

# Soil metabolic profile as influenced by seasonal and topographical variations in tropical dry deciduous forest: A biolog based approach

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**Abstract:** The soil microbial communities and factors therein play important role in maintaining nutrient turnover in forest ecosystem. A biolog ecoplate based approach was applied to a dry deciduous tropical forest to investigate metabolic profile of soil microbial communities in relation to topography and seasonality. Significant ( $p < 0.05$ ) effect of seasonality on metabolic profile was observed. The average well color development (AWCD), rate of change ( $r$ ) and metabolic diversity indices (functional diversity indices) were recorded maximum in rainy season followed by winter and summer indicating maximum microbial activity in rainy season. Principal component analysis revealed seasonality to be the major determinant of soil metabolic profile, a key aspect of ecosystem functioning.

**Index Terms:** Ecoplate, Metabolic profile, Functional diversity, Tropical dry deciduous forest, Seasonality.

## I. INTRODUCTION

The importance of understanding regional pattern of microbial metabolic profiling is becoming ever more urgent as the ecosystems and communities are continuously being transformed by habitat fragmentation, species loss and climate change [1]. Increased understanding of microbial community level physiological profiling of relatively undisturbed landscapes is a pre-requisite for the generality of pristine data comparison and for the advancement of our knowledge on basic and applied aspects of landscape ecology. Considering carbon metabolism as a key indicator of soil quality, community-level physiological profiling (CLPP), based on sole carbon source utilization patterns, is employed to characterize metabolic versatility of microbial communities under different environments including

forest soils [2], [3], [4]. Carbon-substrate relations of microbial communities reflect their *in situ* activity and associated differences in the nature, abundance and bioavailability of carbon sources [5], [6]. Indian tropical forests accounts ~ 3.5 % of the tropical forest cover of the world [7] and the tropical dry deciduous forests of Vindhyan constitute ~ 53 % of total forest cover in India. This region have specific feature such as heterogeneous plant communities, marked seasonal changes, varied topography, and variable patches of nutrient impoverished soils [8]. Studies so far, conducted in the region have focused on N-mineralization, microbial biomass and microbial respiration in relation to land-use [9], vegetation cover [10], seasonal changes [11] and wildfire [12]. However, there is general paucity of data on how the microbial communities respond to seasonality and topographical variations in dry tropical forest of India. In particular, to our knowledge, no data are available on metabolic profile of microbial communities in tropical dry deciduous forest of India.

Information on factors that control soil microbial activity and metabolic profile on forest floor are important on which environmentally sustainable forest management practices depend. We expect that, environmentally driven, for instance, seasonally and topographically controlled, shifts in microbial community may change the microbial metabolic profile in forest ecosystems. Here we present the results of our study conducted along different topographical positions to reveal factors of spatio-temporal variability in soil microbial metabolic profile in a tropical dry deciduous forest of India. Our target was to answer, how well can variability in soil microbial profile in these

forests be accounted for seasonality and topography.

## II. MATERIALS AND METHODS

### A. Study site

The present study was conducted during 2016-17 at three sites selected in a tropical dry deciduous forest of Vindhyan plateau in Chandauli District (24° 49' 32.7" N, 83° 15' 03.2" E) of Eastern Uttar Pradesh, India. The climate of the region is tropical monsoonal with extended dry season. The year is divisible into three distinct seasons; rainy (late June–September), winter (October–February) and summer (March–early June). The annual mean precipitation during study period ranged from 900 mm to 950 mm and about 90–95 % rainfall during wet season due to south-west monsoon. Mean monthly temperature varied from a minimum of 14 °C in January to a maximum of 32 °C in June. Soil is silty loam at hilltop (Naugarh) and middle (Jamsoti) and silty clay loam at foothills (Chakia). The soils show acidic pH (6.20–6.82) with 0.50-1.24% total organic carbon, 0.08-0.10% total nitrogen and 43.10–45.60 % water holding capacity.

