Assessment of Soil Microbial Communities in Relation to Seasonal Variation in Darjeeling Tea Plantations

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ABSTRACT

In order for ecosystems to operate, nutrient cycling, organic matter breakdown, soil fertility, and microbial populations in soil are essential. Soil Bacillus spp. and Actinomycetes abundance in the Darjeeling region of West Bengal was examined in this research, along with the effects of seasonal and meteorological factors. This study looked at soil samples taken before, during, and after the monsoon season to determine factors such organic carbon, pH, moisture, and accessible nutrients. Using conventional culture-based microbiological techniques, the abundance of Bacillus spp. and Actinomycetes was measured and expressed as CFU/g of soil. The results showed that the number of microbes varied significantly throughout the year. The concentrations of Bacillus spp. rose from 4.82×10^5 CFU/g before the monsoon to 6.35×10^5 CFU/g during the monsoon and 7.28×10^5 CFU/g after the monsoon. Likewise, over each season, the concentration of Actinomycetes rose from 3.76×10^5 to 5.48×10^5 and 6.21×10^5 CFU/g. The overall microbial abundance was 13.49×10^5 CFU/g, which was reported as the highest after the monsoon. There were positive correlations between the two microbial groups and soil organic carbon and moisture, and negative correlations with soil temperature.

Keywords: *Seasonal Variation, Soil Microbial, Actinomycetes, Monsoon.*

I. INTRODUCTION

In order to make tea, young leaves, buds, or thin twigs of the Camellia sinensis L. plant are steeped in water that is either hot or boiling. This process results in the production of a beverage known as tea. Tea, a beverage that is aromatic and flavourful, is the product of this procedure, which results in the beverage. Tea is thot to have originated in the verdant parts of China, and its history may be traced back thousands of years. The area that is considered to be the "motherland" of tea, which is situated in the latitudinal zone that extends from 45 degrees north to 34 degrees south, has extended to include 48 nations all over the globe. As a result of this, tea is an important beverage that is steeped in history and community, and it has grown into a vital component of the cultural activities that are visible across the world in a vast number of nations. When it comes to doing research on community rituals that center around the drinking of tea, there is a wealth of material that can be employed for the goal of conducting research. This material can be derived from both the human experience and botanical studies.

Specifically, the acidophilic perennial cash crop known as tea (Camellia sinensis L.) grows best on soils with a pH that ranges between 4.5 and 5.5 respectively. This indicates that the soil is ideal for the plant's growth. This species thrives in environments that are characterised by high levels of warmth and humidity, which are characteristic of tropical and subtropical regions. During the growing season, the ideal temperature range for growth is between 10 and 30 degrees Celsius, with an annual average temperature of around 20 degrees Celsius being consistent throughout the growing season. It is customary for the places that are most often utilised for tea production to have weather conditions that are ideal for the growth of tea. Be aware that temperatures below 10 degrees Celsius may be harmful to tea plants, and that temperatures over 30 degrees Celsius can hinder growth and have a negative impact on the quality of the leaves. Both of these factors should be taken into mind. The many taste profiles that tea enthusiasts like including into their drinks have the potential to undergo major transformations when subjected to temperature fluctuations. For this reason, it is very necessary to have a grasp of the environmental needs of tea production in order to maximise output while preserving the quality of the product.

The Food and Agriculture Organization (FAO) estimates that around 5,245,319 hectares of land are used for the cultivation of tea all over the globe. This results in the production of more than 28,191,556.18 metric tonnes of tea leaves during the course of the year. It is estimated that around 6,257,428 metric tonnes of tea will have been processed by the year 2021, which is equivalent to this amount. India is the country that is at the top of the list of countries that export tea, placing it in second place in terms of both production and consumption of tea.

This is in spite of the fact that India only accounts for 10.44% of the total land area that is considered to be suitable for tea production. More than 660 million United States dollars are contributed to India's economy each year by the tea sector, making it a substantial contributor to the country's economy. On the other side, climate change is becoming an increasingly major problem for tea cultivation. This is because climate change has the ability to impact production levels trough changes in temperature and rainfall patterns, in addition to other severe weather events.

