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# Mechanisms by Which Soil Microbial Communities Regulate Ecosystem Multifunctionality in Tea Gardens of Longnan City, China

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## Abstract

Soil microbial communities are fundamental to soil health and ecosystem functioning in agricultural landscapes. This study assessed how soil nutrient variation influences microbial community structure and ecosystem multifunctionality in tea gardens across three counties in Longnan, China. Key findings revealed that Kangxian tea garden soils exhibited 18–25% higher bacterial and fungal richness and diversity indices than Wenxian, which had the lowest values among the three counties. Co-occurrence network analysis indicated a 32% higher proportion of positive (cooperative) interactions among microbial taxa in Wenxian soils. Null model analysis showed that bacterial community assembly was primarily driven by deterministic heterogeneous selection, whereas fungal assembly was governed by stochastic ecological drift. Functionally, Wenxian soils demonstrated 22% higher carbon sequestration, 19% higher nutrient storage, and 17% higher nutrient supply than the other counties ($p < 0.05$), while Kangxian soils had 21% greater nutrient cycling and overall ecosystem multifunctionality. Soil C/P and N/P ratios significantly influenced carbon sequestration, nutrient storage, and multifunctionality (explaining up to 48% of the variance), while soil pH was a key driver of carbon sequestration, nutrient supply, and cycling. Both bacterial and fungal community structures significantly impacted nutrient storage and multifunctionality. Regional differences in soil nutrients, shaped by tea garden management, directly influence microbial community traits and ecosystem multifunctionality. Targeted nutrient management and enhanced microbial diversity are key to improving soil multifunctionality and sustainability in tea agroecosystems.

**Keywords:** tea garden; soil microbes; ecosystem multifunctionality; co-occurrence network; community assembly

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Academic Editor: Beniamino T. Cenci-Goga

Received: 8 July 2025
Revised: 8 August 2025
Accepted: 14 August 2025
Published: 27 August 2025

**Citation:** Nian, L.; Li, J.; Tang, Y.; Haider, F.U.; Wang, Z.; Dong, L.; Zhang, J.; Long, Q.; Wang, W.; Zhao, X. Mechanisms by Which Soil Microbial Communities Regulate Ecosystem Multifunctionality in Tea Gardens of Longnan City, China. *Microbiol. Res.* **2025**, *16*, 192. https://doi.org/10.3390/microbiolres16090192

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## 1. Introduction

China is recognized as one of the primary centers of origin for tea plants and boasts a rich, longstanding tradition of tea cultivation [1]. As of 2022, China's total area of tea cultivation reached approximately 3.2 million hectares, with annual tea production surpassing 2.93 million tons [2]. The tea industry has continued to flourish in recent years,

steadily expanding tea plantation areas across the country [3]. Nitrogen (N) is a critical nutrient for the growth and development of tea plants, exerting a profound influence on yield and quality [4]. However, the excessive application of N fertilizers has been shown to accelerate soil acidification in tea gardens, leading to the deterioration of soil structure and a decline in soil fertility [5]. Furthermore, the decomposition of tea litter releases a suite of organic substances, such as polyphenolic compounds and organic acids, while tea roots secrete a variety of secondary metabolites [6]. These compounds continuously modify the physicochemical properties of tea garden soils. The cumulative effects of these biochemical processes not only intensify the trend of soil acidification but also profoundly reshape soil microbial activity, community composition, and structural characteristics, ultimately giving rise to a tea garden ecosystem with unique properties [7]. Within this specialized system, the biogeochemical cycling of key elements—particularly carbon (C) and N—differs markedly from that observed in other terrestrial ecosystems [8].

Microorganisms are widely acknowledged as the principal drivers of change within soil ecosystems [9]. Their metabolic activities underpin essential ecological processes, including nutrient cycling, organic matter decomposition, maintenance of soil structure, suppression of plant diseases, and enhancement of plant productivity [10]. The composition and diversity of microbial communities, as integral components of soil function, are extensively employed as indicators of soil health [11]. Given their dominance in soil biological activity and pivotal role in many soil functions, microorganisms are regarded as the main agents orchestrating changes in soil ecosystems [12]. Contemporary research on soil microorganisms has predominantly focused on elucidating how microbial communities respond to environmental changes [13]. Accumulating evidence suggests that microbial communities serve as sensitive indicators of environmental perturbations and can actively facilitate the restoration of degraded ecosystems [14]. Consequently, a growing body of research is dedicated to harnessing microorganisms as mediators of ecosystem modification [15], with particular emphasis on enhancing crop yields and promoting the restoration of arid lands [16]. Nevertheless, there remains a paucity of studies examining the differences in soil microbial communities and ecological functions as carbon and nitrogen cycling among tea gardens in different regions [17]. Moreover, the intricate relationships between the structure and function of soil microbial communities in tea gardens and the surrounding soil environmental factors warrant further in-depth investigation to identify the key determinants shaping microbial community structure and function [18].

Soil multifunctionality (SMF), a vital component of overall ecosystem multifunctionality, has garnered considerable attention recently [19]. Rather than focusing solely on individual soil ecological functions, SMF emphasizes the integrated performance of multiple soil ecosystem functions [20]. In soil ecology, SMF fundamentally refers to the ecosystem's capacity to simultaneously provide various services and functions, such as microbial activity, nutrient cycling, nutrient storage, N transformation, and C sequestration [21]. Research on SMF has been concentrated primarily in farmland and grassland ecosystems, with a particular focus on the interactions between soil microbial diversity and environmental factors [19]. Soil biodiversity is widely considered a principal driving force for community structure and ecosystem function within soil systems [22]. Empirical studies have demonstrated that biodiversity loss and the simplification of biological communities can lead to declining ecosystem functionality [23]. Moreover, a significant positive correlation between soil microbial diversity and SMF has been reported [24], although some studies have observed that SMF may increase as microbial diversity declines [25]. These contrasting findings indicate that the relationship between SMF and microbial diversity remains unresolved, and the mechanisms by which different microbial communities influence SMF across various ecosystems are still not fully elucidated. Tea gardens represent a unique

ecosystem characterized by substantial differences in soil properties and microbial communities across regions. However, research on SMF within tea garden ecosystems remains limited, and the mechanisms through which tea gardens in different areas affect SMF are still unclear.

In light of these considerations, tea gardens across Wenxian, Wudu, and Kangxian counties in Longnan City were investigated to elucidate how soil nutrients influence the relationship between microbial diversity and ecosystem multifunctionality. Moreover, this study assessed key soil physicochemical properties, microbial diversity indices, and a suite of indicators related to carbon sequestration, nutrient supply and storage, and nutrient cycling. It was hypothesized that (1) soil C sequestration, nutrient storage, and nutrient supply functions exhibit synergistic relationships with ecosystem multifunctionality, with each function contributing to enhanced overall multifunctionality, and (2) soil nutrients shaped by tea gardens in different regions indirectly influence microbial diversity, which in turn affects ecosystem functions. To test these hypotheses, high-throughput sequencing technology was employed to assess the diversity and structure of soil microbial communities in tea gardens from different regions. Co-occurrence network analysis was used to examine relationships among microbial communities, while ecosystem multifunctionality and key environmental drivers were quantified using the averaging method. The findings of this study provide a theoretical foundation for understanding soil microbial processes and promoting the sustainable management of tea gardens.