The natural vegetation constitutes tropical mixed deciduous forest [13] with dominance of woody perennials such as *Butea monosperma* (Lam.) Taub., *Boswellia serrata* Roxb. ex Colebr, *Tectona grandis* L.f., *Madhuca longifolia* var. *latifolia* (Roxb.) A.Chev. *Acacia catechu* (L.f.) Willd. , *Ziziphus oenoplia* L. Mill. and *Ziziphus glaberrima* (Sedgw.) Santapau etc.

### B. Experimental design and Soil sampling

A study transect was established along an elevation gradient and three sites were selected with lower end (Chakia forest) at 97m above msl, the upper end (Naugarh forest) at 276m and a transition zone (Jamsoti forest) at 182m above msl. At each study site, 1ha plot was established and five locations in each plot were selected randomly for sample collection. From each location, soil samples (0-10cm depth) were collected in triplicate using corer (5-cm diameter) and mixed to form composite sample. For soil sampling, August was considered to represent rainy, January to winter and May to represent summer season. Soil samples were transported to laboratory within 12 hrs, large plant parts were removed and samples were sieved using 2mm mesh to remove fine roots, and aliquots into two; one part for metabolic profile (4°C), other for analysis of soil attributes. Soil characteristics such as texture, organic C, total N, soil pH, were determined following standard methods [14].

### C. Metabolic profile

The patterns of C source utilization by microbial communities was assessed using Biolog Ecoplate system (Biolog Inc., Hayward, CA, USA) containing triplicates of 31 different environmentally relevant carbon substrate and control well. Soil suspension was prepared by agitating the soil (10 g) in Ringer's solution (0.25 %) at 150 r.p.m. at room temperature for 1 h. The

supernatant was mixed with distilled water and aliquots (150 µl) of 10<sup>-5</sup> dilution were directly inoculated into each well of Biolog Ecoplates and incubated in dark (28° C) for one week. Absorbance recorded at 590 nm with a Biolog microplate reader (Biolog microstation system) at 24 h intervals during entire incubation period. The average well color development (AWCD) values were calculated for each sample by dividing the sum of the optical density data by 31 (number of substrates) using following equation:

$$AWCD = \frac{\sum(C - R)}{n} \quad [1]$$

Where, C is the measure for color production within each well, R is the absorption value of the control well of plate and 'n' is the number of substrates.

### D. Kinetic analysis

To make AWCD data more comprehensive, a kinetic model was fitted to curve of OD vs incubation time using a density dependent logistic growth equation [15].

$$Y = AWCD = \frac{K}{1 + e^{-r(t-s)}} \quad [2]$$

Where, K is the maximum degree of AWCD (OD), r is the exponential rate of OD change (h<sup>-1</sup>), t is the time following inoculation of the microplates (h) and s is the time when AWCD reaches half of its maximum.

