Conjunction of Soil Biota to Green Vegetation in Western Ghats Forests of Coorg, India

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Manuscript received January 1, 2024; revised March 14, 2024; accepted May 11, 2024; published August 16, 2024

Abstract—Forests are the lungs of our planet making them a very essential part of all the living organisms. For the very first time this kind of research study has been carried out in Coorg (Kodagu), also called as the Scotland of India, which has the largest green cover in the state, to quantify the effects of soil microbes on the lush green forests. The results showed an overall positive curvature. Different soil sample analysis of different forest patches showed that all the factors are interrelated in such a way that only those forests that have a healthy soil cover, which received a good amount of sun light, show a good co-relation with all the enzymatic activities. In this research we studied that soil microbial carbon, nitrogen and phosphorus are the main factors regulating the good forest health, all the results showed a descent similarity except in the land slide area and the river bank. In addition, more positive results were found in the forests having mixed vegetation rather than monotonous plantations. The results also emphasized the poor soil quality and lesser Water holding capacity at the land slide sites providing substantial evidence for the recent August 2020 land slide that occurred at Talakaveri in Kodagu.

Keywords—enzyme activity, forest, Sahyadri, soil organic matter, Talakaveri

I. INTRODUCTION

Soil is such a medium that gives life to many plants and microorganisms, it also holds a major portion of dead organisms that degrade in soil after decaying. The western ghats famously known as the Sahyadri's habitats for many creatures, making them unique in all the aspects to conduct research studies. Soil contains many components which are directly linked to the greenery and health of the forests and the organisms depending directly or indirectly on soil. Soil consists of lignocellulosic biomass that includes agricultural leftovers, leaf litter and wood chips [1]. Soil microorganisms play an important role in the ecosystem which directly affects the soil fertility and its dynamics [2]. According to the Economic Survey 2022-23 India ranks third in the net gain in average annual forest area in the last decade. The forests of the Western Ghats are famous for sheltering a lot of microbes and other living organisms that produce enzymes for various purposes. Many of the Bacillus and Aspergillus species isolated from the soils of the forests of the Western Ghats are good sources of cellulolytic enzymes [3]. The western ghats forest soils are rich in their biodiversity. A good combination of biological and physicochemical factors is required to maintain the strength of soil. Soil microorganisms and microbial processes are controlled by factors such as organic matter content, texture, pH and soil moisture, all of which also exhibit spatial heterogeneity [4].

Kodagu, also known as Coorg, is one of the most beautiful hill stations in Karnataka. The word 'Kodagu' is derived from

'Kodimalenad', which means dense forest land on steep hills. The total extent of reserved forests in Coorg is 67,262.45 hectares. However, besides Reserved Forests, there are other tree-clad areas that come under various categories, such as Protected forests, devarakadu (Sacred grooves), paisaries, Section IV notified areas, unredeemed lands and Bane lands, etc. The total extent of such lands, including the reserved Forests, is 1,46,513.84 hectares. A large portion of Coorg is under coffee estates, which complement the green landscape, giving the impression that the entire land is forested [5]. For the very first time this kind of research study has been done in the western ghats forests of Coorg, India. The main objective of this research is to study the co-relation between the chemical and biochemical factors of soil biota in this particular Coorg Forest and try to understand the growth pattern of the forests of different patches of western ghats, which further provides as a base for other research studies, academics, forest operations and details of some land slide prone areas.

II. LITERATURE REVIEW

The microbial growth, mainly fungus starts before the dead leaf reaches the soil, but the decomposition starts after the dead leaf reaches the earth [6]. Many studies have reported the decomposition rate of biopolymers in the eco system goes chronologically from fungi to bacteria and actinobacteria [7]. The microbes present in soil regulate the solidity, productiveness and functions of the ecosystems and are used to evaluate the soil superiority among various foliage types [8]. The Soil flora contains many microbes related to different groups. The Carbon to Nitrogen ratio for each microbial group is different and it ranges from nearly 3 to 5 for bacteria and for fungi it ranges from nearly 4 to 15 [9].