## 2. Materials and Methods

### *2.1. Study Area Overview*

The research was conducted in three representative tea-producing towns, Bikou (Wenxian County), Yangba (Kangxian County), and Yuhe (Wudu District), which are all situated within Longnan City, Gansu Province, China. These locations collectively offer diverse topographical and climatic conditions ideal for tea cultivation. Bikou Town, located in the southeast of Wenxian County (32°41′37″ N, 105°17′43″ E; elevation 1194 m), experiences a subtropical monsoon climate. The area is characterized by a mean annual temperature of 14.8 °C, a growing season of approximately 260 days, a yearly sunshine duration of 1596.5 h, and an average annual precipitation of 451.6 mm. Precipitation is predominantly concentrated between June and August, with July being the wettest month. The soils in this area are mainly red clay and yellow-brown soils, classified as transitional yellow-brown soils. They exhibit characteristics of both aluminization and clay accumulation. The parent material is weathered rock, from which clay particles are leached and deposited in lower soil layers, resulting in a relatively heavy clay texture. Yangba Town, in the southern part of Kangxian County (33°2′31″ N, 105°40′27″ E; elevation 1660 m), also falls within the subtropical monsoon zone. The average annual temperature is 12.6 °C, with a growing season of about 200 days, an annual sunshine duration of 1787.4 h, and a mean annual precipitation of approximately 1000 mm. Rainfall is primarily distributed in July and August. The local soils are mainly composed of dark loam, yellow-black soils, and sandstone-derived soils, which belong to the acidic red-yellow soil type and have a relatively high sand content. Yuhe Town, situated in the southeast of Wudu District (32°59′21″ N, 105°29′55″ E; elevation 963 m), is characterized by a subtropical humid climate with distinct seasonal variation: mild springs and autumns, hot and rainy summers, and dry winters. The average annual temperature is 14° C, with a growing season of around 240 days and a frost-free period of approximately 250 days. The area receives an average of 1920 h of sunshine annually and about 980 mm of precipitation, distributed over roughly 140 rainy days per year, with the majority of rainfall from May to September and peaking in August. The soils in this area belong to the acidic red-yellow soil category. The parent materials mainly consist of

valley alluvium (clay loam) and limestone residuals (sandy clay). Overall, Longnan City exhibits a warm, humid climate with abundant rainfall and extended growing seasons, providing optimal ecological conditions for tea plant growth. The elevation, temperature, and precipitation diversity across these sites offer a valuable natural gradient for investigating the interactions between soil properties, microbial communities, and ecosystem multifunctionality in tea gardens.

### *2.2. Soil Sample Collection*

Soil sampling was initiated on 6 August 2024, across nine tea gardens distributed among Bikou Town (Wenxian County), Yangba Town (Kangxian County), and Yuhe Town (Wudu District), with three tea gardens selected per location to serve as biological replicates. Five tea plants were randomly selected within each tea garden, and rhizosphere soil samples were collected from the 0–20 cm depth using a soil auger. The samples from each garden were homogenized to form a single composite sample. Following collection, extraneous materials such as stones and plant debris were promptly removed. Each composite sample was subdivided into three portions for subsequent analyses. One portion of each composite sample was stored at −80 °C for total soil DNA extraction. A second portion was kept at 4 °C for the determination of soil nitrate nitrogen ($NO_3^-$-N), ammonium nitrogen ($NH_4^+$-N), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN). The remaining portion was air-dried and used for the assessment of fundamental soil physicochemical properties, including soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK).

### *2.3. Methods for Determining Soil Physicochemical Properties*

Soil pH was measured using a pH meter (Mettler Toledo, Switzerland) at a soil-to-water ratio of 1:2.5. Soil organic carbon (SOC) was determined by the external heating potassium dichromate method [26]. TN was quantified using the semi-micro Kjeldahl method [27], while TP was assessed via the ammonium molybdate spectrophotometric method [28]. Urease (UE) activity was measured using the indophenol blue colorimetric method [29], and acid phosphatase (ACP) activity was determined with the p-nitrophenyl phosphate method [30]. Polyphenol oxidase (PPO) activity was evaluated by quantifying the rate of oxidation of phenolic substrates (such as catechol and o-diphenol) to quinone products [31]. Catalase (CAT) activity was determined by titration [32], and cellulase (CL) activity was measured using the 3,5-dinitrosalicylic acid method [33].

### *2.4. Soil Microbial High-Throughput Sequencing*

According to the manufacturer's protocol, total soil DNA was extracted from 0.25 g of fresh soil using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). Each sample was subjected to three independent DNA extractions, and the resulting extracts were pooled to minimize technical variability. DNA quality and concentration were assessed by electrophoresis on a 1% agarose gel and quantified using a NanoDrop UV-Vis spectrophotometer (ND-2000c, NanoDrop Technologies, Wilmington, DE, USA). For bacterial community analysis, the V3–V4 region of the 16S rRNA gene was amplified using primers 515F (5′-GTGCCAGGCGCCGCGCGGTA-3′) and 907R (5′-CCGTCAATTCCTTGAGTTT-3′) [34]. For fungal community analysis, the internal transcribed spacer (ITS) region was amplified using primers ITS5-1737F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2-2043R (5′-GCTGCGTTCTTCATCGATGC-3′) [35]. Polymerase chain reaction (PCR) products were sequenced on the Illumina MiSeq platform (Majorbio Bio-Pharm Technology Co., Shanghai, China) for high-throughput sequencing.

### *2.5. Determination of Ecosystem Multifunctionality*

Soil multifunctionality (SMF), with a particular emphasis on nutrient-related processes, was assessed by categorizing functions into four key subsets: carbon sequestration, nutrient storage, nutrient supply and nutrient cycling. Following established protocols and the recent literature [36,37], SOC was selected as the indicator for C sequestration; TN, TP, and TK were used to represent nutrient storage; available phosphorus (AP) and available potassium (AK) were chosen as proxies for nutrient supply; and the activities of UE, PPO, ACP, CAT, and CL characterized nutrient cycling. Ecosystem multifunctionality was quantified using the averaging approach [38,39]. All 14 functional indicators were standardized as follows:

$$f_{ij} = \frac{x_{ij} - min_{ij}}{max_{ij} - min_{ij}}$$

where $f_{ij}$ is the standardized value of the j-th function in the i-th tea garden, $x_{ij}$ is the observed value, and $min_{ij}$ and $max_{ij}$ are the minimum and maximum values of the j-th function across all sites, respectively.

A single-function index (EF) was calculated as the mean of standardized values for each function:

$$EF_{ij} = \sum_{j}^{n} \frac{f_{ij}}{n}$$

where $n$ is the number of variables associated with each function. The overall ecosystem multifunctionality index (EMF) for each tea garden was determined by averaging the standardized values across all measured functions:

$$EMF_i = \frac{1}{N}\sum_{1}^{N} f_{ij}$$

where $N$ is the total number of ecosystem functions evaluated. This integrative approach enables robust comparisons of soil multifunctionality across different tea garden soils, providing insight into the combined effects of nutrient dynamics and microbial processes on ecosystem functioning.

### *2.6. Calculation of Soil Element and Enzyme Stoichiometry*

Soil elemental stoichiometry was used to characterize nutrient limitation in soils, while the vector angle (VA) and vector length (VL), calculated from the stoichiometry of soil enzyme activities, were used to reflect the nutrient limitation status of soil microorganisms [40]. The equations are as follows:

$$VL = SQRT\left[\left[\frac{CEs}{PEs}\right]^2 + \left[\frac{CEs}{NEs}\right]^2\right]$$

$$VA = Degrees\left[Atan2\left[\frac{CEs}{PEs}\right]^2, \left[\frac{CEs}{NEs}\right]^2\right]$$

where $CEs$ represents the activity of soil carbon-degrading enzymes (including catalase, polyphenol oxidase, and cellulase), $NEs$ represents the activity of soil nitrogen-degrading enzymes (urease), and $PEs$ represents the activity of soil phosphorus-degrading enzymes (acid phosphatase). A longer VL indicates stronger carbon limitation for microorganisms, while a VA less than 45° and greater than 45° indicates nitrogen and phosphorus limitation, respectively.

### 2.7. Data Analysis

Microbial community diversity and structural composition were analyzed using the Majorbio Cloud Platform (https://cloud.majorbio.com/, accessed on 15 March 2025). Soil physicochemical properties were statistically evaluated with Microsoft Excel 2016, and the relative abundance of microbial taxa at the phylum level was visualized accordingly. One-way analysis of variance (ANOVA) was performed in SPSS 25.0 (IBM, Chicago, IL, USA), with significant differences among groups determined by the least significant difference (LSD) test at $p = 0.05$. Microbial co-occurrence networks were constructed in R (version 1.6.0, R Core Team, Vienna, Austria) and visualized using Gephi (version 0.10.1, Gephi Consortium, Paris, France). Redundancy analysis (RDA) was conducted with Canoco 5 (Microcomputer Power, Ithaca, NY, USA). Community assembly processes were assessed in R, and graphical representations, including bar and box plots, were generated using Origin 2021 (OriginLab, Northampton, MA, USA). Partial least squares path modeling (PLS-PM), a correlation-based structural equation modeling approach suitable for exploratory research and datasets of varying sizes without the requirement for multivariate normality [41], was employed to elucidate the influence pathways of soil multifunctionality across tea gardens in different regions. PLS-PM analyses were conducted using the 'plspm' package in R.