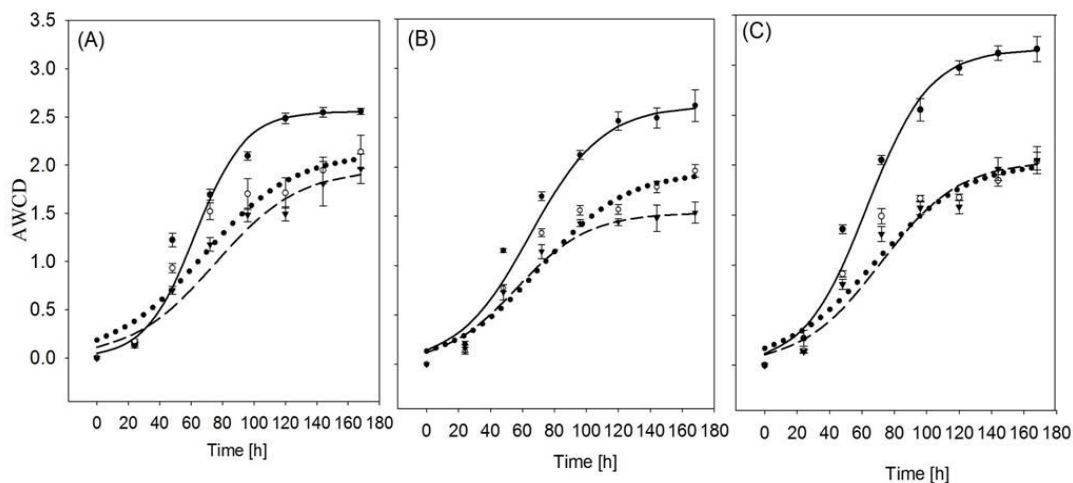
### E. Statistical analysis

The OD values at 96 h were used for calculation of diversity indices and Principle Component Analysis (PCA), Shannon's diversity index (H), substrate richness (S) and substrate evenness (E) were calculated [16]. PCA was performed using PAST v 2.17c software. Data were normalized before PCA analysis, the OD values of each well were divided by AWCD to minimize influence of inoculum density differences between plates [17]. Analysis of variance (ANOVA) used to determine the effects of topography and season IBM SPSS statistics v20).

**Table 1.** Kinetic parameters of the fitted logistic growth equations. (K, the asymptote; s, the time when y = K/2; r, the exponential rate of AWCD change; r<sup>2</sup>, correlation coefficient).

| Study sites | Season | K    | s     | r    | r <sup>2</sup> |
|-------------|--------|------|-------|------|----------------|
| Hilltop     | Rainy  | 2.56 | 62.61 | 0.06 | 0.98           |
|             | Winter | 2.07 | 68.09 | 0.03 | 0.97           |
|             | Summer | 1.90 | 75.39 | 0.04 | 0.98           |

|                 |        |      |       |      |      |
|-----------------|--------|------|-------|------|------|
| <b>Middle</b>   | Rainy  | 2.59 | 64.77 | 0.05 | 0.99 |
|                 | Winter | 1.91 | 71.01 | 0.04 | 0.97 |
|                 | Summer | 1.52 | 72.13 | 0.04 | 0.99 |
| <b>Hillbase</b> | Rainy  | 3.15 | 63.13 | 0.05 | 0.99 |
|                 | Winter | 2.01 | 67.73 | 0.04 | 0.96 |
|                 | Summer | 1.98 | 71.79 | 0.04 | 0.98 |



**Fig. 1.** Kinetics of average well color development (AWCD) of soil microbial community with incubation time (h) at three forest sites (A):Hilltop; (B):Middle; (C):Hillbase. The symbols of filled circle, open circle and filled triangle represents rainy, Error bar represent  $\pm$ SE

**Table 2.** Soil microbial community metabolic diversity as estimated by the Shannon’s diversity index (H), substrate richness (S) and substrate evenness (E) in the Biolog EcoPlate incubated for 96 h. Values are means of three replicates  $\pm$  SE

| Study sites | Season | Moisture (%)    | H               | S                | E               |
|-------------|--------|-----------------|-----------------|------------------|-----------------|
| Hilltop     | Rainy  | 21.25 $\pm$ 0.8 | 3.02 $\pm$ 0.02 | 25.25 $\pm$ 0.97 | 0.82 $\pm$ 0.02 |
|             | Winter | 8.60 $\pm$ 0.4  | 2.89 $\pm$ 0.01 | 21.75 $\pm$ 0.24 | 0.83 $\pm$ 0.01 |
|             | Summer | 4.50 $\pm$ 0.5  | 2.78 $\pm$ 0.01 | 20.50 $\pm$ 0.32 | 0.79 $\pm$ 0.01 |
| Middle      | Rainy  | 18.50 $\pm$ 0.6 | 2.95 $\pm$ 0.01 | 24.50 $\pm$ 0.43 | 0.79 $\pm$ 0.01 |
|             | Winter | 4.60 $\pm$ 0.5  | 2.84 $\pm$ 0.02 | 22.50 $\pm$ 0.60 | 0.77 $\pm$ 0.02 |
|             | Summer | 3.20 $\pm$ 0.4  | 2.85 $\pm$ 0.01 | 21.75 $\pm$ 0.72 | 0.80 $\pm$ 0.02 |
| Hillbase    | Rainy  | 23.05 $\pm$ 0.5 | 3.06 $\pm$ 0.02 | 28.25 $\pm$ 0.52 | 0.76 $\pm$ 0.00 |
|             | Winter | 6.30 $\pm$ 0.5  | 2.88 $\pm$ 0.01 | 23.00 $\pm$ 0.74 | 0.78 $\pm$ 0.02 |
|             | Summer | 5.20 $\pm$ 0.4  | 2.87 $\pm$ 0.02 | 22.25 $\pm$ 0.52 | 0.79 $\pm$ 0.01 |

