility, it plays a key role in the decomposition of litters and fast release of available nutrients. Leaf litters of leguminous and non-leguminous species in alone and mixed form were applied as treatments in the soil to observe the changes in the magnitude of soil microbial biomass. Soil microbial biomass C and N were determined by chloroform fumigation extraction method. Increment in the concentration of microbial biomass C and N was higher in the treatments with leguminous leaf litter (497 - 571 μgCg^{-1}, 48 - 55 μgNg^{-1}) than the non-leguminous one (256 - 414 μgCg^{-1}; 22 - 36 μgNg^{-1}). However, when non-leguminous litters were mixed with leguminous litters then the values increased distinctly (350 - 465 μgCg^{-1}, 28 - 48 μgNg^{-1}). On the basis of increment in soil microbial biomass, leaf litters of the species considered potential to improve soil nutrients are—*Cassia siamea* and *Dalbergia sissoo* from leguminous trees, *Anthocephalus* + *Cassia* and *Shorea* + *Dalbergia* from mixed form of non-leguminous and leguminous one and *Eichhornia crassipes*, an alien aquatic macrophyte. The leaf litters of these species can be used as source of organic matter to improve the crop yield.

Keywords

Leaf Litter of Leguminous Trees, Non-Leguminous Trees, Soil Organic Matter, Soil Microbial Biomass

1. Introduction

Management of microbial community in soil through residue placement has a great potential for organic matter and nutrient management in natural and agro-ecosystem. Leaf litter of different tree species have different decomposition rate. Generally, leaf litters of leguminous trees have fast decomposition rate than non-leguminous leaf litters because of having low C:N ratio [1]. If non-leguminous leaf litter is mixed with leguminous leaf litter, then the decomposition rate may be faster. High nitrogen concentration of leguminous litter makes close C:N ratio which is required to enhance the rate of decomposition. Further, nitrogen immobilization and mineralization are mainly controlled by initial chemical composition of the plant residue [2].

Soil microbial biomass plays a key role in the decomposition of complex organic matter of plant litters. Organic inputs as leaf litters in the soil increase the level of soil microbial biomass [3]. It represents an active fraction of soil organic matter due to its rapid turnover rate and fast release of available nutrients [4]. The size and turnover of microbial biomass affects the quantity of plant available nutrients. An increase in its size indicates the improvement of nutrients in the soil. Thus, it has been used as an index of soil fertility [5]. Further, it is considered as an important early indicator of changes that may occur in the long term and constitutes a source and sink of nutrients [6].

There is an increasing global concern about the decreasing trend of soil organic matter and plant available nutrients due to addition of inorganic fertilizer alone. To improve the yield and achieve sustainable production, use of organic resources is recommended. Several works in this direction have resolved this conclusion [7] and [8]. Further, proper combination of high quality litter (low C:N ratio) and low quality litter (high C:N ratio) decomposes at a rate such that timing of nutrient release synchronizes with plant demand [9].

In the present study, an attempt has been made to assess the effect of leguminous, non-leguminous and its mixed form of leaf litter placement on the level of soil microbial biomass. It may help to manipulate the potential litter quality for the improvement of soil nutrients.

2. Materials and Methods

2.1. Profile of the Experimental Area

The study was conducted at Post Graduate Campus in Biratnagar. Biratnagar is a metropolitan city of Nepal. It lies in Morang district between 26˚23' and 26˚30'N latitude and 86˚14' and 87˚18'E longitude. It is situated at an altitude of 72 m above mean sea level. Study was conducted from the rice cultivating cropland soil of Post Graduate Campus, Biratnagar.

The climate is tropical monsoon type. There are three distinct seasons in a year. A warm and wet rainy season (June to October), a cool and dry winter (November to February) and hot and dry summer (March to May). Mean monthly minimum temperature ranged between 9.17˚C and 25.96˚C, while maximum temperature ranged between 21.52˚C and 33.44˚C. The average annual rainfall was 1821.35 mm out of which 95% was received in rainy season. Highest relative humidity (86%) was recorded in rainy season and lowest (60%) in dry summer season.

Biratnagar is a part of Gangetic plain. Soil of this region is essentially made up of materials transported and deposited in the recent times by the tributaries of the Ganga. In general, soil is pale yellow to dark greyish brown colour. The textural class of the soil is loam.