## 3. Results and Analyses

### 3.1. Soil Microbial Community Structure

High-throughput sequencing analysis of soil microbial communities (Figure 1) revealed that *Actinobacteriota*, *Acidobacteriota* and *Proteobacteria* were the dominant bacterial phyla. Wudu County exhibited the highest total relative abundance of these dominant phyla (67.19%). However, its bacterial community richness and diversity indices were not the highest—these were observed in Kang County. In contrast, Kang County had the lowest total relative abundance of dominant bacterial phyla (61.92%) but the highest bacterial richness and diversity. This trend suggests a potential trade-off in Longnan tea garden soils, where a higher total abundance of certain dominant bacterial groups may be associated with lower overall microbial diversity, possibly due to competitive exclusion limiting the survival of other bacterial taxa. Additionally, the relative abundances of *Proteobacteria* and *Actinobacteriota* were lowest in Kang County (26.65% and 14.38%, respectively) and highest in Wen County (34.26% and 17.34%, respectively). In contrast, *Acidobacteriota* had the highest relative abundance in Kang County (20.90%) and the lowest in Wen County (14.34%).

*Ascomycota*, *Basidiomycota* and *Mortierellomycota* were the dominant fungal phyla, with total relative abundances of 92.86% (Wen County), 86.97% (Wudu County) and 86.22% (Kang County), respectively. This indicates that the relative abundance in Wen County was significantly higher than in the other two counties. Meanwhile, Wen County also exhibited the lowest fungal richness and diversity indices among the three regions. This further supports the observation that extremely high dominance by fungal phyla corresponds with a significant reduction in fungal community diversity. The fungal community in Wen County was mainly dominated by *Ascomycota* (52.56%) and *Mortierellomycota* (25.96%), and their extremely high combined abundance (92.86%) may have led to a simplified community structure.

Similarly, fungal richness and diversity indices were higher in Wudu and Kangxian compared to Wenxian. Similarly, significant differences were observed in the co-occurrence network characteristics of soil microbial communities among tea gardens in different regions of Longnan City (Figure 2, Table 1). Analysis of the bacterial co-occurrence networks revealed that the tea gardens in Wudu exhibited the highest number of edges (9259), number of nodes (284), average weighted degree, and network density, indicating a higher

complexity and more intricate interspecies interactions within the bacterial community compared to the other regions. In contrast, the proportion of positive links in the Wenxian bacterial network was higher, while the proportion of negative links was lower than in the other two counties, suggesting stronger cooperative interactions among bacterial taxa in Wenxian tea garden soils. Correspondingly, to identify the drivers underlying differences in soil microbial community structure among tea gardens in different regions of Longnan City, a null model analysis was employed to elucidate the intrinsic assembly mechanisms shaping microbial community distribution patterns. The null model analysis for soil bacterial communities (Figure 2) indicated that deterministic processes predominated in community assembly across Wenxian, Wudu, and Kangxian, with heterogeneous selection being the primary deterministic factor structuring bacterial communities. In contrast, the null model analysis for soil fungal communities revealed that stochastic processes were dominant in all regions, with ecological drift serving as the main stochastic force governing fungal community assembly. Furthermore, correlation analysis (Figure 3) revealed that bacterial βNTI was significantly positively correlated with total phosphorus and soil pH, while fungal βNTI showed a significant positive correlation with total phosphorus.

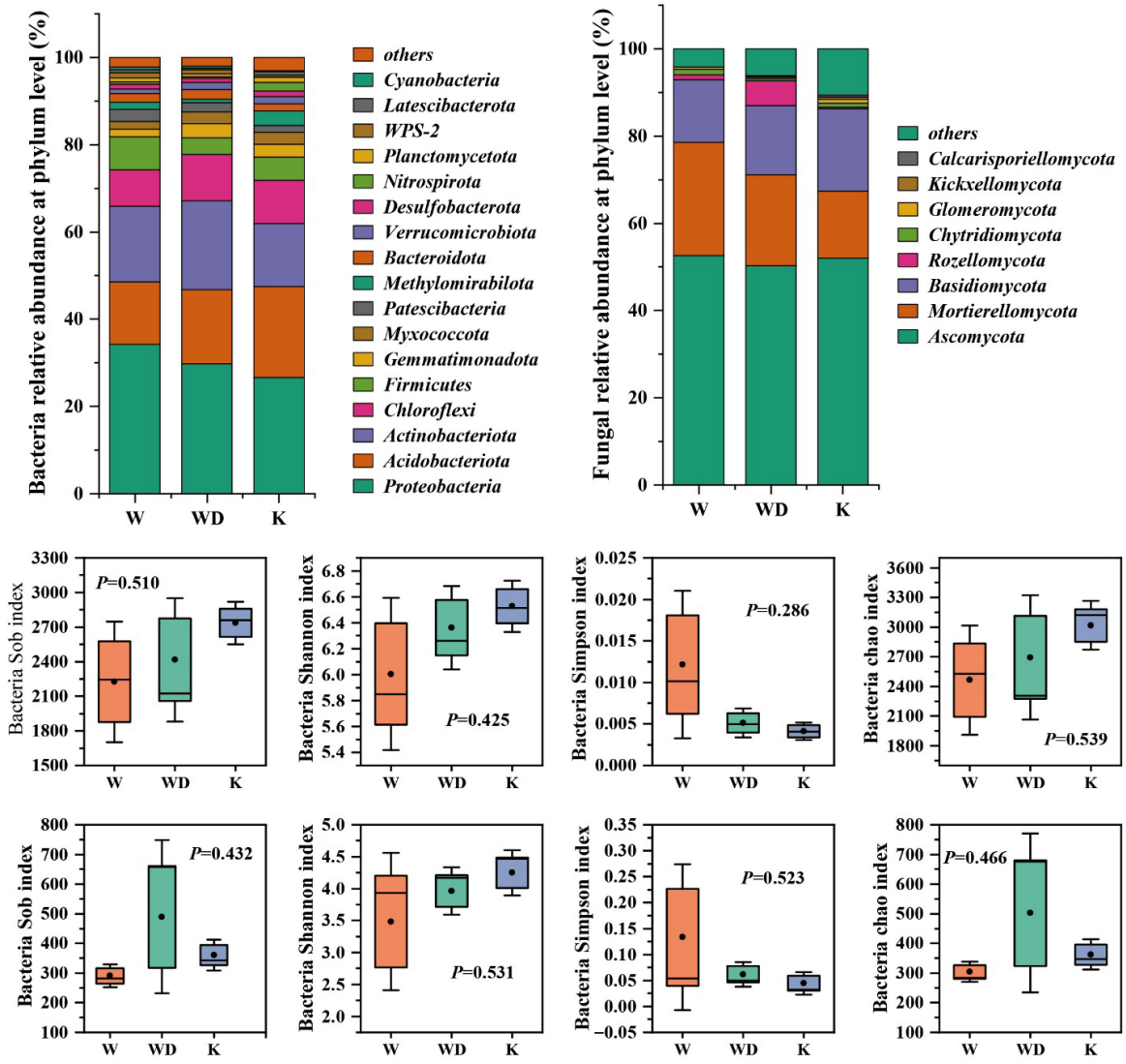



**Figure 1.** Composition and alpha diversity indices of soil microbial communities in tea gardens from different regions of Longnan City, China. Wenxian (W), Wudu (WD), and Kangxian (K) represent the three counties within Longnan City.