### III. RESULTS

#### A. Soil physico-chemical analysis

Soil moisture ranged from 18.5 to 23.05 % (rainy), 4.6 to 8.8 % (winter) and 3.20 to 5 % (summer) across the sites. Soil of the area is silty loam at higher topography (Naugarh and Jamsoti), silty clay loam at foot hills (Chakia) with slightly acidic pH (6.2–6.82), 0.5–1.24 % total organic carbon, 0.08–0.10 % total nitrogen and 43.1–45.6 % water holding capacity.

#### B. Kinetic analysis

Microbial community took less than 25h to start utilizing the substrate as indicated by color development in the micoplate wells. The lower “s” value and higher “r” of rainy season irrespective of sites showed highest utilization rates. The average values of well color development (AWCD) showed (K) and rate change (r) to range from 1.52 to 3.15 and 0.03 to 0.06, respectively (Fig 1). The K and r showed significant ( $p < 0.05$ ) effect of seasonality (Table 1). However, we did not observed significant effect of topography on K and r ( $p > 0.05$ ). ANOVA

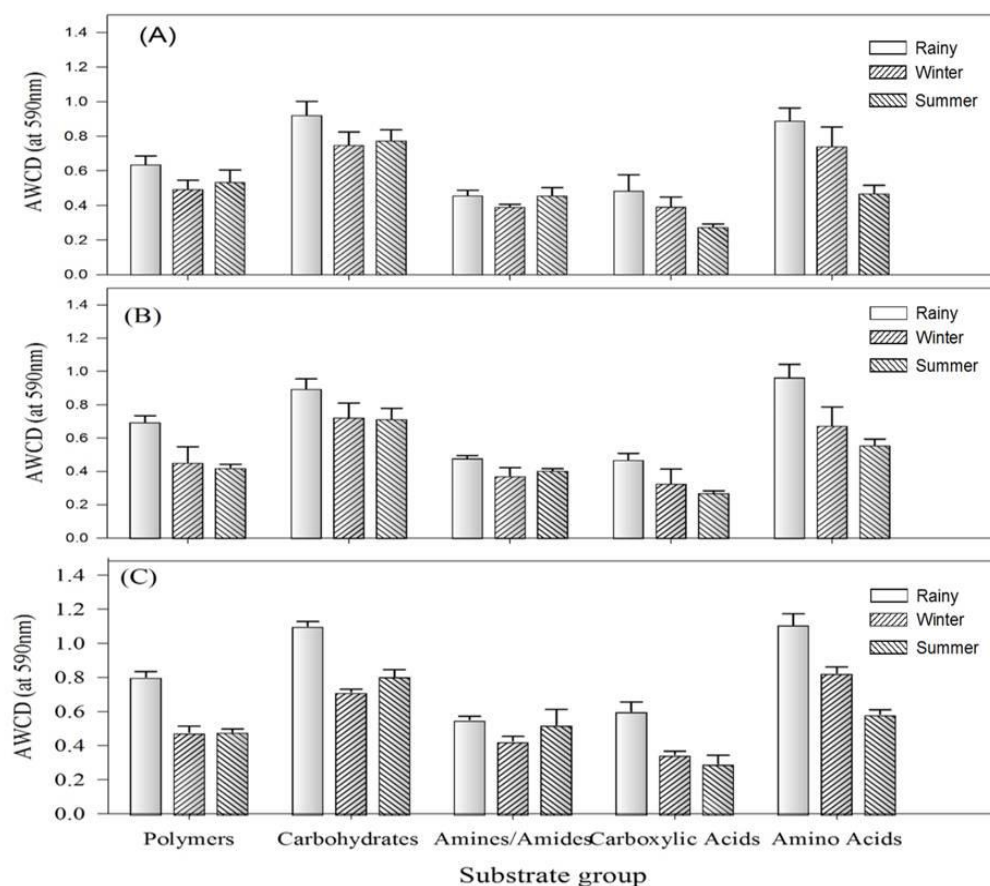
indicated significant difference ( $p < 0.05$ ) in Shannon's diversity index ( $H$ ) and richness in due to season at all sites.

