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Temporal Changes in Soil Microbial Diversity of Tropical and Sub-Tropical Forests of Mizoram, Northeast India

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ABSTRACT

Microbes are ubiquitous in the soil and abundantly available even in a small amount of soil and are represented by group of bacteria, fungi and actinomycetes. Studies are more abundant on bacterial and fungal population while actinomycetes are less studied. We studied seasonal changes in all three groups of soil microbes in tropical (~100 m altitude) and subtropical (~1350 m altitude) forest settings. Soil samples were collected from two forests during June 2015 to May 2016 every three months interval based changes in temperature and precipitation. Using serial dilution Method, different agar media was prepared separately for assessing bacteria, fungi and actinomycetes, for example, nutrient agar with nystatin and actidione for bacteria (using dilution 10^{-5} to 10^{-7}), potato dextrose agar (PTA) with rose bengal, antibiotic (i.e.penicillin and chloramphenicol) for fungi (10^{-3} to 10^{-5}) and starch case in agar (SCA) mixed with nystatin were used for isolation of actinomycetes (10^{-4} to 10^{-6}). For each group of microbes, 1 ml diluted liquid was added into petri-plates containing solid media (triplicates) and incubated in BOD at different temperature. Soil microbial population in the present study varied significantly with respect to changes in abiotic variables. Significantly higher microbial populations were recorded during rainy season compared to other seasons. The soil microbial diversity in the present study changes as function of rainfall and its associated variables. For instance, in sub-tropical forest, rainfall accounted for 97% variability in fungal population and 94% in the actinomycetes population. However, in tropical forest, rainfall and soil moisture together accounted for 99% variability in the population of actinomycetes. We suggest more frequent recording of microbial population and abiotic variables for studying the impact of climate variables on soil microbial diversity in these forests.

Keywords : Microbial population, Fungi, Four season, Environmental factor, Bacteria.

INTRODUCTION

Soils are colonized by range of microbial groups, responsible for the breakdown of organic matter, and play an important role in nutrient recycling in forest ecosystem (Hauchhum and Tripathi 2017, Zhang et al. 2017). Thousands of various species of microbes are present within a gram of soil (Cachuela Palacio 2006), out of which only few microbes (<1%) can be isolated and cultivated as the majority of them are still unnamed (Joshi et al. 2015).High soil mi-

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crobial diversity is associated with large variability within gene, species and even among same functional group (Tiwari et al. 2009) . Torsvik et al. (1990) stated that soil microbial diversity is extremely high (i.e.~ 4×10^3 – 7×10^7 microbes within a gram of fresh soil), which are influenced by various factors like climatic, edaphic and quality of organic matter (Harrison 1979) as a result of changes in vegetation types and microclimatic condition (Torsvik and Øvreås 2002). The availability and activities of soil microbial flora and fauna depends seasonal changes (Dilly et al. 2004). In nutrients rich soil, microbes are more active compare to stress condition (Pascoal and Cássio 2004). Microbes also depend upon soil moisture and temperature, when temperature increase with moisture, microbial activity shows significantly higher (Peterjohn et al. 1994). Changes in microbial activities may affect soil biochemical process that alters availability of nutrient status (Yang et al. 2018). Higher soil microbial diversity also depends upon suitable climate, moisture condition and substrate deposited above the soil surface (Singh and Gupta 1977). There are number of soil microbes which enhance nutrient recycling but still remain unnamed. Among the soil microbes, several bacteria and fungi were studied in different environment condition related to litter (Rosenbrock et al. 1995).

Mizoram a northeastern state of India has wide variations in altitude that has led to wider variations in precipitation and temperature in various topography regions. The state is part of the Indo-Burma biodiversity hotspots and is characterized by various climatic conditions due variation in altitude and vegetation. The annual temperature varies from 12°C to 30°C, mean annual precipitation was varied from 2160 mm to 3500 mm. About 88.93% (ISFR 2015) of total geographical area of the state is under forest. Due to rich in forst diversity with different forest types in this region, microbial population may be vary between two different elevations. The relationship between vegetation and seasonal climatic changes in forest types with soil microbial population (bacteria, fungi and actinomycetes) has not been studied in this region. Therefore, this study was conducted in tropical and sub-tropical forest settings to understand the changes in soil microbial population during four seasons (summer, autumm, winter and spring) and to assess the role of abiotic variables (i.e. rainfall, soil temperature and moisture) on soil microbial diversity.

