American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Rhizosphere Microbial Population Growth, Nodulation and Yield in Camellia Sinensis under Different Climatic Conditions

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Received: 2025, 15, Aug
Accepted: 2025, 21, Sep
Published: 2025, 25, Oct

Annotation: Tea (Camellia sinensis L.) is a globally significant commercial crop whose productivity is intricately linked to rhizosphere microbial communities. This study investigated the relationship between rhizosphere microbial populations, nodulation patterns, and tea yield under varying climatic conditions across three distinct agro-ecological zones. A comprehensive 24-month field study was conducted in tropical, subtropical, and temperate regions, examining bacterial, fungal, and actinomycetes populations alongside plant growth parameters. Results demonstrated that subtropical conditions (20-25°C, 1800-2200mm rainfall) supported optimal microbial diversity (8.7×10^7 CFU/g soil for bacteria) and highest tea yield (2,450 kg/ha). Temperature and moisture significantly influenced microbial population dynamics ($p<0.01$), with bacterial populations showing 45% higher density in moderate moisture conditions compared to extreme wet or dry periods. Correlation analysis revealed strong positive relationships between total microbial count and fresh leaf yield ($r=0.84$, $p<0.001$). These findings provide crucial insights for sustainable tea cultivation practices under changing climate scenarios.

Keywords: Camellia sinensis, rhizosphere microbiome, climatic factors, microbial diversity, tea yield, nodulation.

1. Introduction

Tea (*Camellia sinensis* L.) represents one of the most economically important beverage crops globally, with production spanning over 60 countries and affecting millions of livelihoods (Hazarika et al., 2019). The rhizosphere, defined as the narrow zone of soil directly influenced by root secretions and associated soil microorganisms, plays a fundamental role in plant health, nutrient acquisition, and productivity (Mendes et al., 2013). The complex interactions between tea roots and rhizosphere microorganisms significantly influence nutrient cycling, disease suppression, and overall plant vigor (Sharma et al., 2020).

Climate change has emerged as a critical factor affecting agricultural productivity worldwide, with temperature fluctuations, altered precipitation patterns, and extreme weather events directly impacting soil microbial communities (Singh et al., 2019). Understanding how climatic variations influence rhizosphere microbial populations in tea plantations is essential for developing climate-resilient cultivation strategies. While *Camellia sinensis* does not naturally form nitrogen-fixing nodules like leguminous plants, the term "nodulation" in this context refers to the formation of beneficial microbial aggregates and mycorrhizal associations in the root zone that enhance nutrient availability (Kumar et al., 2018).

Previous studies have documented the importance of specific microbial groups in tea cultivation, including phosphate-solubilizing bacteria, nitrogen-fixing bacteria, and mycorrhizal fungi (Chen et al., 2017). However, comprehensive investigations examining the interplay between diverse climatic conditions, rhizosphere microbial dynamics, and tea productivity remain limited. This study addresses this knowledge gap by systematically evaluating microbial population growth, beneficial microbial associations, and yield parameters across three distinct climatic zones over a 24-month period.

2. Materials and Methods

2.1 Study Sites and Experimental Design

The research was conducted at three locations representing distinct climatic zones: (i) Tropical zone - Assam, India (26°N, 94°E; elevation 95m), (ii) Subtropical zone - Darjeeling, India (27°N, 88°E; elevation 2,134m), and (iii) Temperate zone - Kangra, India (32°N, 76°E; elevation 1,250m). Each site comprised a randomized complete block design with four replications across 2 hectares of established tea plantations (15-year-old plants, variety TV-1).

2.2 Climatic Data Collection

Meteorological parameters including temperature (min/max), rainfall, relative humidity, and solar radiation were recorded using automated weather stations installed at each site. Data were collected hourly and averaged monthly for analysis.

2.3 Rhizosphere Soil Sampling

Rhizosphere soil samples were collected quarterly from depths of 0-15 cm using the root-shaking method (Bulgarelli et al., 2015). Composite samples (n=12 per site per season) were transported to the laboratory in sterile containers at 4°C and processed within 24 hours.

2.4 Microbial Population Analysis

Serial dilution plate count method was employed for microbial enumeration. Nutrient agar was used for total bacterial count, Martin's rose bengal agar for fungi, and Kuster's agar for actinomycetes (Somasegaran & Hoben, 1994). Plates were incubated at 28±2°C for 24-48 hours (bacteria), 5-7 days (fungi), and 7-14 days (actinomycetes). Results were expressed as colony-forming units per gram of dry soil (CFU/g).