A perfect combination of soil physicochemical and biological factors is necessary for the soil to be healthy enough to maintain its rich diverse biota. Soil microbes regulate the perish rate, soil organic matter and the complete chemistry that controls the richness of the forest ecosystems [10]. Worldwide, the forests are formed by natural regeneration and by many human interventions or natural calamities [11]. The forests play a larger role in carbon sequestration by increasing atmospheric CO₂, as they cover a huge portion of Earth's land coverage [12]. Soil systems are highly dynamic as they are dependent on substrate availability and microbial activity [13]. Many of the research conducted on forest soils confirm that lot of biotic and abiotic factors influence the microbial biomass, composition and activity [14]. An extreme pH condition, high or low can damage microbial cells and thus decrease microbial abundance [15].

204

III. MATERIALS AND METHODS

The study was done in the Western Ghats forests of Coorg, Karnataka, India. The study locations were full of thick forests with a variety of vegetation. The samples were collected at 10 different locations from the whole of Coorg (Kodagu) district forests which include Madikeri, Virajpet, Ponnampet, Somwarpet and Kushalnagar taluk forests, respectively (Fig. 1). With reference to the standard classification of forests by Champion and Seth, the forests of

Coorg fall among the following six forest types: Moist tropical wet-evergreen forests- dominated by species such as *Calamus nagabethai, Vateria indica, Artocarpus integrefolia* and many Bamboo species; Moist tropical Semi-evergreen forests with species such as *Dipterocarpus indicus, Hopea parviflora*; Moist deciduous forests with species as *Dalbergia latifolia, Tectona grandis, Lagerstroemia lanceolata* and the Sholas/ Grass lands termed as "Savannahs" with *Strobilanthes ciliatus* species.

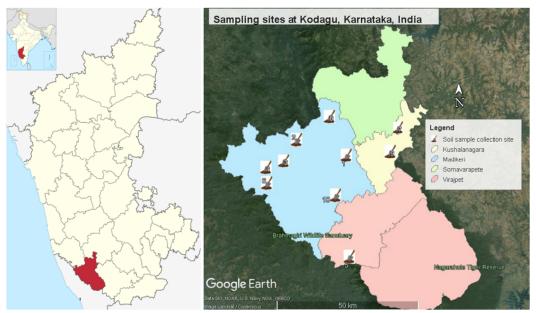


Fig. 1. Study and sampling sites at Kodagu, Karnataka, India.

Table 1. Geographic variables of selected forests

Forest types	Dominant plant species	Latitude	Longitude	Elevation (m)
Moist tropical wet-evergreen forests	Calamus nagabethai, Vateria indica, Artocarpus integrefolia, Bambusa bamboo	N 12.40770°	E 75.55123°	600–1500
Moist tropical Semi-evergreen forests	Dipterocarpus indicus, Hopea parviflora	N 12.106820°	E 75.763419°	500-1000
Moist deciduous forests	Dalbergia latifolia, Tectona grandis	N 12.437745°	E 75.896215°	600-1200
The Sholas/ Grass lands	Strobilanthes ciliatus	N 12.545399°	E 75.699206°	>1200

The temperature maintained during the study period from minimum 11 °C to 18 °C and the max varied from 24 °C to 32 °C in the forest. The recorded highest average rainfall in July was 73.4 centimeters and lowest average rainfall in December was 1.27 cm Additionally, the geographic factors and prominent flora details of the different types of forests from where sampling was done, are presented in Table 1. In total 10 soil samples were collected from the whole of the Coorg region, as described in [16]. Kodagu is a chunk of western ghats that has four different type of forest patches that carry different flora and fauna within it as shown in Table 1. To find out the chemical and biochemical relationships of the samples a quadrate method of sampling was performed. Sampling was done at a depth of 5 to 10 cm in two quadrates. The geographical features and other details such as soil sampling date, climatic condition, topography(elevation), and physical geography of the location are given in Table 2. Nearly 250 g soil samples were collected and clubbed together to obtain a single sample of soil from each quadrate. A portion of the collected sample was stored at -20 °C for analysis of microbial diversity and the rest of the soil samples were stored at 4 °C for physicochemical analysis.