### C. Metabolic diversity and profile

The ( $H$ ) index followed a trend as rainy>winter>summer (Hilltop and Hillbase sites) and as rainy>summer>winter (Middle site) (Table 2). Trends in substrate richness and

evenness showed synchrony to  $H$  index. All the five classes were of substrate utilized by the microbial community present in the soil (Fig. 2). At 96 h the carbohydrates followed by amino acids were found to be most preferable substrates utilized by the microbial community irrespective of season and site (Fig. 2). Carboxylic acids were the least utilized substrate in summer and winter seasons across all sites while amine/amides were the less preferred substrates in rainy season.

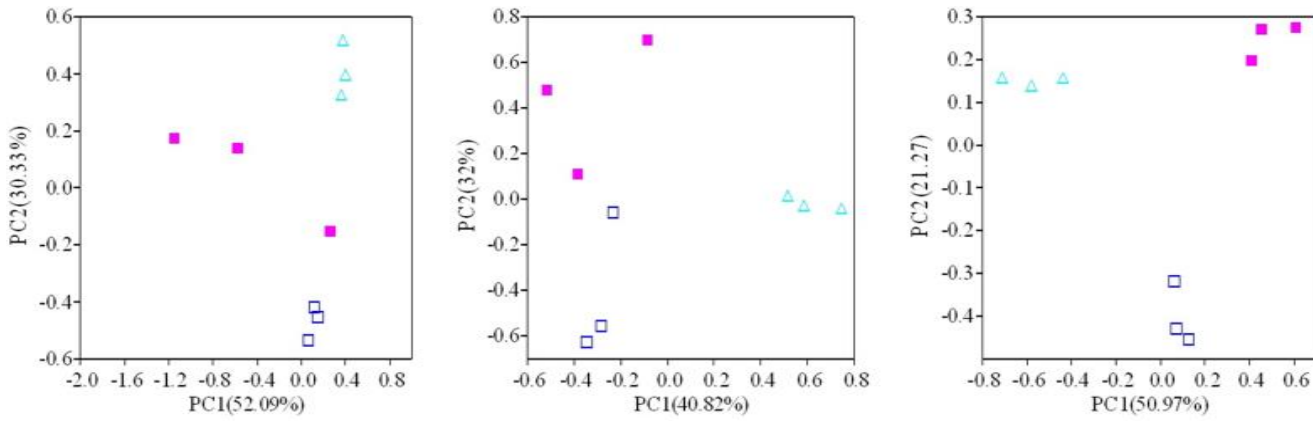
**Fig. 2** Substrates group utilization pattern at Hilltop (A), middle (B) and Hillbase (C) sites in different seasons. Value are the mean of three replicate, error bars represent  $\pm$ SE.



