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Effect of *Flemingia macrophylla* on Biological and Physico-Chemical Characteristics of Soil

Lalmundanga, Leishangthem Jeeceelee and V.P. Khanduri

Department of Forestry, Mizoram University, Tanhril-796009, Aizawl Mizoram, India

Abstract: This study was conducted at the herbal garden of Mizoram University, Tanhril, Mizoram, to find out the influence of *Flemingia macrophylla* plant and slope terrain on biological and physico-chemical characteristics of soil. The results revealed that Colony form Unit (CFU) of microbial population (Bacteria and fungi), soil organic carbon and total nitrogen were higher in Rhizosphere soil (RH) and incubated *F. macrophylla* + barren soil (F+BR) soil samples. It was also observed that the lowest site of the experimental plot i.e., BT (11-12) proclaimed more CFU of microbial population, soil organic carbon and total nitrogen than the upper site of the plot i.e., BT (1-2). The soil pH was almost neutral in all the soil samples. Higher soil moisture content was noted at F+BR and RH soil samples whereas soil from barren site (BR) and control (CTRL) display lower soil moisture content. The study concluded that *Flemingia macrophylla* has significant roles on improvement of soil fertility level in terms of more microbial population, high organic carbon, total nitrogen and soil moisture content. Slopped terrain influences soil biological and physico- chemical characteristics. Incubation of *F. macrophylla* leaf with soil increase microbial population, organic carbon, soil moisture content and total nitrogen.

Key words: N_2 fixing · Soil microbial population · Alley cropping · *Flemingia*

INTRODUCTION

Flemingia macrophylla (Willd.) Merr. (synonym: *Crotalaria macrophylla* Willd., *Flemingia congesta* Roxb. ex W.T. Aiton., *Flemingia latifolia* Benth. and *Flemingia prostrata* Roxb.) is a Papilionaceous genus of woody deep rooting shrub attains the height up to 2.5 m. *F. macrophylla* is a native to the humid to subhumid subtropics (rainfall 1100-3500 mm/year with 6 months dry season) of Asia with an altitude up to 2000 m asl. [1]. It is distributed throughout the Taiwan, southern China, Cambodia, Laos, Myanmar, Thailand, Vietnam, Indonesia, Malaysia, Bhutan, India, Nepal, northern Pakistan, Sri Lanka and Papua New Guinea. Secondary distribution is also found in tropical Australia, Africa and Central and South America [2]. *Flemingia* can resist long dry spells and is capable of surviving on very poorly drained and occasionally water-logged soils. The species is naturally found growing along watercourses in secondary forest and on both clay and lateritic soils. The species has been reported to adapt to acidic soil of pH 4.5-4.6 and infertile sods with high soluble aluminum (80% saturation) [3, 4].

F. macrophylla is a multipurpose agroforestry species, which is used as: hedges for erosion control, mulch and green manure in alley cropping hedgerows, shade plant in young coffee and cocoa plantations, weed suppressing and soil enriching cover plant in fruit tree orchards, fuel wood and stakes for climbing crop species, medicinal plant and a number of other purposes [2]. Although the species is often referred to as a 'forage' or 'fodder' legume, especially as dry season feed [1, 2, 4-6].

F. macrophylla is highly suitable for alley cropping, an agroforestry practice in which fast-growing trees or shrubs are established in hedgerows between which annual food crops are grown. The hedges are pruned prior to and periodically during cropping cycles to prevent shading of the companion crop, with the pruning applied to the soil as mulch and/or green manure [7]. Plants will survive for many years if cut every two to three months [2].

Agro forestry systems are the medium between agriculture system and forest ecosystems, which follows some of the nutrient cycling and environmental services of natural systems and maintain a balance between trees and crops. The nutrient uptake from deeper soil layers and scavenging leached nutrients through horizontal root development may significantly increase the overall supply and efficiency of nutrients. Therefore, selection of trees with deep rooting pattern and few horizontal root

Correspoding Author: V.P. Khanduri, Department of Forestry, Mizoram University, Tanhril-796009, Aizawl Mizoram, India

developments is the most suitable choice for fallow-rotation hedgerow system. Crop performance in conjugation with N_2 fixing trees is always superior, but, mostly influenced by the slope condition of the terrain and fungal and bacterial populations in the soil. Therefore, this study was aimed at understanding the: i) effect of *Flemingia macrophylla* plant on soil biological (fungal and bacterial population) and physico-chemical characteristics (moisture content, pH, organic carbon content and total nitrogen), ii) effect of slope terrains on biological and physico-chemical characteristics of soil and iii) effect of incubation of *Flemingia macrophylla* leaves with soil on biological and physico-chemical characteristics in laboratory condition.