A. Physicochemical Analysis

The soil pH was determined using a potentiometer at the soil to water ratio of 1:2.5 [17]. The electrical conductivity of soil suspension (soil: water, 1:2) was found using a conductivity meter. Deionized water was added to the samples and levels of water-soluble cations such as (Ca²⁺, Mg²⁺, K⁺) were estimated from the filtrate collected after 19 hours of shaking [18]. The other parameters, like Water Holding Capacity (WHC), Bulk Density (BD), Particle Density (PD) and porosity were analyzed by the method described by [19]. Total organic carbon was estimated using [20] method. Available and Total Nitrogen (AN and TN) was assessed using the specified methods given by [21] and Kjeldahl nitrogen by [22]. Available and total phosphorous were estimated by Olsen method [23] and the stannous chloride method given by [24]. Available sodium, potassium and the calcium concentrations were measured by flame photometry [22]. Soil is made up of solids and pore spaces in it, the weight of both solids and pore spaces is called bulk density, whereas the weight of only solid soil particles without pores is particle density. Porosity is a measure of how porous soil is, it relies on the percentage of sand present in the soil.

Table 2. Physical geography and other details of sampling sites

Sample No.	Name of the location	GPS coordinates	Date of sample collection	Climatic condition	Elevation (m)	Physical geography
1	Madikeri East RF	N 12.425912°	08 Jan 2023	Winter	1,134	Too Hilly
		E 75.753774°				
2	Sampaje RF	N 12.475196°	08 Jan 2023	Winter	133	Valley
		E 75.592670°				
3	Bhagamandala Forest	N 12.40770°	14 Jan 2023	Winter	1,091	Too Hilly
		E 75.55123°				
4	Talakaveri land slide area	N 12.386428°	14 Jan 2023	Winter	1,221	Hilly, land slide area
		E 75.493479°				
5	Mahakutta	N 12.106820°	15 Jan 2023	Winter	334	Valley
		E 75.763419°				
6	Nalknadu RF	N 12.337141°	14 Jan 2023	Winter	1,124	Too Hilly
		E 75.496599°				
7	Yadavanadu RF	N 12.513709°	22 Jan 2023	Winter	860	Slight hilly
		E 75.920841°				
8	Mandalpatti	N 12.548092°	26 Jan 2023	Summer	1,165	Too Hilly grassland
		E 75.699331°				
9	Anekadu RF	N 12.437745°	29 Jan 2023	Summer	886	Slight hilly
		E 75.896215°				
10	Vatekadu River bank	N 12.299352°	29 Jan 2023	Summer	871	River bank
		E 75.719078°				

B. Determination of Soil Enzymes

For the determination of the enzymes the crude enzyme portion was prepared by mixing the collected soil sample with the respective substrates and incubating, later measuring the absorbance with respect to the standard protocol.

The acid phosphatase and β -glucosidase activity were determined as given in [25] para nitro phenyl phosphate and para Nitro Phenyl-b-D-Glucopyranoside (pNPG) were used as substrate, the amount of para nitrophenol released during the incubation period of the sample with substrate at 37 °C for 1 hour was later measured at 400 nm.

Protease activity was measured using tyrosine and the quantity of amino-acids released after incubating the soil sample with casein for 2 h. at 50 °C was quantified as shown in [26] and the absorbance was recorded at 690 nano metres.

The dehydrogenase activity was found using [27], the reduction of 2, 3, 5-Triphenyltetrazolium Chloride (TTC) to triphenyl formazan was measured by incubating at 30 °C for 24 h and the absorbance was measured at 485 nm.

Fluorescein Diacetate (FDA) hydrolysis was found by the method prescribed by [28]. Finally, 2 gm of sample was combined with 15 mL of phosphate buffer (60 mM, pH 7.5) and the FDA solution (prepared in acetone), slowly heated at 30 °C, with continuous stirring. The reaction was halted after 25 min by adding chloroform and methanol solution in the ratio of (2:1). Later, the suspension was centrifuged and the supernatant's absorbance was taken at 500 nano meters. The activity of enzymes was measured in micrograms of the respective substrate per dry weight per duration of incubation.