MATERIALS AND METHODS

Study site and the climate

The study sites are shown in Fig. 1. In one of the study site, a Sub-Tropical Forest (STF) at Hmuifang (Fig.1) was selected around 50 km away from the Aizawl city towards south. This is a reserve forest situated at 23° 27.2′ N latitude and 92°45.0′ E longitude with elevation of about 1450 m amsl. STF of Hmuifang posses semi-evergreen vegetation with climate characterized by cool and low to moderate temperature throughout the year. In another site, a Tropical Forest (TF) was selected (Fig.1) at Sairang with contrast vegetation from the previous site. It was located at 23°49.2′ N latitude and 92°39.5′ E longitude with the elevation of about 100 m amsl. This forest site is characterized by humid and warm to hot climate. Both forest sites were located within Aizawl district and have elevation difference of about ~1350 m amsl with considerable variations in vegetation composition.

Daily rainfall data was collected from June 2015

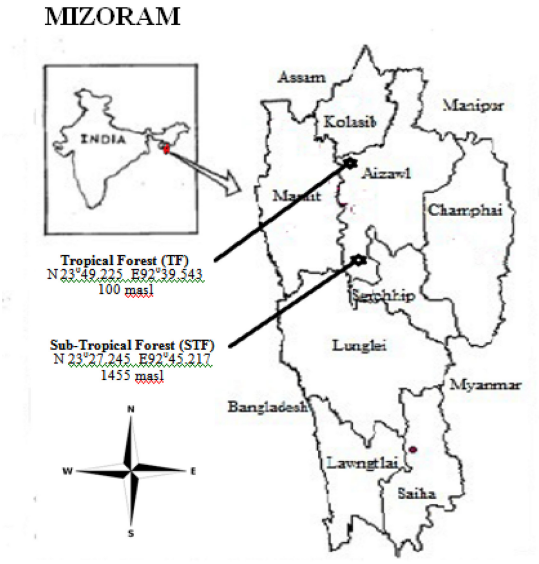

Fig. 1. Mizoram map showing location of study sites.

to May 2016 from Agriculture Department (crop husbandry) Sialsuk branch (~7 km apart from STF) and Lengpui (~4 km apart from TF). Total annual rainfall was marginally higher in site TF (3084 mm) compared to STF (2958 mm). Initially, average seasonal rainfall was significantly higher in STF site, whereas average rainfall in TF site increases in later months (Fig. 2). The area has four distinct seasons e. g. S1-warm humid monsoon (June–September), S2 – post monsoon (autumn, October—November), S-3 cold and dry **(**winter, Decmber–February) and S4-warm pre-monsoon (summer, March—May) as described by Lalnunzira and Tripathi (2018). Soil Temperature (ST) was recorded mercury there mometer (ranges –10 to 110) at 10 cm depth in the soil. Soil was collected randomly during second month of each season from both the forest sites with three different depths (i.e. 0–10 cm, 10–20 cm and 20 – 30 cm) using soil corer (4.2 cm dia). Soil texture was determined using hydrometer methods as described by Gee and Bauder (1986). In brief, 50 g of oven dry soil was taken in 250 ml of lid beaker and mixed with distilled water than add 20 ml 30% hydrogenperoxide, 2 g sodium hexametaphosphate and transfer to 11 measuring cylinder. The reading of hydro meter and temperature was recorded at 4 min and 2 h. Soil Moisture (SM) contnt was detrmined gravimetrically by oven drying the soil samples at 105^{o}C for 48 h to constant weight (Anderson and Ingram 1993). Soil pH was determined using standard pH meter (Mettler Toledo, Switzerland) in 1:2.5 soil/water suspensions. Bulk Density (BD) (g cm^{-3}) was estimated using known volume of soil corer (4.2 cm dia×10 cm height) and expressed as dry weight soil per unit volume as described by Brady (1984).