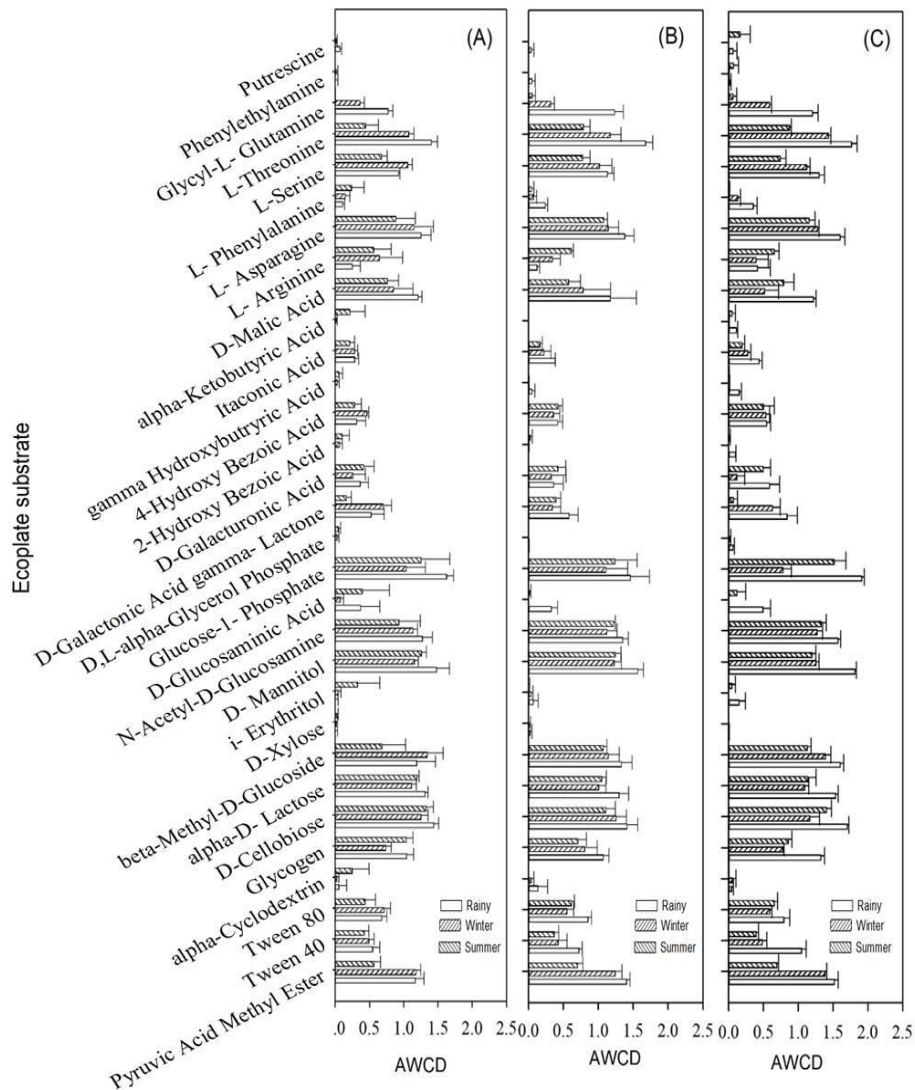
PCA analysis segregated seasons into three different group among all soils tested for a site (Fig. 3). For all sites almost similar pattern of clustering was obtained. PC1 accounted for 40.82 %, 52.09 %, 50.97 % while PC2 32 %, 30.33 %, 21.27 % variance at hilltop, middle and hillbase sites, respectively. The Individual substrate utilization profile (Fig. 4) showed that among 31 substrate types,  $\alpha$  Cyclodextrin, D-Xylose, i-

Erythritol,  $\alpha$ -Ketobutyric acid, D-L- $\alpha$ -Glycerol Phosphate, 2-Hydroxy Bezoic Acid, phenyl ethyl-amine, Putrescine and  $\gamma$  Hydroxy butyric Acid were either less utilized or remain unutilized in each season. Itaconic acid, 4-Hydroxy Benzoic acid, Tween 40 and Tween 80 appeared to be moderately utilized.