2.2. Litter Sampling

Leaf litter of leguminous and non-leguminous tree species was collected from Humse-Dumse community forest of Jhapa District. The forest is located at 4 km north-west to Damak Bazar, covering an area of 627.5 hectare. The vegetation of the forest is dominated by Sal (*Shorea robusta* Gaertn.). The main associated species are *Lagerstroemia parviflora* Roxb., *Dillenia pentagyna* Roxb. and *Terminalia alata* Heyne ex Roth. Collection of freshly fallen leaf litter of tree species (Table 1) was done randomly from the forest floor. Leaf litter of two weed species e.g. *Eichhornia crassipes* (Mart.) Solms and *Ipomea carnea* Jacq. were collected from road side ditches of Biratnagar.

2.3. Soil Sampling

Soil was collected from the rice cultivating cropland of Post Graduate Campus, Biratnagar in January 2013 at the fallow period. Soil was collected randomly from three plots. At each plot soil was collected from three pits (10 cm × 10 cm × 15 cm depth), mixed together and pooled as one replicate. Three replicates of soil samples were brought to the laboratory. After removing the surface organic materials and fine roots, it was sieved through a 2 mm mesh screen. Physico-chemical analysis of the soil was done before litter treatment to represent the control. For the purpose of litter treatment, the soil was filled in earthen pots and three pots for each treatment were used.

Table 1. Plant species selected for leaf litter collection.

Plant Species	Local name	Family
Leguminous Trees		
Dalbergia sissoo Roxb. ex DC.	Sissoo	Leguminosae
Cassia siamea Lam.	Tapre	Leguminosae
Non-leguminous Trees		
Anthocephalus chinensis (Lam.) A. Rich ex Walp.	Kadam	Rubiaceae
Syzygium cumini (L) Skeels	Jamun	Myrtaceae
Shorea robusta Gaertn.	Sakhuwa	Dipterocarpaceae
Lagerstroemia parviflora Roxb.	Bote Dhayaro	Lythraceae
Terminalia alata Heyne ex Roth	Saj	Combretaceae
Dillenia pentagyna Roxb.	Tantari	Dilleniaceae
Tectona grandis L.f.	Tick	Verbenaceae
Non-leguminous Shrub		
Ipomea carnea Jacq.	Besaram	Convolvulaceae
Aquatic macrophyte		
Eichhornia crassipes (Mart.) Solms	Jalkumbhi	Pontederiaceae

2.4. Experiment Design

Treatments

Leaf litter treatment was applied as follows:

1) Leguminous, non-leguminous, aquatic macrophyte and non-leguminous shrub in alone condition: 50 g of the leaf litter was used for each species in triplicate.
2) Combination of leguminous and non-leguminous in mixed condition: 25 g of leguminous and 25 g of non-leguminous leaf litter was used for each combination in triplicate.
3) Three replicates were used as control.

Leaf litters were kept in earthen pots at the sub-surface layer of the soil and then covered with soil to regulate the decomposition. To enhance the decomposition, water was added for three months maintaining the moisture level. After 3 months, soil of each earthen pot was poured on paper separately and mixed to make uniformity in the soil sample and soil microbial biomass was determined in these samples.

2.5. Soil Physico-Chemical Analysis

Physico-chemical properties of the untreated soil were analyzed at the beginning of the experiment. Particle size analysis was done by sieve method. Water holding capacity (WHC) was determined by perforated circular brass box and soil pH was measured by using a glass electrode (1:5, soil: water) [10]. Soil organic carbon was analyzed by dichromate oxidation in a reflux system and titration with ferrous ammonium sulphate [11]. Total nitrogen was estimated by micro Kjeldhal method [12].

Soil microbial biomass C and N were estimated by chloroform fumigation extraction method [13] and [14]. Soil samples (25 g) were saturated with purified liquid $CHCl_3$ for 24 hr. After 24 hr, the $CHCl_3$ was removed by evacuation and the soil was extracted with 0.5 M K_2SO_4 (1:4, soil: extractant) for 30 minutes. This represented the fumigated samples. Another set of un-fumigated soil samples were also extracted with 0.5 M K_2SO_4. Biomass C and N were estimated from these fumigated and un-fumigated soil extracts.

Soil microbial biomass C was determined in the soil extracts of fumigated and un-fumigated samples by dichromate oxidation in a reflux system and titration with ferrous ammonium sulphate. Biomass Carbon (MB-C) was then estimated from the equation: $MB - C = 2.64\ EC$ [13]. Where EC is the difference between C estimated from fumigated and un-fumigated soils both expressed as μgCg^{-1} oven dry soil.

Soil microbial biomass N (MB-N) was determined in the same soil extracts of fumigated and un-fumigated samples using Kjeldahl digestion method [14]. The MB-N value obtained for the un-fumigated soil extract was subtracted from the value obtained from that of fumigated soil extract. The difference in value of N thus estimated was divided by a K_N value of 0.54, assuming that 54% of the biomass N was extracted in K_2SO_4 by $CHCl_3$ treatment.