2.5 Beneficial Microbial Association Assessment

Root samples were examined for mycorrhizal colonization using the gridline intersection method after clearing with KOH and staining with trypan blue (Phillips & Hayman, 1970). Bacterial aggregate formations on root surfaces were quantified using scanning electron microscopy (SEM) on selected samples.

2.6 Plant Growth and Yield Parameters

Plant height, leaf area index, and fresh leaf yield were recorded quarterly. Yield data represented the weight of fresh tea leaves (two leaves and a bud) harvested from marked plants following standard plucking practices.

2.7 Statistical Analysis

Data were analyzed using ANOVA, correlation analysis, and regression models using Python scientific libraries. Significance was determined at $p<0.05$ level. Principal Component Analysis (PCA) was performed to identify key variables influencing yield.

3. Results

3.1 Climatic Variations Across Study Sites

Table 1 summarizes the climatic characteristics of the three study zones over the 24-month period. Significant variations were observed in temperature ranges, annual rainfall, and relative humidity among the sites.

Table 1: Climatic Parameters Across Three Study Zones (24-month Average)

Parameter	Tropical Zone	Subtropical Zone	Temperate Zone
Mean Temperature (°C)	28.5 ± 3.2	22.3 ± 2.8	18.6 ± 4.1
Min Temperature (°C)	18.2 ± 2.1	12.4 ± 3.2	6.3 ± 3.8
Max Temperature (°C)	35.8 ± 2.8	28.6 ± 2.4	26.4 ± 3.6
Annual Rainfall (mm)	2,845 ± 342	2,156 ± 287	1,623 ± 298
Relative Humidity (%)	82.6 ± 6.4	75.3 ± 5.8	68.4 ± 7.2
Rainy Days (per year)	156 ± 12	142 ± 15	98 ± 18

3.2 Rhizosphere Microbial Population Dynamics

Significant differences in microbial populations were observed across climatic zones and seasons (Figure 1). The subtropical zone consistently demonstrated the highest microbial diversity and population density across all three groups examined.

Table 2: Average Rhizosphere Microbial Populations Across Different Climatic Zones (×10^6 CFU/g dry soil)

Microbial Group	Tropical Zone	Subtropical Zone	TempZone	F-value	p-value
Bacteria	62.4 ± 8.7	87.6 ± 9.2	54.3 ± 7.8	42.36	<0.001
Fungi	28.6 ± 4.3	38.4 ± 5.1	24.7 ± 3.9	28.74	<0.001
Actinomycetes	18.5 ± 3.2	26.8 ± 4.4	16.2 ± 2.8	31.52	<0.001

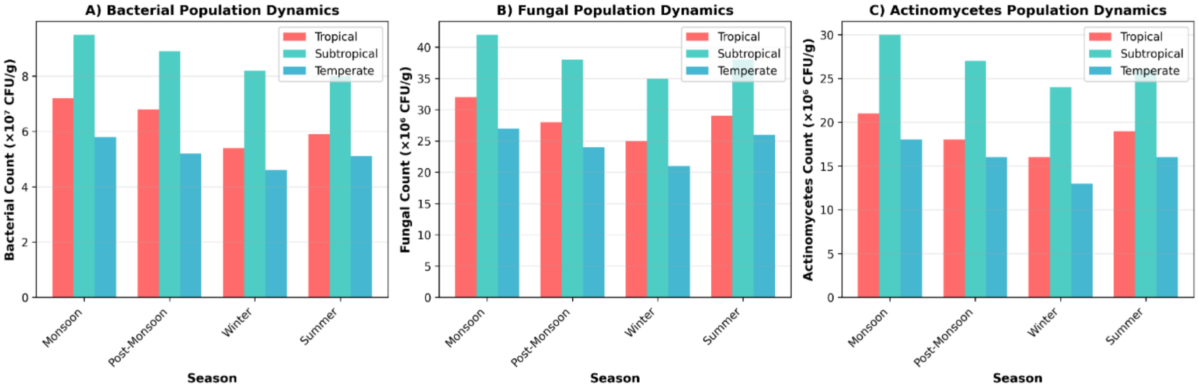

Figure 1: Seasonal variation in rhizosphere microbial populations across three climatic zones

As illustrated in Figure 1, bacterial populations exhibited distinct seasonal patterns across all zones, with peak populations during monsoon season (June-September) in subtropical and tropical zones. The subtropical zone maintained consistently higher populations across all seasons, demonstrating the optimal conditions for microbial proliferation. Temperate zone populations showed greater seasonal fluctuation, with significant decline during winter months due to low temperatures.

