

Article

Gradient Organic Fertilizer Substitution Reshapes Soil Microecology and Microbial Carbon Utilization in Tea Plantations

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Abstract

Organic fertilizer substitution is widely used to improve acidified tea plantation soils, but the coupled effects of substitution gradients on soil aggregates, microbial carbon utilization, and microbial C–N–P limitation remain insufficiently quantified. This study aimed to determine how gradient organic fertilizer substitution regulates soil physicochemical properties, aggregate-size distribution, extracellular enzyme stoichiometry, and microbial carbon-source utilization in a subtropical tea plantation, and to identify a suitable substitution range under the experimental conditions. A long-term field experiment was conducted in an acidic red-soil tea plantation in Wuyishan, Fujian Province, China. Five treatments were established: pure chemical fertilizer (CF) and organic fertilizer substituting 33% (OF33), 50% (OF50), 67% (OF67), and 100% (OF100) of chemical fertilizer nitrogen. Tea growth indices, soil physicochemical properties, aggregate mechanical stability, microbial biomass C, N, and P (MBC, MBN, and MBP), extracellular enzyme activities, and BIOLOG-ECO carbon-source utilization profiles were measured. Results showed that OF33 and OF67 increased soil pH by 0.46–0.60 units relative to CF. OF33 generally improved available N and K and aggregate stability indices, whereas OF50 increased 100-bud weight and microbial carbon-source richness. OF100 increased MBC but also increased vector length and vector angle, indicating stronger relative C and P limitation. BIOLOG-ECO profiles separated treatments, and moderate substitution (OF33–OF50) broadened the carbon-source utilization niche. However, different indicators peaked under different treatments; therefore, 33–50% substitution should be regarded as a suitable range under the conditions of this experiment rather than a universally optimal rate. These findings provide a site- and cultivar-specific basis for reducing chemical fertilizer use in red-soil tea plantations.

Keywords: tea plantations; organic fertilizer substitution; soil aggregates; ecological enzyme stoichiometry; BIOLOG; soil nutrient limitation



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1. Introduction

The tea plant (*Camellia sinensis*) is an iconic perennial economic tree in China's subtropical red soil regions, and the tea industry is a core sector supporting income growth for farmers in the southern hilly and mountainous areas. In pursuit of high yields of fresh

early-spring buds, the long-term, excessive, and single-component application of nitrogen, phosphorus, and potassium chemical fertilizers has become the mainstream cultivation practice in tea plantations. However, the continuous use of chemical fertilizers over many years has exacerbated a series of ecological risks, including red soil acidification and soil fertility decline. According to statistics, the soil pH in more than 65% of China's mountainous tea plantations is below 4.5. The inherent high leaching characteristics of red soil, combined with the accumulation of hydrogen ions from chemical fertilizers, lead to the leaching of large amounts of active aluminum ions, which in turn inhibit the development of lateral roots in tea plants and the expression of nutrient transporter proteins in the root system. At the same time, soil active organic carbon continues to deplete, the quantitative ratio of carbon, nitrogen, and phosphorus elements is severely imbalanced, rhizosphere microbial community diversity declines, and nutrient transformation functions weaken. Ultimately, this results in reduced hundred-bud weight and chlorophyll content in tea leaves, while the risk of non-point source nitrogen and phosphorus runoff continues to rise [1–3]. Partial replacement of chemical fertilizers with organic fertilizers is a core ecological soil-improvement technology for mitigating soil acidification in tea plantations, rebuilding soil carbon pools, and restoring the root-zone microecosystem; however, there remains a lack of systematic, integrated analysis of the differential regulatory effects of organic fertilizer substitution gradients on soil physicochemical properties, aggregate structure, and microbial carbon metabolism, as well as of the underlying multiscale coupling mechanisms [4,5].