2.6. Data Processing

MS-Excel and SPSS were used to process and analyze the data.

3. Results

Physico-chemical characteristics of cropland soil used in the pot experiment is presented in Table 2. Texture of the soil was loam with 59% water holding capacity and 6.6 pH. Cropland soil was poor in organic carbon (0.82%) and total nitrogen content (0.08%). After treatment with leaf litter residues, a distinct variation in soil microbial biomass carbon and nitrogen was observed over the control.

3.1. Changes in Soil Microbial Biomass Carbon (MB-C)

ANOVA indicated that variation in soil microbial biomass carbon due to treatment with leguminous and non-leguminous leaf litter was significantly different with that of control ($p \le 0.001$). Increment was always higher in leguminous treatment than non-leguminous (Tables 3-6).

Table 2. Physico-chemical characteristics of the cropland soil used in the pot experiment.

Soil Properties	**Value (Mean ± SE)**
Soil texture	
Sand (%)	47.2 ± 3.8
Silt (%)	28.0 ± 2.5
Clay (%)	23.0 ± 2.0
Textural class: Loam	
Water Holding Capacity (%)	59.0 ± 5.3
pH	6.6 ± 0.7
Organic Carbon (%)	0.82 ± 0.9
Total Nitrogen (%)	0.08 ± 0.01
Organic matter (%)	1.41 ± 0.11
C:N	10.3

Table 3. Soil microbial biomass C, N, their percentage of soil organic C, total N and their ratio in the soil treated with leguminous leaf litter (Mean ± SE).

Treatments (Leaf Litter)	Microbial Biomass ($\mu g g^{-1}$)		Microbial Biomass as % of		MBC:MBN
	Carbon	Nitrogen	Organic C	Total N	
Control	182 ± 12	14.5 ± 1.7	2.22	1.81	12.5
Dalbergia sissoo	497 ± 46	48.0 ± 5.2	6.06	6.00	10.3
Cassia siamea	571 ± 48	55.0 ± 5.7	6.96	6.87	10.4

Table 4. Soil microbial biomass C, N, their percentage of soil organic C, total N and their ratio in the soil treated with non-leguminous leaf litter (Mean ± SE).

Treatments (Leaf Litter)	Microbial Biomass (μgg^{-1})		Microbial Biomass as % of		MBC:MBN
	Carbon	Nitrogen	Organic C	Total N	
Anthocephalus chinensis	414 ± 28	34.0 ± 3.1	5.05	4.25	12.2
Dillenia pentagyna	402 ± 32	35.0 ± 2.8	4.90	4.37	11.5
Lagerstroemia parviflora	380 ± 22	36.0 ± 2.7	4.63	4.50	10.5
Shorea robusta	256 ± 28	22.0 ± 2.5	3.12	2.75	11.6
Syzygium cumini	346 ± 35	31.0 ± 2.8	4.20	3.87	11.1
Tectona grandis	389 ± 27	32.0 ± 2.5	4.74	4.00	12.2
Terminalia alata	288 ± 25	30.0 ± 3.5	3.51	3.75	9.6

Table 5. Soil microbial biomass C, N, their percentage of soil organic C, total N, and their ratio in the soil treated with mixed leguminous and non-leguminous leaf litter (Mean ± SE).

Treatments (Leaf Litter)	Microbial Biomass (μgg^{-1})		Microbial Biomass as % of		MBC:MBN
	Carbon	Nitrogen	Organic C	Total N	
Anthocephalus + *Cassia*	465 ± 56	48 ± 4.1	5.67	6.00	9.7
Dillenia + *Cassia*	389 ± 36	35 ± 3.2	4.74	4.37	11.1
Lagerstroemia + *Cassia*	397 ± 37	43 ± 3.7	4.84	5.37	9.2
Shorea + *Cassia*	381 ± 36	32 ± 3.5	4.64	4.00	12.0
Shorea + *Dalbergia*	460 ± 48	45 ± 3.8	5.61	5.62	10.2
Syzygium + *Cassia*	371 ± 33	32 ± 3.7	4.52	4.00	11.6
Tectona + *Cassia*	350 ± 34	28 ± 3.0	4.26	3.50	12.5
Tectona + *Dalbergia*	388 ± 40	37 ± 4.0	4.73	4.62	10.5
Terminalia + *Cassia*	358 ± 38	30 ± 2.7	4.36	3.75	12.0

Table 6. Soil microbial biomass C, N, their percentage of soil organic C, total N and their ratio in the soil treated with *Eichhornia* and *Ipomea* (Mean ± SE).