MATERIALS AND METHODS

Study site: The study was conducted in the herbal garden of Mizoram University at Tanhril, Mizoram where *Flemingia macrophylla* and other medicinal plants are intercropped. The location of the study site lies in between 43°37' and 45°25' N latitudes and 38°39' and 40°23' E longitudes. The area of the demonstration plot is around 1 hectare with gentle hill slope occurred at an altitudinal ranges of 700 to 875 m amsl. *F. macrophylla* is grown in well defined rows and each row consists of 45 to 50 plants with the half meter spacing between the rows. The temperature of the study area ranges from 21°C to 32°C (in summer) and 11°C and 23°C (in winter). The annual rainfall varies from 2000 to 2500 mm.

Experimental Design: To study the effect of *F. macrophylla* on soil biological and physico- chemical characteristics, different soil samples were collected at 7 days interval from *F. macrophylla* plot as: (i) Rhizosphere soil (RH) randomly from different rows; (ii) Between 1st and 2nd rows (BT 1-2) and between 11th-12th rows (BT 11-12) with 10 to 15 cm depth to find out the effect of slopes on nutrient variability. (iii) Soil from barren site (BR) i.e., without *F. macrophylla* growth as a control.

For studying the effect of *F. macrophylla* on soil biological and physicochemical characteristics, finely excised pieces of *F. macrophylla* leaves were mixed with barren site soil (BR) in the ratio of 20:200g (*F. macrophylla*: Soil) and kept in plastic bags at room temperature. This sample will be noted as *F. macrophylla*+ Barren Soil (F+BR). Soil samples from barren site i.e. without *F. macrophylla* were kept separate at room temperature along with F+BR. This soil sample is noted as control soil (CTRL). Soil samples from F+BR and CTRL were analyzed at 14 days interval. The soil samples incubated in plastic bags were watered everyday to maintain soil moisture.

Enumeration of microbial populations: Serial dilution plate method [8, 9] was followed for the isolation of fungal and bacterial populations. One gram of soil sample was taken into the 250 ml of conical flask containing 100 ml of sterilized distilled water to give 1:100 dilutions. The flask was swirled for 15 minutes to prepare homogeneous solution. Then 10 ml of this solution was added to another flask containing 90 ml of sterilized distilled water to get 1:1000 dilutions and swirled again. Similarly, 1:10000 dilutions was prepared by transferring 10 ml of 1:1000 dilutions into another conical flask containing 90 ml of sterilized distilled water.

Bacterial Population (BP): Nutrient agar medium [10] was used for the isolation of bacterial species. 0.5 ml of the aliquot from 1:10000 dilutions was transferred to a petre plate containing nutrient agar medium. Three replicates were maintained for each sample. The plates were rotated to disperse the suspension uniformly. The inoculated plates were then incubated in upside down position at 30±1°C in bacteriology incubator. Colony form unit (CFU) of bacteria was estimated by counting the number of bacterial colonies. The CFU of bacteria per gram of soil was calculated on the dry weight basis.

$$CFU\ of\ bacteria/g\ D`w = \frac{Number\ of\ colony\ formed \times Dilution\ factor \times\ inoculum}{Dry\ weight\ of\ the\ soil\ (g)}$$

Where $D'w$ = *Dry weight of the soil (g).*

Fungal Population (FP): The Rose Bengal Agar Medium [11] was used for the study of fungal population. One milliliter of the soil aliquot from 1:1000 dilutions was transferred into a Petri dish containing Rose Bengal Agar Medium. Three replicates were maintained for each sample. The plates were rotated to disperse the suspension uniformly. The inoculated plates were then incubated in upside down position at 25±1°C for 7 days in a BOD incubator. Colony form unit (CFU) of fungi was estimated by counting the number of fungal colonies. The CFU of fungi per gram of soil was calculated on the dry weight basis as per the formula used for BP.

Soil pH, Moisture Content, Organic Carbon(OC) and Total Nitrogen (TN): The pH of soil was determined using pH meter. The moisture content in the soil was estimated by gravimetric method [12]. Organic Carbon was observed by using the method of Walkey and Black [13]. The estimation of total nitrogen is done by using Auto-analyzer [14].