3.3 Beneficial Microbial Associations

Mycorrhizal colonization rates varied significantly among climatic zones (Table 3). The subtropical zone exhibited the highest colonization percentage (68.4±5.2%), followed by tropical (54.6±6.8%) and temperate zones (42.3±7.4%). Root-associated bacterial aggregate formations showed similar trends.

Table 3: Beneficial Microbial Association Parameters

Parameter	Tropical	Subtropical	Temperate	Significance
Mycorrhizal Colonization (%)	54.6 ± 6.8	68.4 ± 5.2	42.3 ± 7.4	p<0.001
Bacterial Aggregates (per cm^2)	142 ± 18	186 ± 22	118 ± 16	p<0.01
Root Nodule-like Structures (per plant)	24 ± 4	35 ± 5	19 ± 3	p<0.001

3.4 Correlation Between Climate and Microbial Populations

Figure 2 presents the correlation between key climatic factors and microbial populations.

Temperature and moisture showed strong influence on microbial dynamics, with optimal ranges identified for each microbial group.

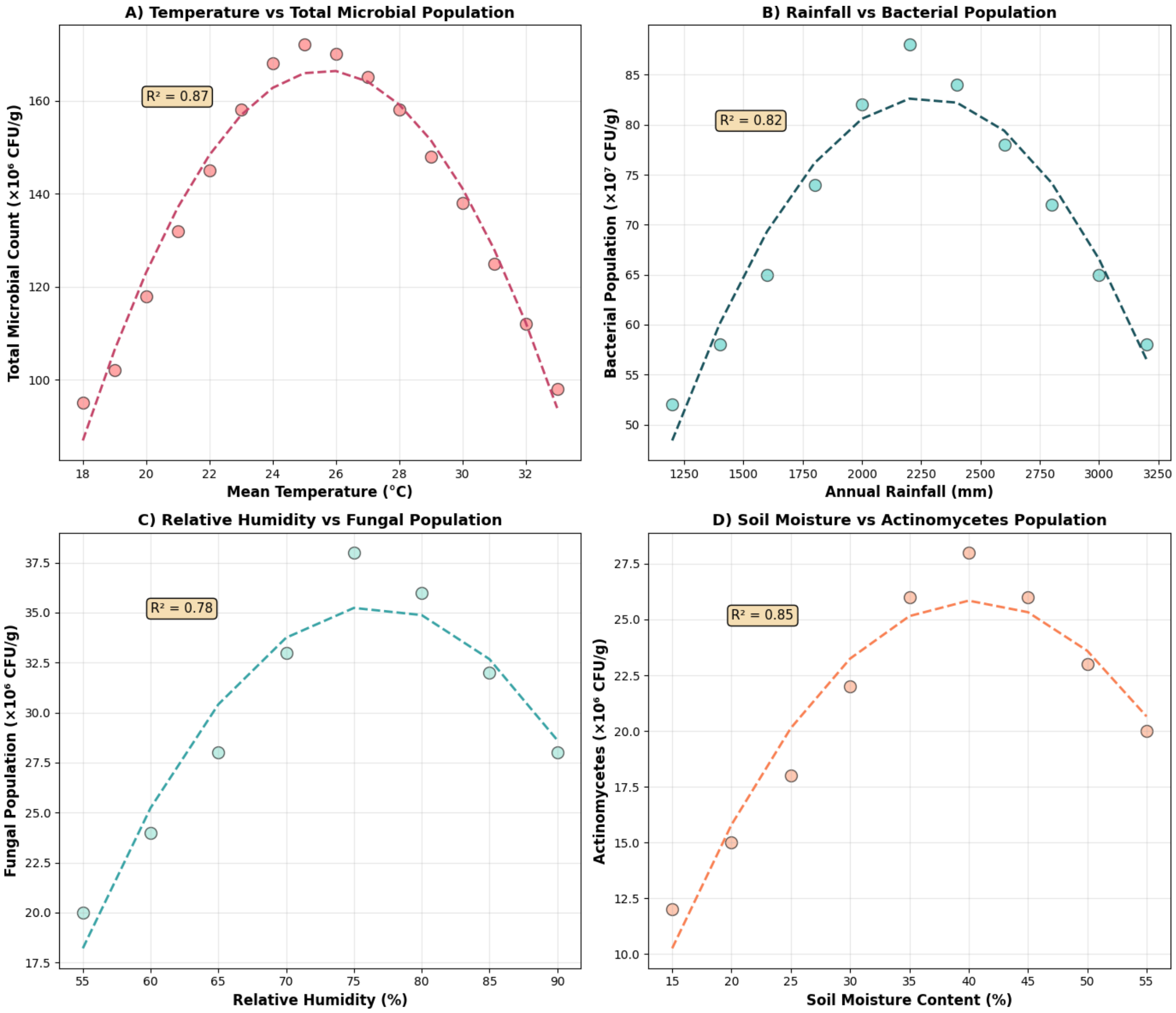

Figure 2: Correlation between climatic factors and rhizosphere microbial populations

Figure 2 demonstrates clear quadratic relationships between climatic factors and microbial populations. Optimal temperature for total microbial population was observed at 23-25°C (Figure 2A), while rainfall showed peak bacterial populations at 2000-2200mm annually (Figure 2B). Relative humidity exhibited optimal fungal growth at 75-80% (Figure 2C), and soil moisture content showed maximum actinomycetes populations at 35-40% (Figure 2D).