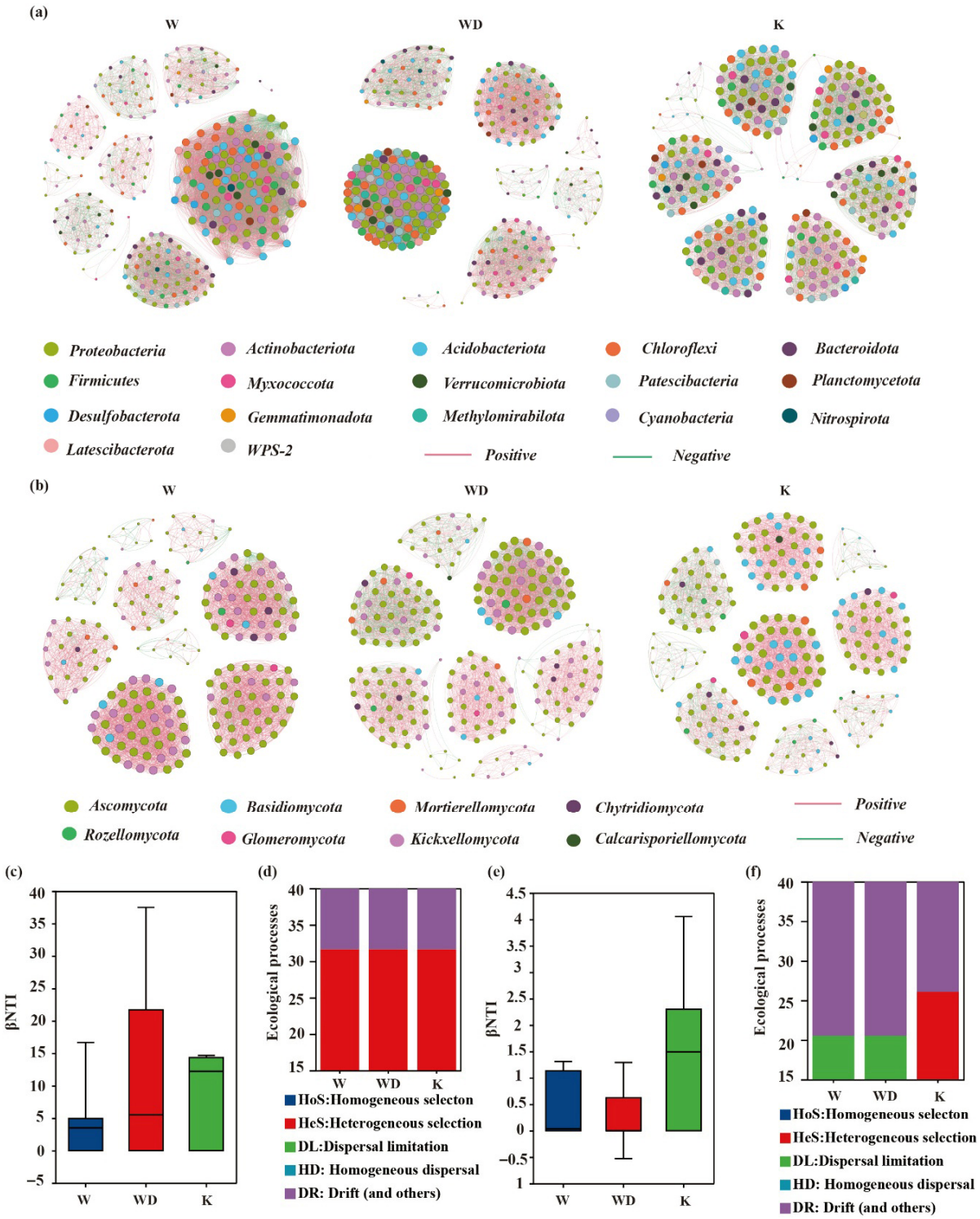



**Figure 2.** Co-occurrence network and assembly processes analysis of soil microbial communities in tea gardens from different regions. (**a**) Bacterial co-occurrence network; (**b**) fungal co-occurrence network; node size represents the degree (i.e., the number of connections per node). Red edges indicate positive correlations, while green edges indicate negative correlations. Different node colors correspond to bacterial and fungal taxa classified at the phylum level. Panels (**c**,**d**) represent the assembly processes of bacterial communities, while panels (**e**,**f**) depict the assembly processes of fungal communities. Values represent means ± standard deviation (n = 3 biological replicates per region). Wenxian (W), Wudu (WD) and Kangxian (K) denote the three counties included in this study.

**Table 1.** Topological parameters of soil bacterial co-occurrence networks in tea garden soils from different regions of Longnan City, China.

| Topological | Bacteria | | | Fungi | | |
|---|---|---|---|---|---|---|
| **Parameters** | **W** | **WD** | **K** | **W** | **WD** | **K** |
| Nodes | 288 | 284 | 281 | 211 | 232 | 224 |
| Links | 8623 | 9259 | 5624 | 3328 | 3913 | 3205 |
| Positive links % | 68.76% | 60.16% | 57.52% | 87.80% | 77.31% | 85.87% |
| Negative links % | 31.24% | 39.84% | 42.48% | 12.20% | 22.69% | 14.13% |
| Average degree | 59.882 | 65.204 | 40.028 | 31.545 | 33.733 | 28.616 |
| Grpah density | 0.209 | 0.23 | 0.143 | 0.15 | 0.146 | 0.128 |
| Modularity | 0.474 | 0.515 | 0.834 | 0.768 | 0.778 | 0.825 |

Note; Wenxian (W), Wudu (WD), and Kangxian (K) represent the three counties within Longnan City.