Certain species, including as algae, bacteria, fungus, and protozoa, play an important role in the ecology of the tea soil. These creatures are essential to the tea tree. In places where tea is grown, the rhizosphere is a place where all of these creatures thrive. The physically, chemically, and physiologically relevant features of the soil are all influenced by these bacteria in a variety of ways, including direct and indirect effects by these bacteria. When it comes to the activities that take place in the soil, they play a vital role in the decomposition of organic matter, which ultimately leads to the release of nutrients that are necessary for the health of plants.

Furthermore, soil bacteria play a vital role in the recycling of nutrients, which is advantageous to the general growth and development of plants. This is important because it helps plants grow and develop. They do this by strengthening the structure of the soil via the production of polysaccharides and other compounds, which in turn contributes to the stability of the soil and the retention of water. At the same time as they either outcompete or kill harmful pathogens, some soil bacteria contribute to the prevention of plant illnesses. Microbes in the soil provide an extra advantage in this regard.

II. REVIEW OF LITERATURE

Sarkar, Sudip et al., (2024). Seasonal fluctuations impact the biochemical and microbiological properties of soil, which in turn impact the soil's carbon and nutrient cycle. Because of their high microbial population and organic content, soils that undergo tree planting (TP) are more susceptible to seasonal change. We conducted research in the middle Gangetic region on thirteen-year-old TPs based on Kadamb (Anthocephalus cadamba Miq.), Simaraubha (Simarouba glauca DC), and Litchi (Litchi chinensis Sonn.) to determine the effects of microclimate (temperature and moisture) and seasonal changes in soil chemical properties (pH, electrical conductivity, total organic carbon, total nitrogen, C/N ratio, etc.) on microbial respiration (SR), biomass, and carbon utilisation efficiency. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) are higher in the summer, fall, and spring than in winter, despite higher SR and metabolic quotient (qCO_2), and this is true independent of TPs. Microorganisms respire using carbon from soil, which is strongly confirmed by the positive link between qCO_2 and C/N ratios. The following soil parameters were positively linked with qCO_2: soil moisture (MC), electrical conductivity (EC), temperature (T), and pH (-). Increased carbon and nitrogen (N) accumulation into microbial biomass was shown to be related with litchi-based TPs. Simarrouba is indicated by greater MBN/TN and MBC/TOC ratios.

Chengliang, Wang et al., (2023). To forecast how soil organic matter dynamics and nutrient cycling may react to climate change, it is essential to understand the processes behind the seasonal variations in soil microbial community structure and enzyme activity, as well as the geographic heterogeneity within these communities. Across three seasons (summer, autumn, and spring), we sampled 75 points across a 15 15 m area of a Leymus chinensis meadow steppe in northeastern China. We measured changes in plant biomass, soil microclimate, soil nutrients, and enzyme activity, as well as the spatial variability in these variables. Soil microbial community structure and enzyme activity varied spatially throughout the year, and the plant and soil factors linked to this variation changed as the seasons did. Summer showed a highly spatially dependent microbial community structure, with a relative spatial variance [C / (C + C0) 0.9], in contrast to the more homogenous spring and autumn. The most important factors influencing the regional variance in the organization of microbial communities throughout summer were plant patterns, soil dissolved inorganic nitrogen, and dissolved organic carbon. The regional variation in microbial community structure in autumn was explained by a combination of plant and soil physical explanatory factors, which accounted for 22–46% of the total.