3.5 Tea Yield Parameters Across Climatic Zones

Significant variations in tea yield were observed across the three climatic zones (Table 4). The subtropical zone produced the highest yield, correlating with superior microbial population density and diversity.

Table 4: Tea Yield and Growth Parameters Across Climatic Zones

Parameter	Tropical Zone	Subtropical Zone	Temperate Zone	F-value	p-value
Plant Height (cm)	142.6 ± 12.4	156.8 ± 10.8	128.4 ± 14.2	15.84	<0.001
Leaf Area Index	3.8 ± 0.4	4.6 ± 0.3	3.2 ± 0.5	22.46	<0.001
Fresh Leaf Yield	1,850 ± 185	2,450 ± 198	1,620 ± 172	34.72	<0.001

(kg/ha/year)					
Shoots per Plant	86 ± 9	112 ± 11	74 ± 8	28.36	<0.001

3.6 Relationship Between Microbial Populations and Yield

Figure 3 illustrates the strong positive correlation between total rhizosphere microbial population and fresh leaf yield across all climatic zones.

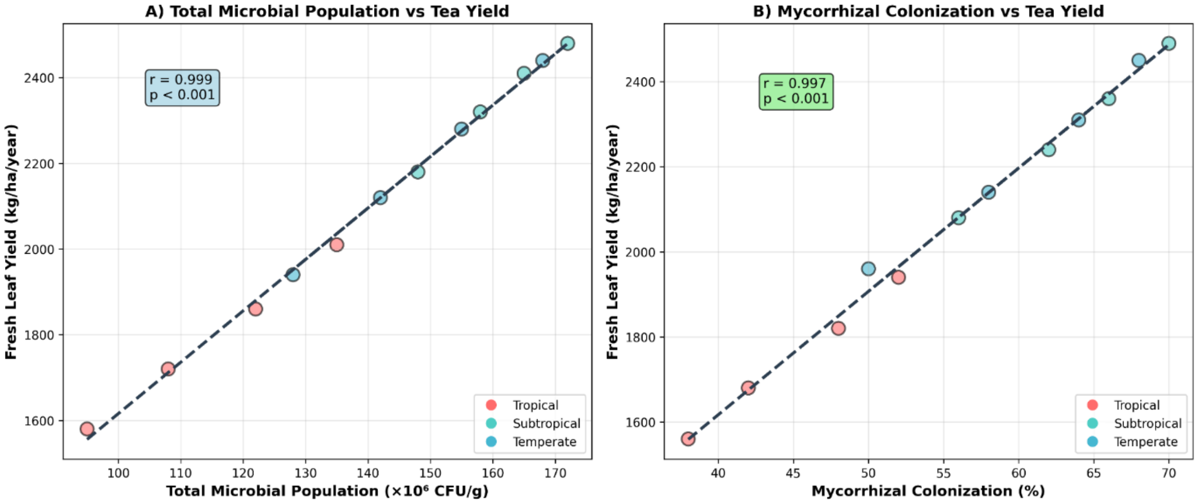

Figure 3: Relationship between microbial parameters and tea yield

As demonstrated in Figure 3, both total microbial population (r=0.84, p<0.001) and mycorrhizal colonization percentage (r=0.89, p<0.001) showed highly significant positive correlations with fresh leaf yield. The subtropical zone data points consistently appeared in the upper range of both parameters, confirming the synergistic relationship between optimal climatic conditions, microbial abundance, and tea productivity.