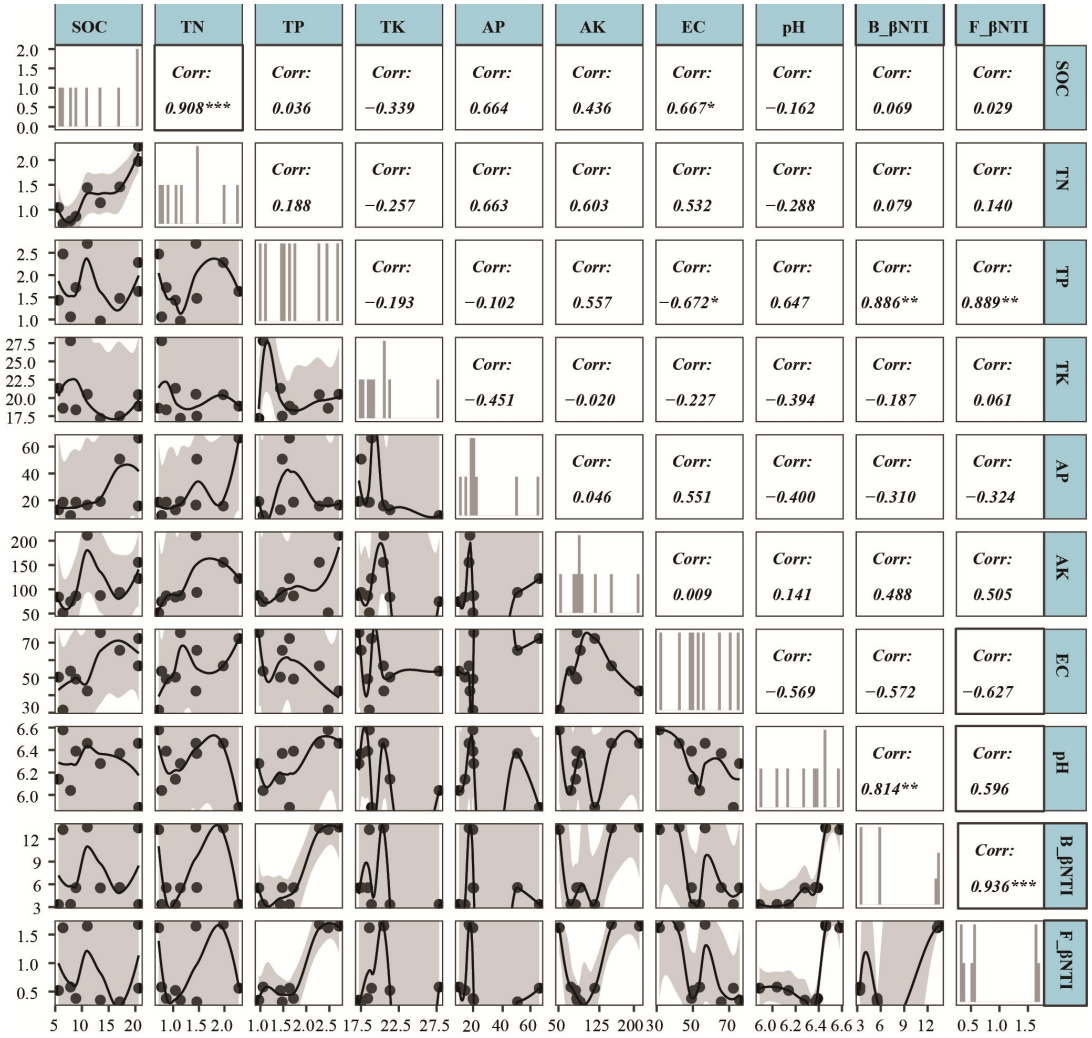



**Figure 3.** Correlation analysis between soil physicochemical factors and β-nearest taxon index (βNTI) of soil microbial communities. The upper panel displays the correlation coefficients and corresponding *p*-values between each pair of variables. Numerical values within the boxes indicate correlation strength, and significance levels are marked with asterisks: *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$. Non-significant values are left unmarked. The middle panel shows density plots for each individual variable, illustrating the distribution of data; the location of peaks on the X-axis indicates where the majority of values are concentrated. The lower panel presents line plots for each variable, showing their variation trends across samples. "B_βNTI" represents the β-nearest taxon index (βNTI) of bacterial communities, and "F_βNTI" represents that of fungal communities.

### 3.2. Soil Ecosystem Multifunctionality

As shown in Figure 4, apparent regional differences were observed in the stoichiometric characteristics of soil nutrients and enzyme activities among the three counties. The C/N ratio in Wudu was higher than in the other two counties, while the C/P and N/P ratios were highest in Wenxian, indicating a greater degree of phosphorus limitation in Wenxian soils. The VL of soil enzyme stoichiometry was used to further assess microbial nutrient limitation, with a longer VL indicating more substantial carbon limitation and a VA greater than 45° reflecting phosphorus limitation. The current results show that Wudu had the highest VL, indicating that microbial carbon limitation was most pronounced in this region. Additionally, VA exceeded 45° in all three counties, suggesting that microbial communities across all sites experienced a certain degree of phosphorus limitation.

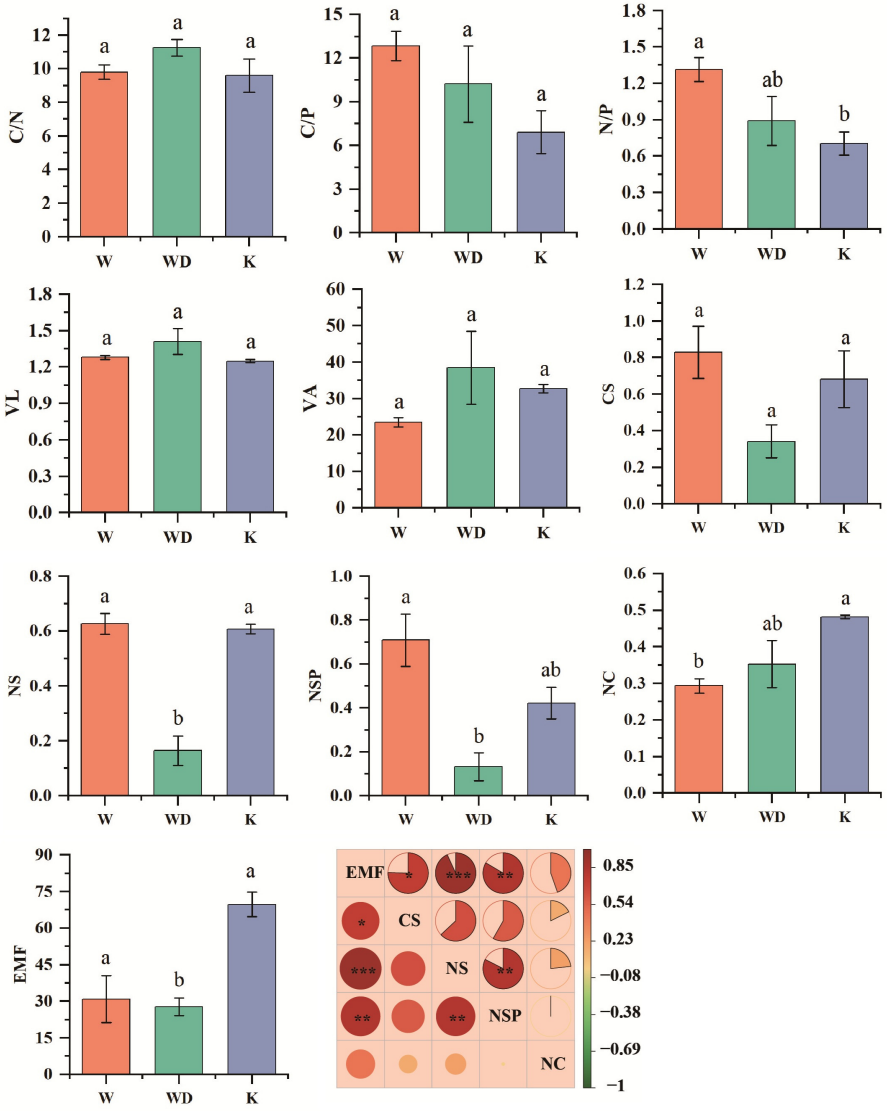



**Figure 4.** Soil chemical properties, enzyme stoichiometric characteristics, and nutrient functions in tea gardens from different regions. Different lowercase letters indicate significant differences among regions ($p < 0.05$). C/N, carbon-to-nitrogen ratio; C/P, carbon-to-phosphorus ratio; N/P, nitrogen-to-phosphorus ratio; VL, vector length; VA, vector angle. CS, soil carbon sequestration function; NS, nutrient storage function; NSP, nutrient supply function; NC, nutrient cycling function; EMF, ecosystem multifunctionality. Values represent means ± standard deviation ($n = 3$ biological replicates per region). Wenxian (W), Wudu (WD), and Kangxian (K) denote the three counties included in this study. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Distinct patterns in soil nutrient-related functions were observed among tea gardens from different counties (Figure 4). The carbon sequestration, nutrient storage, and nutrient supply functions of soils were significantly higher in Wenxian compared to the other counties ($p < 0.05$), whereas nutrient cycling and overall ecosystem multifunctionality were substantially higher in Kangxian ($p < 0.05$). Notably, soil carbon sequestration, nutrient storage, and nutrient supply functions were positively correlated with overall soil multifunctionality, with correlation coefficients of 0.75, 0.93 and 0.83, respectively, indicating that enhancing individual functions promoted overall ecosystem multifunctionality. Structural equation modeling was conducted to elucidate further the pathways influencing ecosystem multifunctionality in tea gardens across Longnan (Figure 5). The results showed that the C/P ratio exerted significant adverse effects on carbon sequestration, nutrient storage, and ecosystem multifunctionality ($p < 0.05$), while the N/P ratio had substantial positive effects on these functions ($p < 0.05$). Soil pH had a significant positive impact on nutrient cycling but a significant adverse effect on nutrient supply ($p < 0.05$). Fungal community structure had substantial adverse effects on soil carbon sequestration, nutrient storage, and ecosystem multifunctionality ($p < 0.05$), whereas bacterial community structure had a significant positive impact on nutrient storage, nutrient supply, nutrient cycling, and ecosystem multifunctionality ($p < 0.05$; Figure 4). Random forest analysis was used to identify the most critical soil and microbial predictors of ecosystem multifunctionality. The results indicated that soil nutrient storage and supply functions were the most important explanatory variables for ecosystem multifunctionality (Figure 6).