C. Soil Basal Respiration

Soil basal respiration was measured using the alkali absorption method using soil sample (moist). 25 g of moist soil sample was incubated for 1 week at 25 °C in an air tight container with 5 mL Sodium hydroxide (0.1 M) in a flask inside it. After incubation, the Sodium hydroxide was titrated with 1 N Hydrochloric acid and later by the addition of 1 mL Barium chloride and two drops of phenolphthalein indicator in Sodium hydroxide. The soil basal respiration was calculated using the difference between the Hydrochloric acid

used during the titration of the above sample and the control. The final value was calculated by the amount of CO^2 evolved from microorganisms present in per gm of soil per hour (μg CO^2 g⁻¹ soil h⁻¹) as mentioned in [2].

D. Soil Microbial Biomass C, N and P

Soil Microbial Biomass Carbon (MBC), Nitrogen (MBN) and Phosphorous (MBP) were measured by the chloroform fumigation-extraction method described by [29] using K₂SO₄ (0.5 M) and NaHCO₃ (0.5 M) as extracting solutions in MBC, MBN and MBP, respectively. For MBC, the extract was filtered and the filtrate was analyzed by the potassium dichromate method given by [30]. Kjeldahl method was used for MBN and for MBP, the filtrate was processed using [23]. Finally, MBC, MBN, and MBP were determined using the differences between fumigated and non-fumigated samples with a conversion factor of 0.33, 0.54 and 0.40 for MBC, MBN and MBP, respectively [2].

IV. RESULT AND DISCUSSION

A. Physicochemical Characters of Soil Samples

The samples of soil showed enormous differences in their physicochemical characteristics (Fig. 2). The content of clay was highest in Bhagamandala forest followed by Sampaje RF (Round the Forest), Madikeri East RF and others; whereas the content of silt was highest in Anekadu RF followed by Madikeri East RF, Yadavanadu RF.

The content of sand was found highest in Vatekadu river bank followed by Talakaveri land slide area. The soil WHC (Water Holding Capacity) and the soil moisture depends on percentage of soil contents i.e., clay > silt > sand. The Higher the percentage of clay, the higher the WHC. The physical characteristics of soil samples such as bulk density, particle density, porosity, WHC, moisture was chalked out through different methods Table 3.

The soil Bulk density was greater in samples of Sampaje RF followed by Bhagamandala forest, and Madikeri East RF; Particle density varied from 2.68 ± 0.45 of Bhagamandala forest to 1.61 ± 0.06 of Vatekadu river side soil samples.

Porosity was greater in Vatekadu river side soil followed by Talakaveri land slide area. The WHC and moisture were greater in Madikeri East RF, Sampaje RF and Bhagamandala forest. The results of physicochemical properties of in Table 4 show that the electrical conductivity ranged from 0.58 to

 $0.67 \mu S$ and the pH varied from 6.3 to 7.2 with sample No. 2 as the highest and sample No. 4 as the least. The levels of available nutrients (N, P, and K) and organic matter were higher in the samples 1, 2 and 3 and the rest of the samples had average levels except sample No. 4 and sample No. 10.

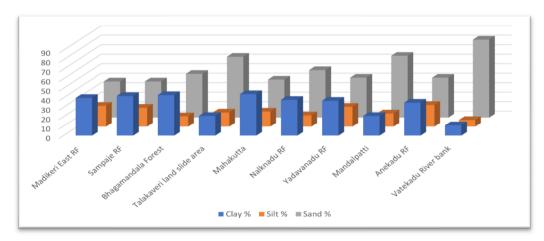


Fig. 2. Percentage of soil contents.