Treatments (Leaf Litter)	Microbial Biomass (μgg^{-1})		Microbial Biomass as % of		MBC:MBN
	Carbon	Nitrogen	Organic C	Total N	
Eichhornia crassipes	380 ± 34	33 ± 2.8	4.63	4.12	11.5
Ipomea carnea	340 ± 36	27 ± 3.1	4.14	3.37	12.6

Within leguminous treatment, MB-C increased by 213% when amended with *Cassia* and by 173% with *Dalbergia* over the control. Similarly, increment in MB-C was also observed when the soil was treated with non-leguminous leaf litter, but it was always lower than leguminous litter. Within non-leguminous tree leaf litter the MB-C value ranged between 256 μgg^{-1} and 414 μgg^{-1}, the minimum in *Shorea* and maximum in *Anthocephalus*. It showed 40% to 127% increment over the control.

In the case of non-leguminous + leguminous mixed treatment, the increment in MB-C was even greater than non-leguminous litter alone which ranged from 350 $\mu g g^{-1}$ to 465 $\mu g g^{-1}$, the minimum in *Tectona* + *Cassia* and maximum in *Anthoceplatus* + *Cassia*.

Treatment with *Eichhornia*, a problematic aquatic macrophyte in pond ecosystem showed increment in MB-C content by 108% while *Ipomea*, a rapidly growing non-legume shrub showed 86% increment in MB-C than control.

3.2. Changes in Soil Microbial Biomass Nitrogen (MB-N)

ANOVA suggested that variation in soil microbial biomass nitrogen due to treatment with leguminous and non-leguminous leaf litter was significantly different with that of control ($p \leq 0.001$). Leguminous litter amendment showed always higher value than non-leguminous leaf litter (**Tables 3-6**).

Under leguminous litter amendment, MB-N increased by 279% with *Cassia* and by 231% with *Dalbergia* over the control. It showed that MB-N increased even by greater percentage than MB-C. Within the non-leguminous treatment also the increment was observed but it was always lower than leguminous litter. The value ranged between 22 $\mu g g^{-1}$ and 36 $\mu g g^{-1}$, the minimum in *Shorea* and maximum in *Lagerstroemia*. It showed the increment from 51% to 148% over the control.

As it happened in the case of MB-C, the mixed treatment of non-leguminous+ leguminous litter showed greater increment in MB-N over the treatment only with non-leguminous litter. The value ranged between 28 $\mu g g^{-1}$ and 48 $\mu g g^{-1}$. It increased from 93% to 231% over the control. It showed that in mixed treatment, the higher value (48 $\mu g g^{-1}$) reached to the level obtained in the treatment with *Dalbergia* (leguminous) alone.

In the case of MB-N also, *Eichhornia* and *Ipomea* showed distinct increment (86% - 127%) over the control.

3.3. Microbial Biomass as Percent of Organic C and Total N

MB-C as percent of soil organic carbon and MB-N as percent of total nitrogen showed more than three times increment over the control when it was amended only with leguminous litter (**Tables 3-6**). Further, even in the case of non-leguminous litter these values showed distinct increment (41% - 148%) over the control. Including all treatments, MB-N as percent of total N (increased 52% - 280%) was always higher than MB-C as percent of soil organic C (increased 41% - 214%).

MB-C: MB-N ratio ranged from 9.2 to 12.5 (**Tables 3-6**). In leguminous treatment MB-C: MB-N ratio was lower (10.3 - 10.4) than the control (12.5). In non-leguminous litter treatment the value ranged between 9.6 to 12.2. Narrow MB-C: MB-N ratio was seen in *Terminalia alata* (9.6) and wide MB-C: MB-N ratio was observed in *Tectona grandis* and *Anthocephalus chinensis* (12.2).

In mixed combination of non-leguminous + leguminous litter, MB-C: MB-N ratio ranged from 9.2 to 12.5, the lower in *Lagerstroemia* + *Cassia* and higher in *Tectona* + *Cassia*.

The treatment with *Anthocephalus* alone showed wide C:N ratio (12:2) but became narrow (9.7) when it is mixed with the legume *Cassia*. Similarly, *Lagerstroemia* alone showed C:N ratio as 10.5, but it is lowered to 9.2 when it is mixed with *Cassia* (legume).

4. Discussion

Soil microbial biomass is potentially very important to increase the level of soil fertility. It responds quickly to changes occurring with different types of leaf litter. The decomposition of plant litter plays a crucial role in the nutrient budget of ecosystem. Different plant species affect the soil microbial biomass in different way depending upon their litter quality. In the present study, the variation in the concentration of soil microbial biomass was significant due to litter treatment over the control.