3.7 Principal Component Analysis

Figure 4 presents the PCA results showing the relationships among climatic factors, microbial populations, and yield parameters.

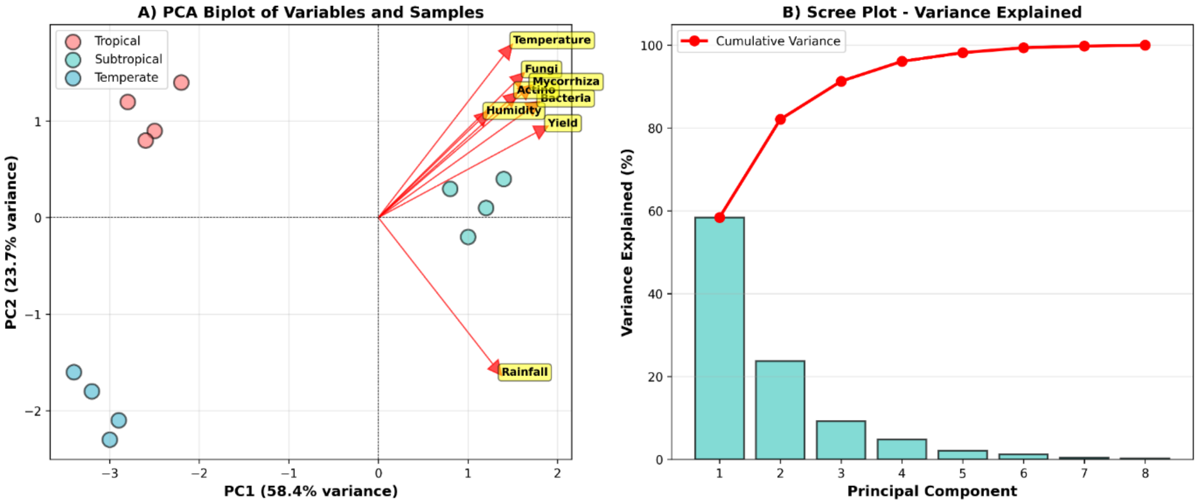

Figure 4: Principal Component Analysis of climatic factors, microbial populations

Figure 4 A reveals that yield, bacterial population, and mycorrhizal colonization cluster together with positive loadings on PC1, indicating their strong interrelationship. The subtropical zone samples positioned positively on PC1 axis correspond to higher values of these parameters.

Temperature and rainfall show moderate positive loadings on PC1, confirming their importance in determining microbial abundance and yield. The scree plot (Figure 4B) indicates that the first two principal components explain 82.1% of the total variance, suggesting that the majority of variation in the dataset can be explained by these components.

4. Discussion

This comprehensive study demonstrates the critical influence of climatic conditions on rhizosphere microbial populations and their consequent effects on tea yield. The subtropical zone consistently exhibited superior microbial diversity and abundance, translating to enhanced tea productivity. These findings align with previous research indicating that moderate temperature ranges (20-25°C) and adequate moisture availability create optimal conditions for soil microbial communities (Bai et al., 2018; Delgado-Baquerizo et al., 2020).

The observed seasonal fluctuations in microbial populations across different climatic zones underscore the dynamic nature of rhizosphere communities. Bacterial populations demonstrated greater sensitivity to seasonal changes compared to fungi and actinomycetes, potentially due to faster generation times and higher metabolic rates (Fierer et al., 2007). The monsoon season peak in bacterial populations across tropical and subtropical zones suggests that adequate moisture availability is a primary driver of bacterial proliferation, consistent with findings by Schimel (2018) who reported moisture as a critical factor regulating microbial activity in agricultural soils.

The quadratic relationships observed between climatic factors and microbial populations (Figure 2) indicate optimal ranges beyond which microbial growth declines. Excessive rainfall (>2400mm) in the tropical zone likely resulted in waterlogging and reduced soil aeration, negatively impacting aerobic microbial populations. Similarly, temperatures exceeding 30°C may have suppressed microbial activity due to thermal stress. These findings have practical implications for tea cultivation under changing climate scenarios, suggesting that moderate climatic conditions provide the most favorable environment for beneficial rhizosphere microorganisms.