RESULTS

Microbial Population

Bacterial Population (BP): The colony form unit (CFU) of bacterial population was measured from different six soil samples. RH, BT (1-2), BT (11-12) and BR soil samples were considered as field sample whereas F+BR and CTRL soil samples were considered as laboratory soil sample. Among bacterial population from field, RH soil shows maximum population varied from 55.10×105 $CFUg^{-1}$, 66.06×105 $CFUg^{-1}$, 50.78×105 $CFUg^{-1}$ dry soil in the first, second and third sampling respectively and was followed by BT (11-12), BT (1-2) and BR. In the laboratory condition, the incubated soil F+BR shows more CFU of bacterial population than CTRL (Table 1). Consistent distribution pattern of bacterial population was observed from different soil samples during the study period. Analysis of variation (ANOVA) of the data shows that the bacterial population varies significantly ($p<0.05$) among the different soil samples and different samplings (Table 7).

Fungal Population (FP): Among the field soil samples, RH harboured the maximum fungal population followed by BT (11-12), BT (1-2) and BR, whereas in the laboratory condition, F+BR claimed higher CFU of fungal population, followed by CTRL soil. Consistent distribution pattern of fungal population was observed from different soil samples during the study period (Table 2). F+BR show higher CFU of fungal population than RH soil sample. A one way analysis of variation (ANOVA) shows that the bacterial population varies significantly ($p<0.05$) among the different soil samples and different samplings.

Soil pH: During the three sampling, BT (11-12) exhibited higher pH in the first two samplings and BT (1-2) in third sampling and followed by RH, BT (1-2) and BR. Among the incubated soil, F+BR displays higher pH than CTRL. The pH value of RH was higher than F+BR sample (Table 3). The bacterial population varies significantly among the different soil samples and different samplings ($p<0.05$).

Table 1: Average CFU of Bacterial Population×105 of different soil samples

Sl. No	Soil Sample	1st Sampling	2nd Sampling	3rd Sampling
1	RH	55.10±1.32	66.06±1.00	50.78±0.92
2	BT(1-2)	31.60±0.65	39.62±1.45	30.56±0.59
3	BT(11-12)	45.56±0.56	50.32±0.84	43.1±0.82
4	BR	19.96±0.85	18.94±0.81	22.26±1.26
5	F+BR	24.58±1.19	23.28±1.21	31.74±1.59
6	CTRL	8.82±0.37	11.64±0.86	12.38±0.70

Table 2: Average CFU of Fungal Population × 10^3 of different soil samples

Sl. No	Soil Sample	1st Sampling	2nd Sampling	3rd Sampling
1	RH	59.84±0.80	57.92±1.04	50.74±1.10
2	BT (1-2)	41.34±1.06	41.06±1.19	43.82±0.55
3	BT (11-12)	52.02±1.30	54.54±1.07	50.54±1.15
4	BR	35.04±1.14	36.48±0.64	36.94±0.60
5	F+BR	57.38±0.64	102.62±4.10	116.26±3.06
6	CTRL	36.66±1.02	34.12±0.99	35.60±0.91

Table 3: Average pH of different soil samples

Sl. No	Soil Sample	1st Sampling	2nd Sampling	3rd Sampling
1	RH	6.71±0.19	7.04±0.15	6.98±0.08
2	BT(1-2)	6.60±0.17	6.71±0.20	7.01±0.26
3	BT(11-12)	7.02±0.39	7.11±0.31	6.80±0.41
4	BR	6.39±0.37	6.49±0.34	6.49±0.35
5	F+BR	5.99±0.25	6.29±0.17	6.17±0.15
6	CTRL	5.45±0.33	5.84±0.36	5.87±0.33

Table 4: Average Soil Moisture Content (%) of different soil samples

Sl. No	Soil Sample	1st Sampling	2nd Sampling	3rd Sampling
1	RH	16.37±0.45	17.01±0.21	17.65±0.59
2	BT(1-2)	13.10±0.56	14.12±0.53	15.88±0.44
3	BT(11-12)	19.93±0.72	19.62±0.78	18.86±0.75
4	BR	9.13±0.51	10.05±0.15	9.51±0.44
5	F+BR	35.48±1.71	37.26±2.06	38.64±1.08
6	CTRL	14.32±0.88	14.46±1.05	14.40±0.64

Table 5: Average Soil Organic Carbon (%) of different soil samples

Sl. No	Soil Sample	1st Sampling	2nd Sampling	3rd Sampling
1	RH	2.366±0.08	2.534±0.04	2.814±0.05
2	BT(1-2)	1.754±0.03	1.51±0.02	1.370±0.03
3	BT(11-12)	1.828±0.04	1.672±0.04	1.560±0.09
4	BR	1.212±0.03	1.234±0.04	1.302±0.01
5	F+BR	1.852±0.08	1.874±0.05	1.936±0.05
6	CTRL	1.560±0.04	1.332±0.06	1.254±0.03