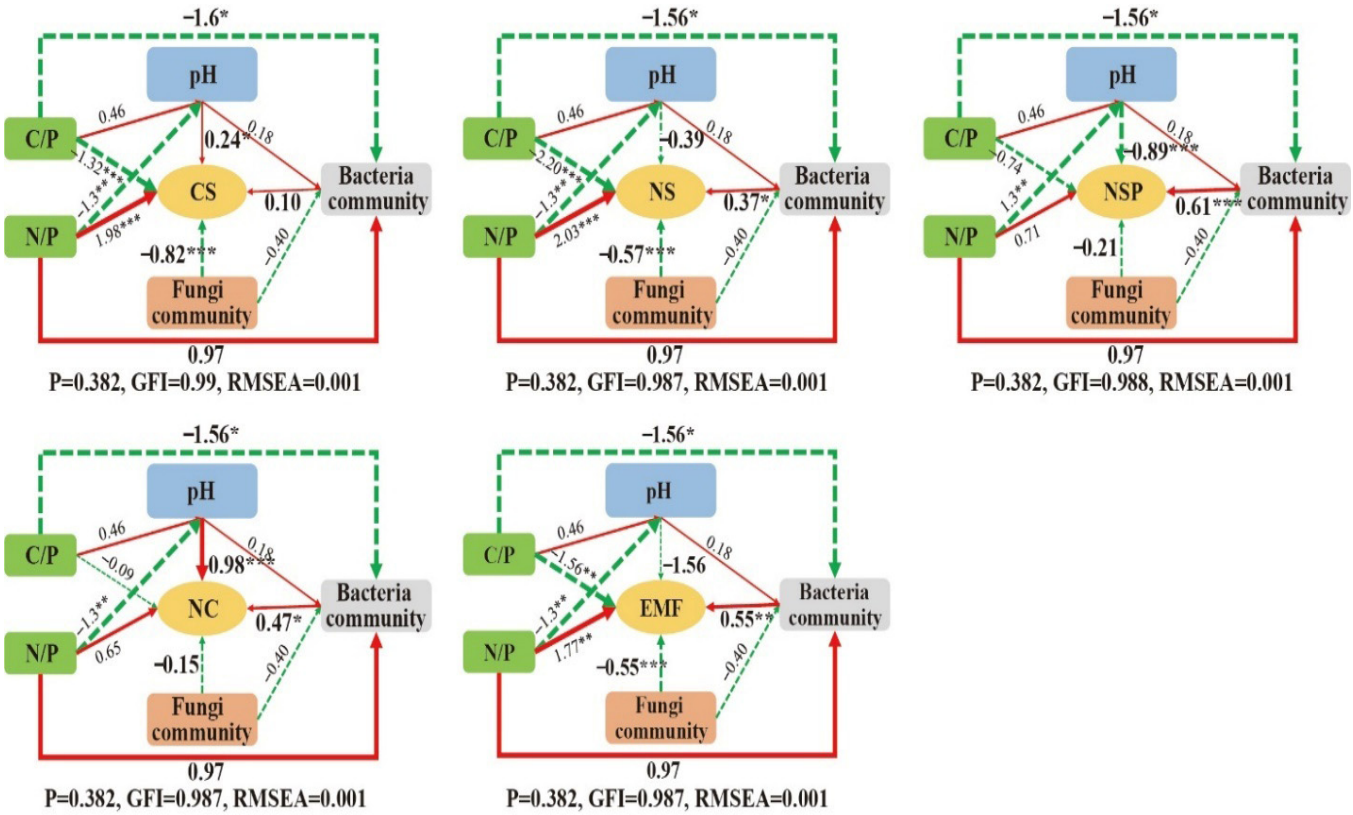



**Figure 5.** Structural equation modeling analysis of the effects of environmental factors on ecosystem functions in tea gardens from different regions. Arrow width is proportional to the strength of the path coefficient. Solid red arrows indicate positive relationships, while dashed green arrows indicate negative relationships. Abbreviations: CS, soil carbon sequestration function; NS, nutrient storage function; NSP, nutrient supply function; NC, nutrient cycling function; EMF, ecosystem multifunctionality. * $p < 0.05$; ** $0.001 < p < 0.05$; *** $p < 0.001$.

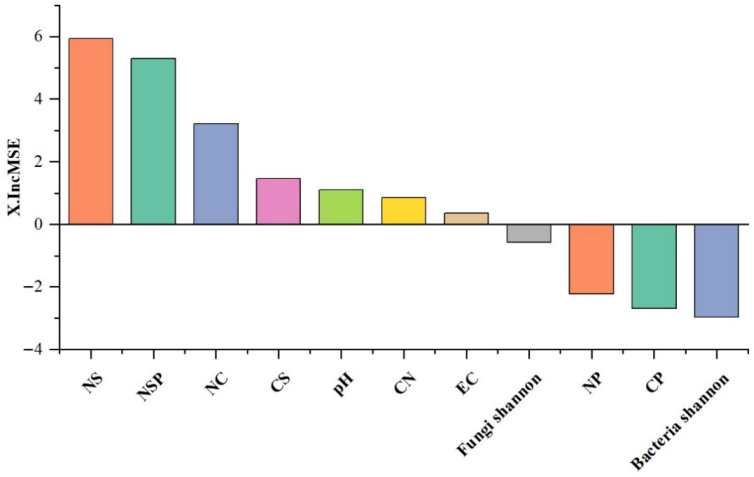



**Figure 6.** Importance of explanatory variables for ecosystem multifunctionality as determined by random forest analysis.

### *3.3. Matching Analysis of Spatial Interpolation Results of Soil Heavy Metals with Irrigation Water Sampling Points*

As shown in Figure 7, the soil physicochemical properties and enzyme activities varied significantly among the three regions. In Wenxian, available phosphorus, total potassium, urease, and polyphenol oxidase activities were higher than those in the other regions, while soil organic carbon, cellulase, pH, and acid phosphatase were comparatively lower. In Wudu, soil organic carbon, cellulase, available phosphorus, and electrical conductivity (EC) were higher, whereas total potassium, total nitrogen, urease, and polyphenol oxidase were lower than in the other regions. In Kangxian, pH, acid phosphatase, catalase, total phosphorus, and available potassium were higher, while available phosphorus and EC were lower compared to the other two regions.

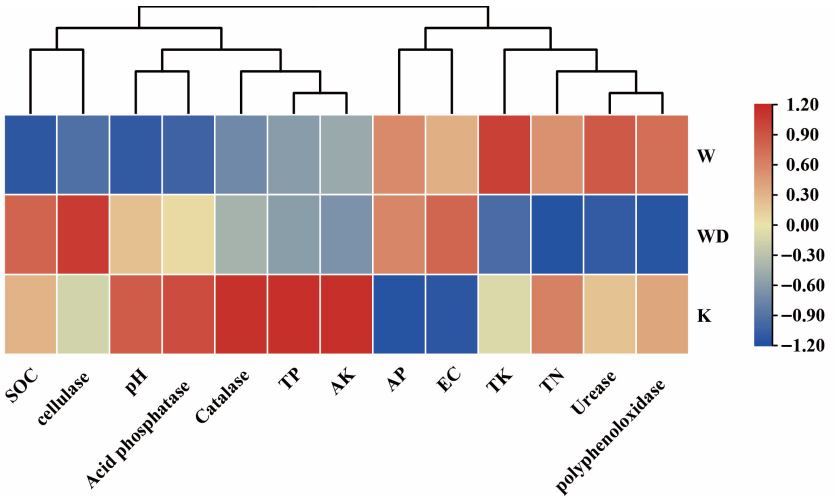



**Figure 7.** Soil nutrient contents and enzyme activities across tea gardens in different regions.

To investigate the key ecological factors influencing the composition of soil bacterial and fungal communities, redundancy analysis (RDA) was performed using the relative abundances of bacterial and fungal phyla as response variables and soil nutrient functions as explanatory variables (Figure 8). The RDA results for the bacterial community indicated that the abundances of *Bacteroidota*, *Proteobacteria*, *Firmicutes*, *Cyanobacteria* and *Latescibacterota* were positively correlated with carbon sequestration (CS), nutrient storage (NS), and nutrient supply (NSP) functions. In contrast, *Gemmatimonadota*, *Chloroflexi*, *Acidobacteriota*

and *Myxococcota* were positively correlated with nutrient cycling (NC) and soil pH. For the fungal community, RDA revealed that *Glomeromycota*, *Basidiomycota*, *Kickxellomycota* and *Chytridiomycota* were positively associated with pH, NC, NS, and NSP functions, while *Ascomycota* and *Calcarisporiellomycota* were positively correlated with CS.