Table 3. Physical characteristics of soil samples

Sample No.	Location	Bulk density (g/cm ³)	Particle density	Porosity (%)	WHC (%)	Moisture (%)
1	Madikeri East RF	1.52 ± 0.14	2.61 ± 0.34	41.76 ± 1.66	50.11 ± 0.76	18.08 ± 0.02
2	Sampaje RF	1.56 ± 0.25	2.63 ± 0.08	40.68 ± 2.54	49.56 ± 0.55	17.85 ± 0.07
3	Bhagamandala Forest	1.54 ± 0.21	2.68 ± 0.45	42.54 ± 2.12	49.68 ± 0.63	17.97 ± 0.04
4	Talakaveri land slide area	0.78 ± 0.34	1.87 ± 0.22	58.29 ± 1.75	42.15 ± 0.14	15.86 ± 0.02
5	Mahakutta	1.37 ± 0.26	2.23 ± 0.47	38.57 ± 2.33	47.24 ± 0.86	18.12 ± 0.04
6	Nalknadu RF	1.41 ± 0.46	2.46 ± 0.08	42.68 ± 3.24	48.51 ± 0.66	17.24 ± 0.05
7	Yadavanadu RF	1.48 ± 0.27	2.54 ± 0.24	41.73 ± 2.64	49.45 ± 0.35	17.66 ± 0.12
8	Mandalpatti	0.99 ± 0.48	2.12 ± 0.32	53.30 ± 4.95	43.12 ± 0.64	17.01 ± 0.08
9	Anekadu RF	1.51 ± 0.23	2.62 ± 0.51	42.37 ± 1.54	48.12 ± 0.42	$17.84 {\pm}~0.18$
10	Vatekadu River bank	0.62 ± 0.32	1.61 ± 0.06	61.49 ± 1.66	41.24 ± 0.32	14.89 ± 0.05

Table 4. Physicochemical properties of soil samples collected from the Western of Coorg region

Sample No.	GPS location	EC (µS)	pН	Organic matter	AN (kg/ha)	AP (kg/ha)	AK (kg/ha)
1	N 12.425912°	0.66 ± 0.01	7.1 ± 0.06	0.86 ± 0.01	490.55 ± 1.25	20 ± 0.06	300.05 ± 2.0
	E 75.753774°						
2	N 12.475196°	0.67 ± 0.02	7.2 ± 0.04	0.88 ± 0.01	485.12 ± 1.59	25 ± 0.06	289.60 ± 1.7
	E 75.592670°						
3	N 12.40770°	0.65 ± 0.03	6.7 ± 0.04	0.86 ± 0.01	486.23 ± 0.64	26 ± 0.11	285.45 ± 0.66
	E 75.55123°						
4	N 12.386428°	0.58 ± 0.01	6.3 ± 0.06	0.57 ± 0.01	211.15 ± 0.65	8 ± 0.06	85.12 ± 0.66
	E 75.493479°						
5	N 12.106820°	0.62 ± 0.02	7.2 ± 0.06	0.89 ± 0.01	460.32 ± 0.52	25 ± 1.11	260.22 ± 0.66
	E 75.763419°						
6	N 12.337141°	0.61 ± 0.01	6.6 ± 0.06	0.86 ± 0.01	456.87 ± 1.42	27 ± 1.55	261.33 ± 1.11
	E 75.496599°						
7	N 12.513709°	0.65 ± 0.01	7.1 ± 0.11	0.80 ± 0.02	460.92 ± 1.88	23 ± 1.33	201.66 ± 1.11
	E 75.920841°						
8	N 12.548092°	0.62 ± 0.01	6.4 ± 0.04	0.81 ± 0.01	457.22 ± 2.18	24 ± 1.11	283.16 ± 1.55
	E 75.699331°						
9	N 12.437745°	0.63 ± 0.01	7.0 ± 0.06	0.80 ± 0.03	460.10 ± 2.44	20 ± 2.0	301.33 ± 2.22
	E 75.896215°						
10	N 12.299352°	0.60 ± 0.03	6.8 ± 0.06	0.75 ± 0.04	380.50 ± 1.77	19 ± 1.13	210.10 ± 1.11
	E 75.719078°						

EC (Electrical Conductivity); AN (Available Nitrogen); AP (Available Phosphorus); AK (Available Potassium). The values represent the average of 03 biological replicas of the collected samples. The values recorded are the mean and standard deviation.