Rhizosphere microorganisms are the core drivers of the biogeochemical cycles of carbon, nitrogen, and phosphorus in soil, and their preferences for carbon substrate utilization and life history trade-off strategies influence whether soil organic matter is mineralized or sequestered. The microbial life-history theory, based on resource availability trade-offs, classifies microbial communities into nutrient-rich acquisition types (copiotrophs, Strategy A) and nutrient-poor sequestration types (oligotrophs, Strategy Y). The amounts of carbon, nitrogen, and phosphorus sequestered within soil microorganisms are referred to as soil microbial biomass carbon (MBC), soil microbial biomass nitrogen (MBN), and soil microbial biomass phosphorus (MBP), respectively. When the supply of soil carbon, nitrogen, and phosphorus deviates from microbial metabolic requirements, the microbial community invests significant energy in synthesizing extracellular enzymes to degrade organic matter and compensate for nutrient deficits, favoring the mineralization-oriented Strategy A; conversely, when nutrient supply matches microbial demand, the community reduces its investment in catabolism and converts more assimilated carbon into microbial residues, thereby activating the sequestration-type Y pathway [6,7]. This theory is also supported by long-term controlled nutrient-supplementation experiments, and related research is currently delving deeper into the mechanisms by which nutrient balance and microbial interactions influence the soil carbon cycle. Ecoenzymatic stoichiometry is a standardized method for quantitatively characterizing microbial carbon, nitrogen, and phosphorus nutrient stress. By analyzing the ratios of enzyme activities for carbon, nitrogen, and phosphorus, as well as the lengths and angles of nutrient limitation vectors, it can intuitively quantify the relative intensity of resource limitations faced by microorganisms under different fertilization gradients [8,9]. BIOLOG-ECO carbon-source fingerprinting enables non-destructive, high-throughput characterization of the carbon substrate utilization profile, functional niche breadth, and functional redundancy of the actively respiring microbial community. The combined application of these two techniques can systematically and comprehensively characterize the entire process by which organic fertilizer input gradients reshape microbial carbon metabolic functions [10,11].

Soil aggregates serve as important physical carriers for microbial colonization and the adsorption and retention of extracellular enzymes. Within aggregates of different particle sizes, aerobic-anaerobic stratified microenvironments form, directly regulating substrate diffusion, oxygen supply, and the assembly patterns of microbial communities; their mechanical stability acts as a key intermediary linking soil physicochemical properties and microbial functions [12,13]. Existing studies in farmland and tea plantations indicate that the application of organic fertilizer promotes the formation of large aggregates. However, although several studies have used only two fertilization levels (chemical fertilizer vs. fully organic fertilizer), the combined assessment of soil aggregates, ecoenzymatic indicators, and microbial carbon-source utilization across a quantitative substitution gradient remains limited. At the same time, existing studies often focus solely on soil nutrients, community structure revealed by high-throughput sequencing, or individual enzyme activity indicators. Very few studies integrate multidimensional indicators—such as soil aggregate structure, physicochemical nutrients, ecoenzymatic metrics, and microbial carbon-source utilization—into a single analytical framework, and the integrated responses of soil physicochemical properties, aggregate microhabitats, and microbial carbon metabolism to organic substitution gradients remain poorly characterized [14,15]. Furthermore, most fertilization studies in tea plantations focus solely on changes at the soil level, lacking quantitative analyses of the relationships between agronomic indicators—such as tea plant photosynthetic characteristics and fresh leaf yield—and the soil microecosystem. Consequently, it is difficult to provide guidance on reducing chemical fertilizer application in the field that aligns with actual production needs [16,17].