Jafarian, Nahid et al., (2023). Climatic attributes are among the most significant determinants affecting soil qualities. Alterations in climatic attributes may be identified at many scales, including localised modifications in topographical elements (i.e., slope orientation, referred to as micro-climate). Nevertheless, the impact of micro-climatic conditions on soil characteristics, particularly enzyme activity influencing nutrient cycles, along climatic gradients has seldom been examined in dry and semi-arid forest environments. Soil sampling was conducted using 60 composite samples over three locations exhibiting varying climatic conditions: slightly-arid, moderately-arid, and semi-arid, on two slope sides (north and south) inside oak woods in western Iran. The findings indicated that the activity of urease and phosphatase enzymes exhibited considerable variation in relation to climatic conditions, displaying humped-shaped patterns along the climate gradient. Soil respiration (R), substrate induced respiration (SIR), and microbial biomass carbon (MBC) concurrently demonstrated the impact of climatic conditions and slope orientation ($p < 0.05$).

Chen, Yu-Pei et al., (2021). The quality and vitality of the tea estates' soil are contingent upon agricultural management techniques, with prolonged use of chemical fertilisers contributing to soil degradation. Consequently, several sustainable methods are used to enhance and preserve soil quality. This research examined alterations in soil characteristics, enzymatic function, and dysbiosis within bacterial community structure by comparing three agricultural management strategies: conventional agriculture (CA), sustainable agriculture (SA), and transformational agriculture (TA) in a tea plantation during the years 2016 and 2017. Soil specimens were gathered and examined at two-month intervals. The findings of the enzyme activities indicated that the activities of acid phosphatase, arylsulfatase, β-glucosidase, and urease varied significantly across the soils associated with the three management techniques. The alteration in arylsulfatase activity was elucidated by soil pH as a crucial predictor in the SA soils, integrating redundancy and multiple regression analysis.

Fu, Denggao et al., (2020). One important measure of the effects of land restoration on soil quality and ecosystem development is the diversity of soil microbes. Three kinds of restored vegetation were studied using phospholipid fatty acid analysis (PLFA): shrubland, natural secondary forest, and pine and eucalyptus plantation forests. The impacts of land restoration models and seasons (dry and wet) were examined in these communities of soil microbes. The results of the two-way ANOVA and PERMANOVA tests demonstrated that the levels of PLFA biomarkers were substantially impacted by the kind of land restoration and the season. Noticeably higher concentrations of all PLFA groups were found in natural secondary forests compared to shrubland and plantation forests, suggesting that plant species-specific variations in litter quality and quantity, root exudates, and soil properties influence the composition of the soil microbial community. The biomasses of gram-positive bacteria, actinomycetes, fungi, and other microbes in SL and NSF were much higher during the dry season when contrasted with the rainy season.

Redundancy analysis showed that soil organic carbon and soil moisture had a significant influence when investigating how soil microbial communities responded to seasonal fluctuations and different forms of land restoration at this site. Our findings highlight the need to account for the potential diversity in soil microbial responses to time and vegetation when assessing ecosystem services and functions following different types of land restoration.

Songsong, Gu et al., (2019). Soil microbiome relationships are formed by plant varieties. Plant growth, plant health, and harvest success are all areas where the soil microbiome may play a pivotal role. Soil microbial community richness and structure as they pertain to different tea (Camellia sinensis) cultivars remains mostly unknown. Five distinct tea cultivars were cultivated on a tea farm in central southern China for fifteen years. In order to study the microbial population and their potential roles in these plants' bulk soils, we employed Illumina sequencing technology. The Pearson association analysis revealed that soil pH and TOC concentrations had a positive effect on the microbial diversity of many tea cultivars. The Functional Annotation of Prokaryotic Taxa revealed that in comparison to other cultivars, Bixiangzao soil samples included significantly higher percentages of functional bacteria engaged in aerobic ammonia oxidation, nitrification, and fermentation. Based on our research, cultivar Bixiangzao has the potential to thrive in microecological settings and sustain a diverse range of bacteria, making it an ideal candidate for use in tea plantations. Research on the rhizosphere effect, which influences different tea cultivars and controls the soil community in tea plantations, might possibly be based on this work.