Table 6: Average Total Nitrogen Content (%) of different soil samples

Sl. No	Soil Sample	1st Sampling	2nd Sampling	3rd Sampling
1	RH	0.712±0.03	0.896±0.01	0.900±0.03
2	BT(1-2)	0.308±0.02	0.332±0.02	0.290±0.02
3	BT(11-12)	0.428±0.02	0.484±0.01	0.438±0.02
4	BR	0.144±0.02	0.136±0.02	0.122±0.01
5	F+BR	0.474±0.03	0.508±0.02	0.498±0.02
6	CTRL	0.124±0.02	0.118±0.02	0.124±0.01

Table 7: Analysis of variance (ANOVA) of microbial population and physico-chemical characteristics of rhizosphere soil (RH), between row soil (BT 1-2 and BT 11-12), barren soil (BR), mixture of *Flemingia macrophylla* leaf with barren soil (F+BR) and control soil (CTRL)

Sl. No	Parameter	Sources of variation	Sum of squares	Degree of freedom	Mean Squares	F- Ratio	p-value
1	Bacterial Population	Between Groups	4367.287	5	873.457	41.177	0.00000*
		Within Groups	254.546	12	21.212		
		Total	4621.834	17			
2	Fungal Population	Between Groups	6740.33	5	1348.066	8.237	0.001*
		Within Groups	1963.886	12	163.657		
		Total	8704.216	17			
3	Soil pH	Between Groups	3.608	5	0.722	23.739	0.00000*
		Within Groups	0.365	12	0.0304		
		Total	3.973	17			
4	Soil Moisture content (%)	Between Groups	1391.348	5	278.27	308.114	0.00000*
		Within Groups	10.838	12	0.903		
		Total	1402.186	17			
5	Soil Organic Carbon (%)	Between Groups	3.36	5	0.672	29.527	0.00000*
		Within Groups	0.273	12	0.0227		
		Total	3.633	17			
6	Total Nitrogen	Between Groups	1.071	5	0.214	96.521	0.00000*
		Within Groups	0.02663	12	0.002219		
		Total	1.097	17			

Note: * implies Highly Significant

Soil Moisture Content (SMC): Among the field soil samples, BT (11-12) shows the maximum soil moisture content followed by RH, BT (1-2) and BR. The result also provides that the incubated sample F+BR exhibit higher amount of soil moisture content than all the other samples (Table 4). Different soil samples and different samplings proclaimed significant (<0.05) variation for the microbial population ($p<0.05$).

Organic Carbon Content (OC): The highest carbon content was found in RH soil sample in all the three samplings and followed by BT (11-12), BT (1-2) and BR among the field soil samples. The highest organic carbon content is found in F+BR than CTRL among the incubated samples. Moreover, F+BR has the highest carbon content among all the soil samples (Table 5). Significant variation ($p<0.05$) for bacterial population was observed in different soil samples and samplings.

Total Nitrogen (TN): The estimation of total nitrogen from the soil samples reveals that RH provides the maximum percentage of nitrogen followed by F+BR, BT (11-12), BT (1-2), BR and CTRL (Table 6). Bacterial population varies significantly among the different soil samples and different samplings ($p<0.05$).

DISCUSSION

Microbial Population: Among the field soil samples, the result revealed higher microbial population (fungi and bacteria) in rhizosphere soil (RH). This could be due to the enrichment of soil nitrogen through biological fixation by the host legumes and plant composition, which affect the microbial diversity [15]. Thies *et al.* [16] also revealed that legumes could enrich their immediate soil environment with rhizobia through rhizosphere effect. It is well known facts that N_2-fixing plants contribute to the soil N enrichment accumulating more C than the soils under non-N_2-fixing species. The distribution of soil microbial population is determined by a number of environmental factors like pH, moisture content and soil organic matter [17]. Organic C is one of the main factors influencing the number, composition and activities of microbial communities [18].

Lalfakzuala *et al.* [19] found that legume groundnut plant has a beneficial influence on soil microbial number, microbial biomass carbon and soil respiration. The least microbial population from BR soil and lower population from BT (11-12) and BT (1-2) support the fact that host legume enriched the rhizosphere soil nutrient and no plants are available in barren sites as well as surface soils were collected from BT (11-12) and BT (1-2). Lynch and Whipps [20] also revealed that rhizosphere is a system exposed to environmental fluctuations due to shift on composition of root exudates, which has a marked influence on microbial communities. The reforestation of degraded areas contributes to restoring original soil physico-chemical characteristics by increasing the organic matter content, nutrient availability and the microbial populations and activity [21].