The soil treated with the leaf litter of *Cassia siamea* showed significant effect and increased the maximum level of soil microbial biomass C and N. Being a legume plant, it has close C:N ratio in its leaf litter which helps in rapid decomposition of litter [1]. Fast decomposing litter releases the nutrients rapidly which helps the microbial growth.

Under the treatment of non-leguminous leaf litter, *Anthocephalus*, *Dillenia*, *Tectona* and *Lagerstroemia* supported to develop the level of MB-C and MB-N in a better way than others. However, non-leguminous litters showed lower value for soil microbial biomass in all cases in comparison to leguminous litter treatment. But, when it is mixed with leguminous litter the level of microbial biomass increased distinctly. The C:N ratio of leguminous plant litter is generally low. The low C:N ratio helps in fast decomposition [7]. The N is immobilized when the C:N ratio is greater than critical ratio and it is mineralized when this ratio becomes narrow. Combination of legume litter with non-legume litter lowered the C:N ratio due to high nitrogen concentration.

Under the treatment with mixed form of non-leguminous and leguminous leaf litter, the combination of *Anthocephalus* + *Cassia* showed maximum level of MB-C and MB-N than other combinations. The treatment with *Eichhornia* leaf litter also showed comparable level of microbial biomass with *Lagerstroemia*. *Eichhornia* is an aquatic weed and cause eutrophication in water bodies. This weed can be used to manage the soil fertility as it enhances the microbial growth in soil and decomposed completely within 90 days [15].

Microbial biomass responds more rapidly to any change in soil environment than soil organic matter because of its high turnover rate. It constitutes a significant part of the potentially mineralizable N and serves both as source and sink of nitrogen. Consequently, the microbial biomass nitrogen has significant impact on nitrogen availability and overall soil nitrogen cycling [16].

Microbial biomass carbon and nitrogen increased by three times over the control due to addition of legume litter and even 1.4 - 2.2 times higher in non-leguminous leaf litter. Growth of microbial biomass on leaf litter treatment enhances the turnover rate of the soil organic matter through concurrent immobilization [17]. The soil microbial biomass C and N increased over the control in fallow based rice-wheat system by applying the residues of different types of green manure legumes [18].

Among the non-legume tree species, treatment with leaf litter of *Shorea* showed comparatively low microbial biomass. This may be due to low nitrogen concentration in leaf litter. Due to higher nitrogen concentration in *Cassia* (1.5%N), it decomposes at fast rate while *Shorea* having low nitrogen concentration in leaf litter (0.82%N) decomposes slowly. Consequently, it releases least amount of nitrogen [19]. This may be the reason that *Cassia* showed maximum value while *Shorea* showed the minimum value of microbial biomass. Nevertheless, combination of non-leguminous leaf litters with the leguminous leaf litter e.g. *Dalbergia* and *Cassia*, have double increment in their microbial biomass. This may be due to the presence of leguminous species where N-mineralization is faster.

As indicated by the status of soil microbial biomass, the leaf litter of *Cassia siamea* can enrich the nutrient level in soil within a short period. On the other hand, among non-leguminous tree species litter of *Anthocephalus chinensis* can be considered as good for nutrient management. Similarly, *Eichhornia crassipes* can also be exploited for nutrient management as it is a problematic aquatic macrophyte, propagating at fast rate and causing eutrophication in the water bodies. Further, in the mixed form of non-leguminous and leguminous litter, *Anthocephalus* + *Cassia* could be the best combination. These are the potential plant species to improve the nutrients level in soil. The leaf litter of these species can be exploited to make compost and even in combination with inorganic fertilizer to achieve sustainable production.

5. Conclusions

On the basis of increment in the concentration of soil microbial biomass, an index of soil fertility, the leaf litter of following plant species considered potential to improve the nutrients level in soil.

1) *Cassia siamea* and *Dalbergia sissoo* from leguminous trees.
2) *Anthocephalus* + *Cassia* and *Shorea* + *Dalbergia* from mixed form of non-leguminous and leguminous trees.
3) *Eichhornia crassipes*, an alien aquatic macrophyte.

Leaf litters of these species can be applied to amend the soil and even to make compost with them. Furthermore, to reduce the negative consequence of inorganic fertilizer the above potential litters can be used as source of organic matter to integrate with inorganic fertilizer to improve the yield and achieve sustainable production.

Acknowledgements

We are grateful to the Campus Chief and the Head, Department of Botany, Post Graduate Campus, Tribhuvan University, Biratnagar, Nepal for providing laboratory and library facilities.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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