**Fig. 3** Principal component analysis (PCA) of Biolog Ecoplate data incubated for 96 h from soil samples of three season at three forest sites Naugarh(A, Hilltop), Jamsoti (B, Middle) and Chakia (C, Foothill). The symbols of open square, filled square and triangle represent rainy, winter and summer season respectively



**Fig. 4** Individual substrates utilization pattern expressed by AWCD at Naugarh (A), Jamsoti (B) and Chakia (C) site in different seasons. Value are the mean of n=3; error bars represent  $\pm$ SE

#### IV. DISCUSSION

The pattern of metabolic profile and the factors that regulate it can be used to more accurately predict the microbial activities and ecosystem functioning in dry tropical forests. The soil moisture content (%) varied significantly ( $p < 0.05$ ) among seasons, with values being highest in rainy season which accounts for 80 % of total rainfall. The AWCD indicate sole-carbon-source utilization ability of the soil bacterial community. The AWCD showed significantly ( $p < 0.05$ ) effect of season. We found significant positive correlation ( $r^2 = 0.732$ ;  $p < 0.05$ ) between AWCD and soil moisture. It seems that seasons drive the pattern of metabolic profile via influencing the moisture content of the soil. Our finding corroborates with reports of Costa et al. [18] which indicated highest microbial activity in rainy season in cork and holm oak trees dominated Montado ecosystem. Li et al. [19] have also showed higher microbial activity in rainy season and reasoned that the induced microbial growth due to increased soil moisture and temperature were the causal factors.

The covariation between AWCD and incubation period allowed us to fit data from each study site to a logistic growth model presenting kinetics of metabolic activity. When AWCD (OD) was modelled as function of incubation period (t), we found significant difference in response variable between three seasons (Fig.1). With respect to site the degree of consistency was remarkable and the pattern of substrate utilization was statistically indistinguishable. Higher value of 'r' across the sites for rainy season suggests that microbial community utilize substrates quickly during this season (Table 2). This function showed an excellent fit to data ( $r^2 > 0.96$ ) and can be used as a likelihood estimate of K to compare key features of functional relationships across systems.

The diversity indices (Shannon index, H; substrate evenness, E; and substrate richness, S) are used to indicate the metabolic diversity of microbial community in soil [20]. Their functional diversity indices varied significantly in association with seasonal changes and revealed a trend as rainy > winter > summer almost synchronous to the variation in metabolic profile. Favorable microclimatic condition coupled with increased below ground plant biomass may lead to more organic C input in soil to support different kinds of C utilizing microbial community in rainy season. Chen et al. [21] reported high Shannon diversity index during wet season due to enhanced substrate inputs and favorable microclimatic conditions in Chinese fir planted fields. With respect to site, individual substrate utilization pattern did not show significant difference in AWCD indicating that in qualitative term the diversity function relationship was consistent across habitats/the elevation gradient ( Fig. 2).

Among different classes of ecoplate substrates [22], the carbohydrates and amino acids were found to be the most preferred substrate irrespective of season and site (Fig. 2). This might be due to the activation of metabolic pathways associated with respiration and mineralization [23]. Higher rate of N-mineralization have also been reported in tropical dry deciduous forest soil [24]. Further, the PCA analysis also identified season as the main determinant of soil microbial community (Fig. 3) Recent study based on PLFA approaches also indicates the seasonal change in total microbial community [25] in the forest soil.

Present study demonstrates that the seasonality drives, through changes in soil moisture content, the microbial activity and metabolic profile in tropical dry deciduous forest soil. Rainy season showed rich metabolic diversity and fast rate of substrate metabolism. The study opens opportunities to pin-point microbial community analyses as influenced by key environmental factors using genomics and proteomics based approaches or methods.

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#### CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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