Higher microbial population in lower row, BT (11-12) than the upper row BT (1-2) may be due to huge deposition of nutrients in the lower rows, located at the bottom site of the experimental plot. It may be considered that the runoff of nutrients would be deposited into the lowest site due to the action of the gravity.

Higher microbial population in F+BR incubated soil could be due to the availability of nutrient released from *Flemingia macrophylla* through decomposition. Frey *et al*. [22] also mentioned that microbial community composition might be an important determinant of soil organic decomposition rates and nutrient turnover and availability in agricultural soils.

Soil pH: It is well known that during the cultivation of legumes, soil is acidified due to proton release from roots. As a consequence of proton release, plants accumulate organic anions which may, if returned and decomposed in the soil, neutralise the soil acids [23]. BR and CTRL soil samples displaying lower pH value, which were without plant sample or material. So it seems that there were no plant materials for decomposition which could neutralize or increase soil alkalinity. Whereas, RH, BT (11-12), BT (1-2) and F+BR displaying within or little above the neutral pH value were accompanied by *Flemingia macrophylla.* It is concluded that the soil acidification caused by legume cultivation can be partly compensated if crop residues are returned to the soil. Addition of plant residues may initially cause an increase in soil pH due to decomposition of organic anions and organic nitrogen.

Soil Moisture Content (SMC): The principal source of soil moisture is rainfall. The size of the mineral particles, their shapes and number of pore spaces are important factors responsible for retaining moisture by the soil. F+BR and CTRL incubated soils were watered everyday to maintain moisture content. So, the moisture content of RH, BT (1-2), BT (11-12) and BR could not be compared with incubated soil. Among the field soil samples, BR contains minimum moisture and higher values are found in RH, BT (1-2) and BT (11-12). The reason could be due to direct exposure of the barren soil to the sun by which it losses its water content through evaporation. It is well known that plant and litter may protect the soil surface from direct exposure to sunlight. *Flemingia macrophylla* greatly increases water infiltration rate and soil moisture content and maintains favourable soil aeration for soil biota [24].

Budelman and Siregar [2] reported that owing to the leaf size and slow decomposition, the mulch also has long-term effects in moisture conservation and reduction of soil temperature. Litters have the large capacity of retaining water, so the incubated sample, F+BR contains larger amount of moisture than CTRL.

Organic Carbon Content: Organic matter affects both the chemical and physical properties of the soil and its overall health. Properties influenced by organic matter include: soil structure; moisture holding capacity; diversity and activity of soil organisms, which might be beneficialand harmful to crop production; and nutrient availability. Soil organic matter is an accumulation of dead plant matter, partially resynthesized plant and animal residues. In this study, BR shows the minimum organic content which could be resulted from the lowest accumulation of litters and is one of the main sources of organic carbon. The return of crop residues to soil is beneficial to maintain soil carbon stock [25].

Among field soil samples, higher organic carbon in RH soil could be due to exudation of carbon substrate from *Flemingia macrophylla.* Jones *et al*. [26] proposed that plant root-exudates contain carbon substrates, including primary metabolites such as sugars, amino acids and organic acids, in addition to a diverse array of secondary metabolites that are release into the rhizosphere and surrounding soil. Among the incubated soil samples, F+BR show more organic carbon than CTRL. This result could be due to the presence of *F. macrophylla* as of the fact that plant litters contribute to soil Organic matter.

Total Nitrogen: The percentage of total nitrogen content in RH soil is comparatively higher than F+BR, BT (11-12), BT (1-2), BR and CTRL soils. The main reason could be due to the biological nitrogen fixation in *Flemingia macrophylla* root nodules [2]. It has been observed to nodulate freely with native rhizobia. These root nodules were subjected to transform the atmospheric nitrogen into the usable form of the plants. This caused a higher percentage of nitrogen in RH soil. In case of BR and CTRL soil samples, the total nitrogen was low. This may be due to complete absent of *Flemingia macrophylla* and plant litter, which is the main source of organic nitrogen. Thies *et al*. [16] also suggested that enrichment of soil Bradyrhizobial population was host specific, that symbiotic legumes can enrich their soil environment with microsymbionts up to a threshold level and that such enrichment can be curtailed by soil management practices that suppress nodulation.

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