B. Activity of Soil Enzymes

Enzymes like acid phosphatase, β -glucosidase, protease, dehydrogenase and fluorescein diacetate are the reason for the nutrient release in the forest soil. Acid phosphatase levels

varied from (800 μg g^{-1} h^{-1} to 2650 μg g^{-1} $h^{-1}),$ β -glucosidase (80 μg μg g^{-1} h^{-1} to 500 μg g^{-1} $h^{-1}),$ protease (1400 μg g^{-1} h^{-1} to 8400 μg g^{-1} h^{-1}), dehydrogenase (30 μg g^{-1} h^{-1} to 510 μg g^{-1} h^{-1}) and fluorescein diacetate (25 μg g^{-1} h^{-1} to 510 μg g^{-1}

h⁻¹). Fig. 3 shows the variance of each enzyme with respect to all the samples. In all the samples the activity of enzymes was highest in sample No. 3 and activity of enzymes was lowest in sample No.10.

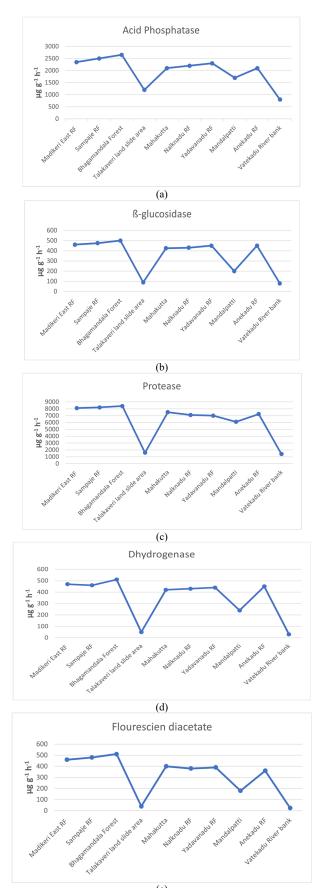


Fig. 3. Enzyme activity of the samples collected (a) Acid phosphatase, (b) β-glucosidase, (c) protease, (d) dehydrogenase, (e) fluorescein diacetate.

C. Microbial Soil Biomass C, N and P in Soil Samples

The microbial soil biomass of all the forest soil samples is summarized in Fig. 4. MBC varied from 120.47 µg g⁻¹ to 356.66 μg g⁻¹, MBN ranged from 28.63 μg g⁻¹ to 73.41 μg g⁻¹ whereas MBP varied from 18.44 μg g⁻¹ to 47.66 μg g⁻¹. In all the cases Sample Nos. 1, 2, 3 showed highest microbial soil biomass and sample No. 4 showed the least which was a land slide area.

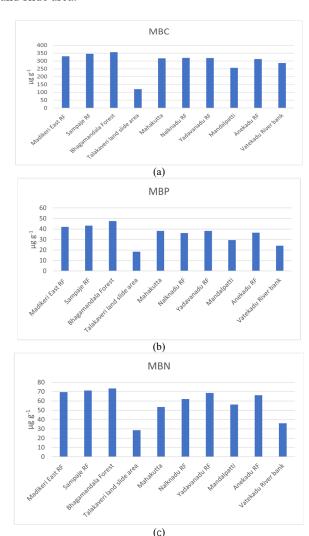


Fig. 4. Microbial biomass of different soil samples (a) carbon, (b) phosphorous, (c). nitrogen.

D. Soil Basal Respiration

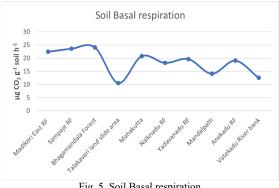


Fig. 5. Soil Basal respiration.

The results of soil basal respiration also matched with other tests showing the sample No. 3 (Bhagamandala forest) as highest with 24.12 μg CO₂ g^{-1} soil h^{-1} followed by samples no. 2 and 1. The least soil basal respiration was recorded in sample No. 4, i.e., 10.54 μg CO₂ g^{-1} soil h^{-1} in Talakaveri land slide area. Fig. 5 represents the graph of soil basal respiration of the samples collected.

E. Effect of Elevation on Soil Biota

In this study we found out that there is an effect of elevation on the soil biota. Fig. 6 shows that as we go to the higher elevation there is a drop in temperature, increase in precipitation, decrease in the abundance of organisms in the soil and the vice-versa at the lower elevation. The soil samples of lower elevation were rich in microbial biomass and contained more carbon and nitrogen, this is in acceptance with the previous studies conducted on the effect of elevation on soil biota [31].