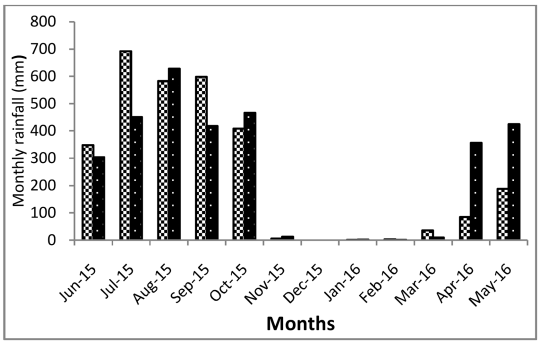

Fig. 2. Monthly rainfall data of both forest sites. Rainfall data was collected from Agriculture Department (crop husbandry) Sialsuk branch (~7 km apart from STF) and Lengpui (~4 km apart from TF) for12 months (June 2015 to May 2016).

Microbial population counts

Freshly collected 1 g of homogenized soil was used for microbial population count. Soil sample was added to the first test tube containing 10 ml (dilution factor 10^{-1}) of distilled water and remaining test tube containing 9 ml of distilled water. Then after mixing thoroughly, 1 ml from the first test tube was transfer to next test tube containing 9 ml of distilled water (dilution factor 10^{-2}) then same serial dilution process continue to obtained 10^{-3},10^{-4},10^{-5},10^{-6} and 10^{-7} (Serial dilution technique by Martin (1950). For Colony Forming Units (CFUs), experiment was based on Dilution Plate Method (Waksman 1922). Different agar media were prepared separately for fungi, actinomycetes and bacteria. For fungi, Potato Dextrose Agar (PTA) added with antibiotic ~ 0.08% of penicillin and chloramphenicol along with rose bengal at the time of preparing. Starch Case in Agar (SCA) mixed with nystatin (~0.08%) used for isolation of actinomycetes. For bacterial counts, media prepare from nutrient agar added with around 0.08% of nystation and actidione. Dilution of 10^{-3} to 10^{-5} use for isolation of fungi, 10^{-4} to 10^{-6} actinomycetes and 10^{-5} to 10^{-7} were used for bacterial isolation. 1ml of each dilution was added into petri-plates containing solid media (triplicates). Then media plates were incubated at 28 ±1^{o}C for fungal growth and 25 ±1^{o}C for both actinomycetec and bacterial growth. After 24 h of incubation actinomycetes and bacteria population started counted and for fungi after 72 h of incubation. The microbial population was expressed in CFU/g of soil. [Note : all glassware's and media used in this experiment were sterile at 120^{o}C for 20 min in autoclave].

Statistical analysis

To understand the effect of various seasons (Sl–S4) on microbial population (fungi, actenomycetes and bacteria) at both forest sites, one way ANOVA was used. After ANOVA test LSD was performed (MS-Excel 7). Pearson correlations were performed among various soil physico-chemical properties (texture,

pH and bulk density) of the two sites using SPSS-18. Soil microbial population was correlated with abiotic factors (i.e. rainfall, soil temperature and moisture). Further, stepwise multiple regression analysis was carried out using minitab-18 software to evaluate the effect of various abiotic factors (i.e. rainfall, soil temperature and soil moisture) on soil microbial diversity in two forests.

RESULTS AND DISCUSSION

Soil physico-chemical properties

Annual ST ranged from 14.8–25.1° C in STF and 15.8–28.3° C in TF sites with a considerably lower mean ST (19.3°C) in STF compared to TF (23.1°C) site. Maximum ST was 25.1°C and 28.3°C, respectively in September and August months of 2015, whereas, minimum ST was 14.8°C and 15.8°C in December and January months of the same year (Fig.3). The SM contents were considerably high in STF compared to TF in all months (Fig.3). Maximum SM contents was observed in S1 (40.7% STF and 30.07% TF) and minimum in S3 (21.4% STF and 16.3 TF). The SM content in STF site of the present study was significantly higher compared to recent reports of SM in rhizosphere and bulk soil of bamboo forest (20.7% –27.2%) by Hauchhum and Tripathi (2017). However, the SM content of TF site was considerably lower as compare to the reports of Hauchhum and Tripathi (2017).

In both forests, Bulk Density (BD) increases with increase in soil depths (STF, 0.55–1.15 g cm^3) and (TF, 0.74–1.17 g cm^3). TF site observed significantly higher ($p < 0.05$) BD compared to STF site (Table 1).