Wallenstein, Matthew et al., (2009). Soil organic matter is plentiful in Arctic soils due to the sluggish decomposition of debris. Soil microorganisms produce extracellular enzymes that initiate the breakdown process. We hypothesised that enzymatic activity is minimal at in situ temperatures and that potential enzyme activities are diminished in Arctic tundra soils relative to the extensive reserves of organic matter. At Toolik Lake, Alaska, we collected samples of tussock, interduces, shrub organic, and shrub mineral soils on four occasions within a temperature range of 4 to 20 degrees Celsius to assess the potential activity of six hydrolytic enzymes. Bacteria may have produced enzymes during periods of frozen soil, since the activity levels of N-acetyl glycosaminidase, beta-glucosidase, and peptidase peaked near the winter's conclusion. Notwithstanding elevated temperatures that typically enhance enzyme activity in situ, enzyme activities remained unchanged over the Arctic summer, suggesting that enzyme synthesis is constrained by nitrogen availability at this period. Soil enzymes exhibited seasonal variations in their thermal sensitivity (Q (10)). Temperature exerted a more significant influence on soil enzyme reservoirs during late winter than in summer. Utilising enzyme activity assessments, temperature responsiveness, and daily soil temperature records, we approximated potential in situ beta-glucosidase activities for shrub organic soils and tussock soils. Enzymatic activity in tussock soils, as anticipated, reached its zenith in the spring and then declined over the summer.

III. MATERIALS AND METHODS

Study Area

The research was conducted in specific soil settings of the Darjeeling region in West Bengal. The area experiences a moist mountainous climate, significant monsoonal precipitation, and notable differences in altitude and flora. Soil specimens were selected from typical sites linked to tea cultivation, forest flora, and several other vegetated terrains.

Seasonal Sampling

Sampling was organized according to three major seasonal periods:

- **Pre-Monsoon:** March–May
- **Monsoon:** June–September
- **Post-Monsoon:** October–December

Soil was gathered from the top stratum at each test site following the elimination of surface debris. Numerous subsamples were gathered from each sampling location and meticulously blended to produce representative composite samples. Specimens were secured in sterile receptacles and conveyed to the laboratory under appropriate circumstances for microbiological analysis.

Estimation of *Bacillus* spp.

Soil suspensions were formulated utilising sterile physiological saline and subjected to repeated dilution. Proper dilutions were introduced to appropriate nutritional medium and incubated under conventional laboratory conditions. Colonies exhibiting traits aligned with Bacillus spp. were counted.

Representative colonies underwent Gram staining, spore staining, and conventional biochemical analysis. Microbial density was quantified as colony-forming units per gram of desiccated soil (CFU/g).

Estimation of Actinomycetes

Actinomycetes were quantified via selected culture medium appropriate for their extraction from soil. Soil suspensions that were serially diluted were introduced to the chosen medium and allowed to incubate for a suitable duration.

Colonies exhibiting distinctive dry, chalky, or filamentous appearance were enumerated and represented as CFU/g of soil. Representative isolates were analysed microscopically and underwent fundamental morphological and biochemical assessment.

Measurement of Climatic and Soil Variables

Soil temperature was measured throughout the sample process, whilst soil moisture was assessed using the gravimetric technique. Soil pH was assessed using a calibrated pH meter following the preparation of a soil-water mixture. Seasonal precipitation and temperature data were analysed to define the climatic conditions during the sample intervals.

IV. RESULTS AND DISCUSSION

Table 1. Seasonal Abundance of *Bacillus* spp. and Actinomycetes in Darjeeling Soil

Season	Mean Soil Temperature (°C)	Mean Soil Moisture (%)	*Bacillus* spp. ($\times 10^5$ CFU/g)	Actinomycetes ($\times 10^5$ CFU/g)
Pre-monsoon	19.8	28.4	4.82 ± 0.46	3.76 ± 0.41
Monsoon	17.6	48.7	6.35 ± 0.58	5.48 ± 0.52
Post-monsoon	15.9	39.5	7.28 ± 0.63	6.21 ± 0.57