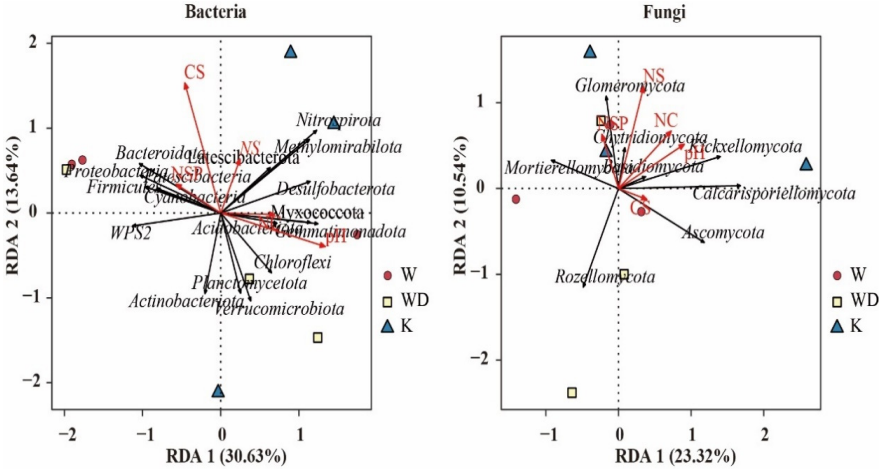



**Figure 8.** Relationships between dominant bacterial and fungal phyla and soil ecosystem functions as revealed by redundancy analysis (RDA). CS, carbon sequestration; NS, nutrient storage; NSP, nutrient supply; NC, nutrient cycling.

## 4. Discussion

The study's findings supported our hypotheses. Significant positive correlations were observed among carbon sequestration, nutrient storage, nutrient supply, and ecosystem multifunctionality, confirming the presence of synergistic relationships among these functions. Additionally, regional differences in soil nutrient status were closely associated with distinct microbial community structures, mediating key ecosystem functions, thus substantiating the second hypothesis.

### *4.1. Drivers of Soil Microbial Community Structure in Tea Gardens*

Soil microorganisms are characterized by their small size, wide distribution, short life cycles, and remarkable diversity, enabling them to respond to environmental disturbances rapidly [42]. These traits allow microbial communities to adapt to changing conditions and maintain ecosystem stability swiftly [43]. In this study, the relative abundances of *Proteobacteria* and *Actinobacteriota* were lowest in Kangxian and highest in Wenxian, while *Acidobacteriota* exhibited the opposite trend, being most abundant in Kangxian and least in Wenxian (Figure 1). This pattern may be attributed to the higher electrical conductivity and available phosphorus content but relatively lower organic C and total N in Wenxian soils. Such nutrient-rich conditions favor the proliferation of copiotrophic taxa like *Proteobacteria* and *Actinobacteriota*, while suppressing oligotrophic groups such as *Acidobacteriota*. Conversely, Kangxian soils, with the highest levels of organic C, total N, and available K but the lowest available P, likely contain organic matter in more recalcitrant forms. This environment is more suitable for oligotrophic *Acidobacteriota* while limiting the abundance of *Proteobacteria* and *Actinobacteriota* [44,45]. For fungi, *Ascomycota* and *Mortierellomycota* were most abundant in Wenxian, whereas Basidiomycota reached their highest abundance in Kangxian and lowest in Wenxian (Figure 1). This may be explained by the pronounced N transformation in Wenxian soils, where the highest urease activity and elevated available P act synergistically to enrich Ascomycota [46]. In Kangxian, high acid phosphatase activity compensates for low available P, creating a P-limited environment that promotes the growth of phosphate-solubilizing *Mortierellomycota* and selects *Basidiomycota*, which

are adept at degrading recalcitrant organic matter. Both groups secrete phosphatases and lignin-degrading enzymes to cope with phosphorus limitation [47].

The highest bacterial richness and diversity indices were observed in Kangxian soils, while Wenxian had the lowest. Similarly, fungal richness and diversity indices were higher in Wudu and Kangxian than in Wenxian (Figure 1). This may be due to frequent fertilization in Wenxian, which creates a nutrient-rich environment (with higher available P than Kangxian) that imposes selective pressure, allowing fast-growing r-strategists (e.g., *Proteobacteria*) to dominate and monopolize resources, thereby reducing microbial diversity. In contrast, P limitation and high-quality organic matter inputs in Kangxian create multidimensional ecological niches, promoting the coexistence of functional microbial groups [48]. The high cellulase activity in Wudu suggests a unique carbon cycling pattern that supports fungal diversity [49]. These findings support the intermediate disturbance hypothesis in soil ecosystems and indicate that reducing fertilizer input and increasing organic matter diversity may help restore microbial ecological balance in Wenxian tea gardens. In comparison, current management practices in Kangxian and Wudu appear more conducive to maintaining microbial diversity [50].

Microbial co-occurrence networks reflect interactions among different taxa within microbial communities and serve as tools to assess community complexity. They have been successfully applied to analyze the effects of environmental factors on microbial communities [51]. Significant differences in soil microbial co-occurrence networks were observed among tea gardens from different counties in Longnan (Figure 2a,b and Table 1). In particular, Wenxian exhibited a higher proportion of positive links, indicating stronger cooperative relationships among bacterial and fungal taxa [52]. This may be closely related to the unique montane climate in the region; the elevation gradient (Wenxian ~1194 m, Kangxian 1660 m) results in substantial temperature differences, with Wenxian's lower elevation providing a warmer growing season (annual mean temperature 2–3 °C higher than Kangxian), thereby promoting cooperative interactions within microbial communities [53].

A null model analysis was conducted to elucidate further microbial community structure drivers [54]. Deterministic processes, specifically heterogeneous selection, primarily governed the assembly of bacterial communities in Wenxian, Wudu, and Kangxian, whereas stochastic processes, particularly ecological drift, dominated fungal community assembly (Figure 2c–f). This can be attributed to the complex topography of the western Qinling Mountains, where local climate (e.g., precipitation, temperature) and soil properties (pH, organic matter, N and P content) vary significantly among counties. Bacterial communities are susceptible to environmental fluctuations and are more readily influenced by abiotic filtering. In contrast, fungal community composition often fluctuates due to biotic factors, such as animal disturbance and plant-root interactions [55]. Correlation analysis revealed that bacterial βNTI was significantly positively correlated with total phosphorus and soil pH, while fungal βNTI was significantly positively correlated with total phosphorus. These results suggest that variations in soil total phosphorus and pH may alter ecological niches in the soil environment, thereby influencing species coexistence and competition dynamics.

### *4.2. Soil Ecosystem Multifunctionality in Tea Gardens*

Cultivating healthy soils is a prerequisite for enhancing ecosystem multifunctionality [56]. In recent decades, increasing attention has been paid to soil functions to optimize land resource allocation and improve ecosystem services. The current findings indicated that Wenxian soils exhibited significantly greater C sequestration, nutrient storage, and supply functions (Figure 3), likely due to favorable hydrothermal conditions promoting organic matter accumulation and the protective effects of clay minerals on nutrients [57]. In contrast, Kang County performed better in nutrient cycling and overall ecosystem multifunctionality,

which is closely related to its higher microbial diversity and more efficient enzyme activities. This is mainly because mutualistic interactions among diverse microbial taxa can enhance the efficiency of complex soil functions. High microbial diversity increases soil resistance and resilience to disturbance, and improves resource use efficiency, thereby enhancing the overall multifunctionality of the soil ecosystem. Therefore, soils with greater microbial diversity tend to have a stronger capacity to simultaneously sustain productivity, nutrient cycling, and environmental regulation functions [58]. Additionally, since nutrient-related functions are largely determined by soil physicochemical properties, regional differences in these properties among tea garden soils ultimately lead to variation in the corresponding ecosystem functions.

Moreover, the current study demonstrated significant synergistic effects among soil carbon sequestration, nutrient storage, nutrient supply, and overall ecosystem multifunctionality [3]. The mutual enhancement of these functions, particularly the substantial contributions of nutrient storage and supply, led to improved soil multifunctionality. This may be because C sequestration provides an essential substrate for nutrient storage, facilitates soil aggregate formation, and enhances nutrient retention [59]. An efficient nutrient storage system ensures sustained nutrient supply, supporting microbial activity and plant growth [60]. Enhanced nutrient cycling further promotes organic matter decomposition and nutrient release, establishing a positive feedback loop [61–63]. Such functional synergy collectively builds a stable soil nutrient reservoir, improves soil physical structure and biological activity, and ultimately enhances overall ecosystem functioning.