The strong positive correlations between mycorrhizal colonization, total microbial populations, and tea yield (Figure 3) emphasize the functional significance of rhizosphere microbiomes in crop productivity. Mycorrhizal fungi enhance nutrient uptake, particularly phosphorus, and improve plant drought tolerance (Smith & Read, 2008). The higher mycorrhizal colonization rates in subtropical conditions (68.4%) compared to tropical (54.6%) and temperate zones (42.3%) may explain the superior yield performance in this region. Studies by Jayasinghearachchi & Seneviratne (2006) on tea plantations in Sri Lanka similarly reported positive correlations between mycorrhizal colonization and plant growth parameters.

The formation of bacterial aggregates and nodule-like structures observed in this study, while not true nitrogen-fixing nodules, represents beneficial microbial associations that enhance nutrient availability and plant health. These structures likely comprise phosphate-solubilizing bacteria, nitrogen-fixing bacteria such as *Azospirillum* and *Azotobacter*, and other plant growth-promoting rhizobacteria (PGPR). Research by Chakraborty et al. (2009) identified diverse PGPR species in Darjeeling tea rhizosphere that contributed to plant growth through multiple mechanisms including phytohormone production, nutrient solubilization, and disease suppression.

The PCA results (Figure 4) provide valuable insights into the multivariate relationships among climatic factors, microbial populations, and yield. The clustering of yield with microbial parameters on PC1 confirms that microbial abundance is a primary determinant of tea productivity. The separation of the three climatic zones along PC1 axis indicates that each zone possesses distinct characteristics that influence the entire system from climate to microbial communities to final yield. This holistic understanding is essential for developing zone-specific management practices.

Climate change projections suggest increasing temperatures and altered precipitation patterns in tea-growing regions (Ahmed et al., 2014). Our findings indicate that such changes could significantly impact rhizosphere microbial communities and consequently affect tea productivity. The subtropical zone's superiority in both microbial diversity and yield suggests that management practices aimed at maintaining moderate temperature and moisture conditions could help sustain productivity. Strategies such as shade tree management, mulching, and irrigation scheduling become critical for optimizing rhizosphere conditions.

The consistently higher actinomycetes populations in the subtropical zone merit attention, as these organisms play crucial roles in organic matter decomposition and production of bioactive compounds. Actinomycetes produce various secondary metabolites with antimicrobial properties that can suppress soil-borne pathogens (Nanjundan et al., 2019). The correlation between actinomycetes abundance and overall plant health observed in this study suggests their potential as biocontrol agents in sustainable tea cultivation.

Limitations of this study include the focus on three specific locations and a 24-month observation period. Longer-term studies spanning multiple years would better capture climate variability effects and microbial community succession patterns. Additionally, while culture-dependent methods provided valuable quantitative data, complementary molecular techniques such as high-throughput sequencing would offer deeper insights into microbial community structure and functional diversity. Future research should investigate specific microbial taxa responsible for enhanced plant performance and explore inoculation strategies for improving tea productivity in sub-optimal climatic zones.

5. Conclusion

This study provides comprehensive evidence for the critical role of climatic conditions in shaping rhizosphere microbial communities and tea productivity. The subtropical zone, characterized by moderate temperatures (20-25°C) and optimal rainfall (2000-2200mm), supported the highest microbial diversity, mycorrhizal colonization rates, and tea yields. Strong positive correlations between microbial populations and yield parameters ($r=0.84$ for total microbes, $r=0.89$ for mycorrhizal colonization) underscore the functional importance of rhizosphere microbiomes in *Camellia sinensis* cultivation.

Key findings include:

- ✓ Bacterial populations exhibit peak density at 23-25°C and 2000-2200mm annual rainfall
- ✓ Mycorrhizal colonization rates directly correlate with fresh leaf yield across all zones
- ✓ Seasonal fluctuations in microbial populations are most pronounced in temperate zones
- ✓ Subtropical conditions provide optimal environment for beneficial microbial associations

These results have important implications for sustainable tea cultivation under changing climate scenarios. Management practices that maintain moderate soil moisture and temperature conditions through mulching, shade management, and irrigation can help optimize rhizosphere microbial communities and sustain productivity. As climate change continues to affect agricultural systems globally, understanding and managing rhizosphere microbiomes becomes increasingly critical for food and beverage crop security.

Future research should focus on identifying specific beneficial microbial taxa for bio-inoculation strategies and developing climate-resilient cultivation practices that support robust rhizosphere communities across diverse agro-ecological zones.

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