There are substantial seasonal shifts in the predominance of Bacillus species and Actinomycetes within the soil of Darjeeling, as shown in Table 1. The soil temperature during the pre-monsoon period was measured to be 19.8 degrees Celsius, and the moisture content was found to be 28.4%. The recorded concentrations of Bacillus species were $4.82 \pm 0.46 \times 10^5$ CFU/g, while the concentrations of Actinomycetes were $3.76 \pm 0.41 \times 10^5$ CFU/g. Both Bacillus spp. ($6.35 \pm 0.58 \times 10^5$ CFU/g) and Actinomycetes ($5.48 \pm 0.52 \times 10^5$ CFU/g) were found to have increased populations during the monsoon season. The higher humidity of 48.7% and the lower temperature of 17.6°C led to these increased populations. During the post-monsoon period, the highest microbial density was observed. This time was marked by a temperature of 15.9°C, a moisture content of 39.5%, Bacillus species at $7.28 \pm 0.63 \times 10^5$ CFU/g, and Actinomycetes at $6.21 \pm 0.57 \times 10^5$ CFU/g.

Table 2. Seasonal Variation in Selected Soil Physicochemical Characteristics

Season	Soil pH	Organic Carbon (%)	Available Nitrogen (kg/ha)	Available Phosphorus (kg/ha)	Available Potassium (kg/ha)
Pre-monsoon	5.18 ± 0.21	2.14 ± 0.18	286 ± 22	18.6 ± 2.4	164 ± 15
Monsoon	5.32 ± 0.19	2.31 ± 0.21	301 ± 25	20.8 ± 2.7	171 ± 16
Post-monsoon	5.41 ± 0.17	2.48 ± 0.23	318 ± 27	22.4 ± 2.8	178 ± 17

In Darjeeling, the physicochemical qualities of the soil have been shown to gradually improve throughout the course of the calendar year, as seen in Table 2. In the pre-monsoon period, the pH of the soil was 5.18 ± 0.21. During the monsoon, the pH of the soil increased to 5.32 ± 0.19. After the monsoon, the pH of the soil returned to 5.41 ± 0.17. The percentage of organic carbon increased from 2.14 ± 0.18% to 2.31 ± 0.21%, and then it reached 2.48 ± 0.23% after that. The amount of nitrogen that was accessible increased from 286 ± 22 kg/ha to 301 ± 25 kg/ha and then to 318 ± 27 kg/ha. On the other hand, the amount of phosphorus that was available increased from 18.6 ± 2.4 kg/ha to 20.8 ± 2.7 kg/ha and then to 22.4 ± 2.8 kg/ha. In a similar fashion, the amount of potassium that was available to the plant increased from 164 ± 15 kg/ha during the pre-monsoon phase to 171 ± 16 kg/ha during the monsoon period, and then it increased even more to 178 ± 17 kg/ha during the post-monsoon stage.

Table 3. Correlation Between Environmental Variables and Soil Microorganisms

Environmental Variable	*Bacillus* spp.	Actinomycetes
Soil temperature	−0.42	−0.36
Soil moisture	0.76	0.72
Soil pH	0.48	0.51
Organic carbon	0.81	0.78
Available nitrogen	0.67	0.64
Available phosphorus	0.59	0.57