This study also examined the mediating roles of soil nutrients and microbial communities in shaping ecosystem functions and multifunctionality. The results revealed that the C/P ratio had significant adverse effects on carbon sequestration, nutrient storage, and ecosystem multifunctionality, while the N/P ratio exerted substantial positive effects (Figure 4). A high C/P ratio reflects relative phosphorus deficiency, which can constrain microbial activity and enzyme function, slow organic matter decomposition and nutrient cycling, and ultimately reduce ecosystem C sequestration and nutrient storage efficiency [64]. Conversely, an optimal N/P ratio favors metabolic activity in plants and microbes and promotes photosynthesis, N fixation, and nutrient use efficiency, thereby enhancing C sequestration, nutrient storage, and overall multifunctionality [65]. Soil pH significantly positively affected carbon sequestration and nutrient cycling but adversely affected nutrient supply. Lower pH can inhibit microbial decomposition, slow organic matter mineralization, and promote organic C accumulation [66]. Additionally, low pH can increase the solubility of toxic elements such as aluminum and manganese, inhibit root growth, and cause essential nutrients (P, K, Ca and Mg) to become fixed or leached, reducing their availability [67].

The fungal community structure exhibited significant negative effects on soil carbon sequestration, nutrient storage, and overall ecosystem multifunctionality, whereas the bacterial community structure had strong positive impacts on nutrient storage, supply, cycling, and multifunctionality. This difference may be attributed to the decomposition strategies of fungi, which tend to accelerate the mineralization of organic matter. Fungi often allocate more carbon substrates to respiration to fuel the synthesis of nitrogen-mineralizing enzyme systems, resulting in 60–85% of organic carbon being mineralized and released as $CO_2$. Consequently, this leads to rapid carbon loss from the soil. Moreover, fungi typically rely on high C/N organic substrates and can intensify nutrient competition, thereby reducing the retention of available soil nutrients [68]. These mechanisms are consistent with our findings in Wen County, where fungal dominance reached 92.86% and bacterial diversity was low—indicating a typical scenario in which fungal-driven mineralization enhances short-term nutrient availability at the expense of long-term carbon and nitrogen storage. In

contrast, bacteria preferentially utilize labile organic matter, promote nutrient mineralization and turnover, and enhance nutrient availability. Certain bacteria (e.g., nitrogen-fixing and phosphate-solubilizing taxa) directly participate in biogeochemical cycles, increasing soil fertility [69,70]. Rapid nutrient cycling driven by bacteria helps maintain high ecosystem multifunctionality. Therefore, fungal and bacterial community structure differences result in distinct regulatory effects on key soil ecological processes.

### *4.3. Limitations and Future Perspectives*

While this study provides new insights into the microbial mechanisms regulating soil multifunctionality in tea gardens (Figure 9), several limitations should be acknowledged. First, the sampling was confined to three counties within a single region and a single season, limiting the generalizability of the findings across broader spatial and temporal scales. Second, while high-throughput sequencing and network analyses provide robust community-level insights, they do not capture functional gene expression or direct microbial activity, which may be further elucidated by metagenomics or transcriptomics in future studies. Third, the study focused primarily on bacteria and fungi; the roles of archaea, viruses, and soil fauna remain unexplored. Future research should expand spatial and temporal sampling, incorporate multi-omics approaches to link community composition with functional potential and activity and investigate the interactions between microbial communities and plant roots, soil fauna and environmental gradients. Long-term field experiments manipulating nutrient inputs and organic matter diversity also help clarify causal relationships and inform sustainable management practices for tea agroecosystems. Overall, the findings of this study demonstrated that regional variation in soil nutrients and microbial community shapes ecosystem multifunctionality in tea gardens. The synergistic interplay among carbon sequestration, nutrient storage, and nutrient supply functions is a key driver of multifunctionality, with bacterial communities playing a central regulatory role. These findings provide a scientific basis for optimizing soil management and promoting sustainability in tea-producing regions.

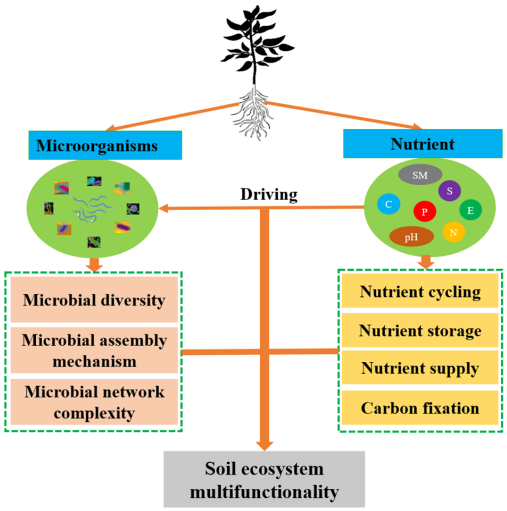



**Figure 9.** Schematic diagram illustrating the regulatory mechanisms by which soil microbial communities and nutrient properties drive ecosystem multifunctionality in tea gardens. The diagram depicts how soil microorganisms, through their diversity, community assembly processes, and network complexity, and key soil nutrient factors (such as carbon, nitrogen, phosphorus, pH, and enzyme activities) jointly regulate essential ecosystem functions, including nutrient cycling, nutrient storage, nutrient supply, and carbon fixation. The synergistic interactions between microbial communities and soil nutrients underpin the overall multifunctionality of tea garden soils, supporting sustainable tea cultivation.

## 5. Conclusions

This study provides robust evidence that soil carbon sequestration, nutrient storage, and nutrient supply functions are significantly higher in Wenxian tea gardens compared to those in Wudu and Kangxian, whereas nutrient cycling and overall ecosystem multifunctionality are markedly greater in Kangxian than in Wenxian and Wudu. The results demonstrate that soil C/P and N/P ratios significantly influence C sequestration, nutrient storage, and ecosystem multifunctionality. Soil pH also plays a critical role, significantly affecting carbon sequestration, nutrient supply, and nutrient cycling functions. Both bacterial and fungal community structures are closely linked to nutrient storage and ecosystem multifunctionality. Moreover, strong synergistic effects were observed among soil carbon sequestration, nutrient storage, nutrient supply, and nutrient cycling functions, collectively enhancing soil multifunctionality. Nutrient storage and supply functions contributed most substantially to overall multifunctionality. These findings offer valuable theoretical support for the sustainable management and utilization of tea gardens in Longnan. They provide new insights into how soil properties and management practices can be leveraged to improve soil ecosystem services and ensure long-term productivity and ecological sustainability in tea-producing regions.

**Author Contributions:** Conceptualization, L.N. and X.Z.; methodology, J.L.; formal analysis, X.Z.; investigation, Y.T. and Z.W.; data curation, X.Z.; writing—original draft preparation, L.N., L.D. and W.W.; writing—review and editing, F.U.H. and X.Z.; visualization, J.Z. and Q.L.; supervision, X.Z.; funding acquisition, X.Z. and Y.T. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the Key Research and Development Program of Gansu Academy of Agricultural Sciences (2024GAAS11); the Central-Guided Local Science and Technology Development Fund Project (23ZYQA0323); the National Key Research and Development Program Subproject (2023YFD1900405-01); the Key Science and Technology Project of the Regional Innovation Center under the Gansu Modern Agricultural Science and Technology Support System (2023GAAS07); the Gansu Provincial Science and Technology Program (25CXGA046); the Key Research and Development Program of Gansu Province (25YFNA021); and the Scientific and Technological Achievement Transformation Project of Gansu Academy of Agricultural Sciences (2024GAAS-CGZH04-1).

**Institutional Review Board Statement:** Not applicable as animal or human study was not involved.

**Data Availability Statement:** The sequence data associated with this project have been deposited in NCBI database under accession number PRJNA1272242.

**Acknowledgments:** We sincerely thank the anonymous reviewers for valuable comments on the manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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