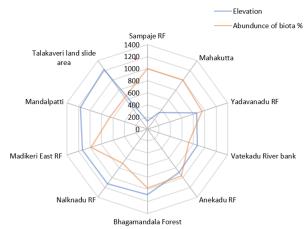


Fig. 6. Effect of Elevation on soil biota.

There were also some exceptional species which did not follow this pattern. However, furthers experiments and research are required to know more about the influence of elevation on different species.

The western ghats of Coorg are popular for their richness of biodiversity and species variety. Many studies have been performed on western ghats but the studies mainly focusing the soil biota of the Coorg region are less known. The zenith of this report highlights that, this is the first case study to chalk out the presence of a variety of soil enzymes, soil microbial biomass, and soil basal respiration in the soil samples of the Western Ghats of Coorg, India.

Among all the tests for physical characteristics of soil samples such as percentage of clay content, Bulk density, particle density, porosity, WHC, moisture etc., the soil samples from Madikeri East RF, Sampaje RF, Bhagamandala forest were in the top position showing, a good homely environment for micro-organisms which symbiotically enriched the forest growth, this is in accordance with the other studies conducted in western ghats [32]. The presence of thick roots on the upper segment of soil and greater organic carbon content result in higher WHC among the forest soil samples [33]. The pH of the entire soil samples was near neutral in nature.

The examined forest soil was good for the bacterial growth when compared to fungal growth. The pH of the soil has much control over the nutrient availability of plant. Soil microbes represents the live portion of organic matter which is responsible for converting complex form of nutrients into

available form of nutrients [34]. Highest enzymatic activity was found in Bhagamandala forest, Sampaje RF and Madikeri East RF which shows the higher metabolic rates of soil microorganisms, higher availability of nutrient in these forests. The enzymatic activity such as \(\beta\)-glucosidase, acid phosphatase, protease, dehydrogenase and fluorescein diacetate all were higher in first three samples and least in the sample of landslide affected area. The higher enzyme activity in these soil samples resulted in a good lignocellulolytic activity and a faster decomposition rate of which in turn made the soil more fertile and humus due to covered canopy along with dampness which overall helped the microbial activity to grow at faster rate [7]. Soil microbial biomass MBC, MBN, MBP are the important parameters that affects the metabolism and functionality of soil.

The soil with microbial biomass, i.e., MBC, MBN and MBP was highly found in Bhagamandala forest, Sampaje RF and Madikeri East RF. The soil organic matter is responsible for its physico-chemical characteristics and provides favourable conditions for the growth of forests. Microbes form an important part of the forest soil ecosystem as they are directly involved in decomposition of organic matter in the soil. Analysing the previous research works and the results obtained in the present work, there is a lot of scope and need of research in this field to know in-depth about the interaction and role of the soil biota with the forests. The western ghats of Coorg region is an important biodiversity hotspots and there is a lot to study and understand about it.

V. CONCLUSION

This research study conducted was a first of its kind in the western ghats of Coorg region. This research, clarifies that the biochemical as well as microbial properties of soil are all interrelated to the growth of forests in that particular landscape. Any change or introduction of new foreign nonnative species will affect the entire ecosystem of that forest and will also affect the biological properties as well as the physiochemical properties of the soil eco system. Due to the variation in climate and complexity of the soil properties it is hard to calculate the parameters and microbial activities precisely. This study gives highlights on the growth pattern of the forests of western ghats of Coorg. This study also provides a continuation and substantial evidence to the upcoming research studies, through this study some of the understandings can be included in the academics as well as in forest cultural operations which will be helpful for the forest department too. This research also throughs light upon the details of poor soil quality of some land slide prone areas which will be very useful in saving the lives of human beings as well as the fauna The overall results also highlighted the poor soil quality and lesser WHC at the land slide sites providing more evidence that the land slide occurred at Talakaveri in Kodagu during August 2020.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

MUK conducted the research; MS analyzed the data; KR guided in the whole research; MUK wrote the paper; KR and

MS both did proof reading; all authors had approved the final version.

ACKNOWLEDGMENT

Authors are grateful to the enormous guidance and support received from the College Principal, Head of Chemistry department and members of the Doctoral committee.

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