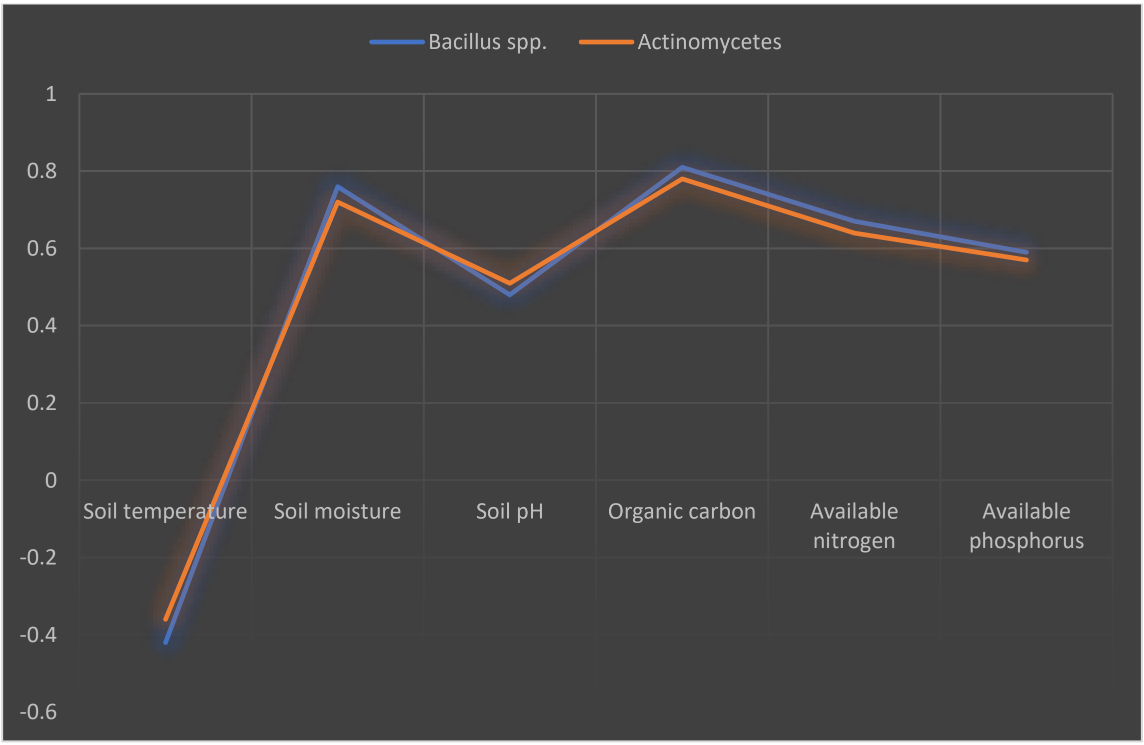

Figure 1. Correlation between Environmental Variables and Soil Microorganisms

Table 3 demonstrates that there were significant positive connections between the two groups of microorganisms and the soil moisture and organic carbon. Bacillus species and actinomycetes were shown to have correlations of 0.76 and 0.72, respectively, with soil moisture. On the other hand, organic carbon was found to have the largest positive correlations, with 0.81 and 0.78, respectively. Additionally, available nitrogen had positive correlations of 0.67 and 0.64, with available phosphorus following closely behind with correlations of 0.59 and 0.57. Bacillus species (0.48) and actinomycetes (0.51) were shown to have somewhat favourable correlations with different soil pH levels. While Bacillus species had a negative correlation of -0.42 with soil temperature, Actinomycetes exhibited a negative correlation of -0.36 with soil temperature.

Table 4. Comparative Seasonal Change in Microbial Abundance

Season	*Bacillus* spp. ($\times 10^5$ CFU/g)	Actinomycetes ($\times 10^5$ CFU/g)	Combined Microbial Abundance ($\times 10^5$ CFU/g)
Pre-monsoon	4.82	3.76	8.58
Monsoon	6.35	5.48	11.83
Post-monsoon	7.28	6.21	13.49
Seasonal mean	6.15	5.15	11.30