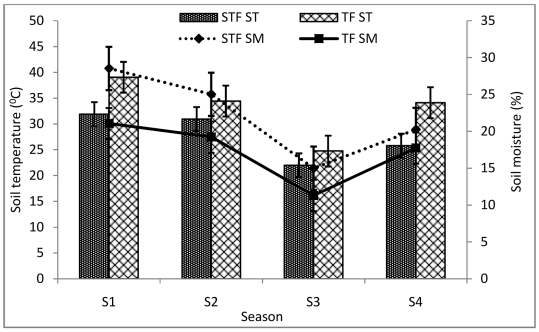

Fig. 3. Seasonal changes in soil temperature (ST) and soil moisture (SM). Soil temperature was significantly higher in all seasons for TF site compare to STF while soil moisture was higher in all seasons for STF compare to TF sites. (Mean ±1SE, n=3).

In soil pH, value ranged from 4.77–4.92 in STF which is slightly acidic compared to TF (4.83–4.94). No significant difference ($p < 0.05$) was observed in pH within sites. Soil texture analysis showed that clay content ranged between 13.6 and 16.3% in STF and 12.6 and 15% in TF site. Clay content did not vary significantly between two sites. Percent variations in sand content were : 64.1–69.3% in STF and 67.1–71.8% in TF. Silt content was significantly higher in STF (17—19.5%) compared to TF (15.4–17.9%) (Table1).

Microbial population counts

Marked seasonal variations were observed in the groups of microbial population counts (Table 2). The seasonal variations in microbial counts were more pronounced in the TF site as compared to the STF site as result of alternating wet and dry conditions in the TF due to fluctuation in temperature. Diversity of fungi (F) was maximum in S1 (21×10^3) season

Table 1. Variation in soil properties in both forest sites, such as bulk density (BD), soil pH, soil texture (sand, silt and clay contents). (Mean ± 1SE, n=3). As per LSD value depth wise differences for soil parameters are not significant except in few parameters.

Site	Depth (cm)	BD (g cm^3)	pH	Clay	Soil texture (%) Sand	Silt
STE	0–10	0.55 ± 0.02	4.77 ± 0.2	14.0 ± 2	67.6 ± 2.4	18.4 ± 1
	10–20	0.82 ± 0.02	4.81 ±0.1	13.6 ± 1.6	69.3 ± 2	17 ± 0.4
	20–30	1.15 ±0.03	4.92 ±0.2	16.3 ± 1.2	64.1 ± 2	19.5 ± 1
	LSD	0.6	0.25	5.7	7.5	3.2
TF	0–10	0.74 ± 0.01	4.83 ± 0.1	14.0 ± 1.1	69.3 ± 1.3	16.6 ± 0.6
	10–20	0.92 ± 0.02	4.80 ± 0.1	15.0 ± 0.5	67.1 ± 0.7	17.9 ± 0.8
	20-30	1.17 ± 0.03	4.94 ± 0.2	12.6 ± 0.6	71.8 ± 0.1	15.4 ± 0.5
	LSD	0.4	0.24	2.9	3.1	2.4

Table 2. Colony Forming Units (CFUs) of various soil microbes (F-Fungi, A-Actinomycetes and B-Bacteria) in different seasons. # Colony count based on triplicate.

	Site					
	STF			TF		
Seasons	F (CFUs/g)	A (CFUs/g)	B (CFUs/g)	F (CFUs/g)	A (CFUs/g)	B (CFUs/g)
S1	21×10^3	44×10^6	78×16^6	43×10^3	76×10^5	128×10^6
S2	19×10^3	34×10^6	87×10^6	39×10^3	84×10^5	87×10^6
S3	17×10^3	65×10^5	98×10^6	15×10^3	48×10^5	69×10^6
S4	18×10^3	59×10^5	72×10^6	15×10^3	75×10^5	65×10^6