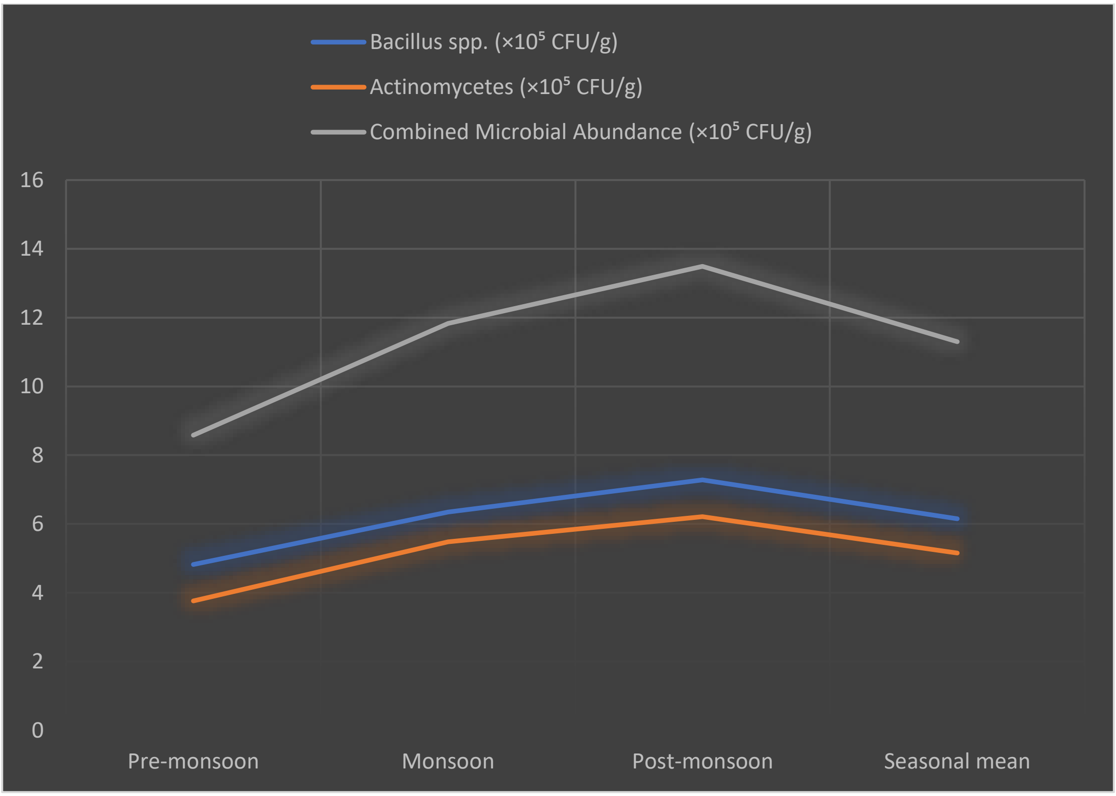

Figure 2. Comparative Seasonal Change in Microbial Abundance

There is a discernible rise in the number of microorganisms during the seasons, as seen in Table 4. The pre-monsoon season witnessed the recording of Bacillus spp. and Actinomycetes, with the former recording 4.82×10^5 CFU/g and the latter recording 3.76×10^5 CFU/g. The total abundance of Bacillus and Actinomycetes was 8.58×10^5 CFU/g. Both of these numbers grew to 6.35 x 10^5 CFU/g and 5.48×10^5 CFU/g during the monsoon season, resulting in a combined abundance of 11.83×10^5 CFU/g after the monsoon season. In the post-monsoon period, the largest abundance of Bacillus species was seen, with a recorded value of 7.28×10^5 CFU/g. Actinomycetes were found to have a value of 6.21×10^5 CFU/g, while the overall microbial abundance was recorded at 13.49×10^5 CFU/g. During the post-monsoon period, the total seasonal mean reached 6.15×10^5 CFU/g for Bacillus spp., 5.15×10^5 CFU/g for Actinomycetes, and 11.30×10^5 CFU/g for combined microbial abundance. These results indicate that the circumstances for microorganisms were good.

V. CONCLUSION

The abundance of *Bacillus* spp. and Actinomycetes varied across the pre-monsoon, monsoon, and post-monsoon seasons, with the highest microbial abundance recorded during the post-monsoon period. This seasonal pattern was associated with variations in soil moisture, temperature, pH, organic carbon, and available nutrients. Soil moisture and organic carbon showed strong positive relationships with both microbial groups, while soil temperature showed a negative relationship. These findings indicate that favourable moisture conditions, nutrient availability, and suitable soil physicochemical characteristics promote microbial proliferation and biological activity. The study highlights the important role of *Bacillus* spp. and Actinomycetes in maintaining soil fertility, nutrient cycling, and ecological functioning in the Darjeeling landscape.

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