and minimum in S3(17×10^3) season in STF. The corresponding values of fungi were (43×10^3) in S1 season and (15×10^3) in S3 and S4 seasons (Table 2). Borneman and Triplett (1997) have reported higher population of soil fungi during autumm season. Similarly, actinomycetes (A) population count was maximum in S1 (44×10^6) and minimum in S4 (59×10^5) in STF. In TF site, maximum A population (84×10^5) was recorded in S2 and minimum in S3 (48×10^5). In contrast, soil bacterial (B) count was maximum in S3 (98×10^6) and minimum in S4 (72×10^6) in STF site. Whereas, maximum (128×10^6) B count was recorded in S1 season and minimum (65×10^6) in S4 (Table 2) in TF site. Maximum bacterial population count was reported in spring and minimum in winter (Borneman and Triplett 1997). Soil bacteria are highly fluctuated with seasonal changes of environmental factors (moisture, temperature and substrate availability Smit et al. 2001). Microbial counts were significantly high in top soil layer which decreases down the soil depths in forest (Shamir and Steinberger 2007). Seasonally, in the present study microbial population counts in top layer of soil were more abundant than other microbial groups(i.e. fungi and actinomycetes). Similar results were reported from eastern Amazonian forest soil by Borneman and Triplett (1997).

Relationship of soil microbial population and environmental factors

Abiotic factors like ST and SM were found to significantly ($p<0.05$) effect the populations of soil microbes (F, A and B). Various environmental factors have reported to strongly affect soil microbial population (Kennedy et al. 2005). Hauchhum and Tripathi (2017) reveal that higher microbial population in rhizosphere soil than bulk soil were due to the availability of favorable substrates for microbes in rhizospheric zone. The present study fungi population was lowercompared to bacterial population; same have been reported in grassland and arable field soils (Hassink et al. 1993). Seasonal variations of soil microbial counts as a result of changes in soil moisture and temperature were in accordance with the report of Yang et al. (2018), (Fig. 4).

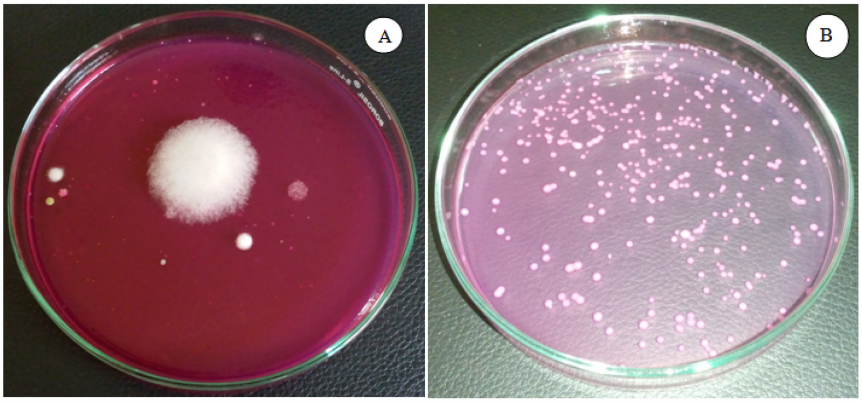

Fig. 4. Showing colonies of fungi (A) and bacteria (B) from forest soil during inoculation.

To assess the effect of environmental variables (rainfall, soil temperature and soil moisture) on microbial population (Fungi, Actinomycetes and Bacteria), we regressed the changes in microbial counts with that of environmental variables. In STF site, RF accounted for about 97.11% variability in fungal and 94.66% variability in the population of actinomycetes, however, none of the environmental variables were able to predict the population bacteria in these forest sites. In TF site, RF and SM together accounted for 99.81% variability in the population of actinomycetes and there was no correlation evironmental variables and fungal population. Equations are as follows :

In STF site,

$$\text{Fungi} = 17.036 + 0.006951\ (RF),$$
$$\text{Actinomycetes} = 34.1 + 0.779\ (RF),$$

And TF site,

$$\text{Actinomycetes} = -78.7 - 0.1780\ (RF) + 7.886\ (SM)$$

CONCLUSION

This study shows that in STF, seasonal changes in microbial populations (fungi and actinomycetes) are more strongly influenced by fluctuations in the rainfall, temperature and their associated variables. However, the above abiotic variables were able only regulate the variability in the population of actimycetes in TF. It is suggested that more frequent data on seasonal abiotic variables (at least monthly) would required to understand the role of abiotic variables on soil microbial population.

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