Redundancy analysis (RDA) is a classic constrained ordination method for analyzing the relationships among multiple environmental factors, crop traits, and microbial functions. It quantifies the explanatory power of different types of environmental variables on microbial carbon utilization functions, thereby elucidating the key drivers of microbial functional changes under graded fertilization [18,19]. To address gaps in existing research, this study utilized a long-term fixed-site fertilization experiment platform in subtropical red soil tea plantations. Five gradient treatments were established, including pure chemical fertilizer (CF) and organic fertilizer substitution rates of 33% (OF33), 50% (OF50), 67% (OF67), and 100% (OF100) based on organic nitrogen replacement. Tea plant growth indices, soil physicochemical properties, aggregate mechanical stability, microbial biomass carbon, nitrogen, and phosphorus, extracellular enzyme activities, and BIOLOG-ECO carbon-source utilization profiles were systematically measured. The overall objective was to evaluate how gradient organic nitrogen substitution regulates the soil microecosystem of tea plantations and to identify a suitable substitution rate under the experimental conditions. Specifically, we aimed to (1) quantify the gradient-dependent responses of soil acidification mitigation, soil nutrient pools, aggregate mechanical stability, and tea growth to organic substitution; (2) characterize the responses of microbial life-history strategies and extracellular enzyme secretion to fertilization-induced nutrient imbalances using ecoenzymatic stoichiometry; and (3) evaluate changes in the microbial carbon-source utilization niche and functional diversity using BIOLOG-ECO combined with RDA and random forest modeling. In contrast to previous studies that used only two fertilization levels or focused on single indicators [14,15], this study combined gradient organic substitution with an integrated framework linking soil physicochemical properties, aggregate microhabitats, and microbial carbon metabolism. We hypothesized that moderate organic substitution would alleviate soil acidification and broaden the microbial carbon-source utilization niche, whereas complete substitution would intensify microbial carbon limitation. These findings provide a theoretical basis for reducing chemical fertilizer use and establishing microorganism-driven green cultivation systems in red-soil tea plantations.

2. Materials and Methods

2.1. Overview of Field Experiments

The experimental site was located in Xingcun Town, Wuyishan City, Fujian Province (27.66° N, 117.89° E). The region has a subtropical monsoon climate with an average annual temperature of 19 °C and an average annual precipitation of 1284 mm. The test soil is mountainous acidic red soil. The field experiment was established in 2021 and has been maintained continuously under the same fertilization regime for 5 years. The tea variety used in the experiment was Huang Guanyin, with trees 25 years old. A double-row planting pattern was adopted, with a row spacing of 1.5 m and a plant spacing of 0.33 m. The experiment employed a completely randomized block design, with three independent plots per treatment serving as biological replicates; each plot covered an area of 13.5 m². The organic substitution rate was calculated on a nitrogen basis as follows: substitution rate (%) = (N supplied by organic fertilizer/total N applied) × 100%. The total nitrogen input was kept equal across all treatments (105 kg N ha⁻¹); because the organic fertilizer contained lower P₂O₅ and K₂O concentrations (3% each) than the compound fertilizer (15% each), the P₂O₅ and K₂O inputs were approximately balanced across treatments (90–105 kg ha⁻¹; Table 1). A total of five fertilization gradients were established, including chemical fertilizer (CF), organic fertilizer substitution rates being 33% (OF33), 50% (OF50), 67% (OF67), and 100% (OF100).

Table 1. Fertilizer application amounts and nutrient inputs of the five treatments.

Treatment	Organic Fertilizer (kg ha ⁻¹)	Compound Fertilizer (kg ha ⁻¹)	Total N (kg ha ⁻¹)	Total P ₂ O ₅ (kg ha ⁻¹)	Total K ₂ O (kg ha ⁻¹)	Supplementary P ₂ O ₅ (kg ha ⁻¹)	Supplementary K ₂ O (kg ha ⁻¹)
CF	0	700	105	105	105	0	0
OF33	990	469	105	105	105	4.95	4.95
OF50	1500	350	105	105	105	7.5	7.5
OF67	2010	231	105	105	105	10.05	10.05
OF100	3000	0	105	105	105	15.0	15.0

Note: CF: chemical fertilizer; OF33, OF50, OF67, and OF100 represent treatments in which 33%, 50%, 67%, and 100% of the nitrogen was supplied by organic fertilizer, respectively. Fertilizer application rates were calculated on an equal-nitrogen basis: the total N input was 105 kg N ha⁻¹ in all treatments (compound fertilizer N-P₂O₅-K₂O = 15-15-15; organic fertilizer N-P₂O₅-K₂O = 3.5-3-3). The P₂O₅ and K₂O supplied by the organic and compound fertilizers were 100.05, 97.5, 94.95, and 90.0 kg ha⁻¹ for OF33, OF50, OF67, and OF100, respectively, i.e., lower than the CF level; the differences (4.95, 7.5, 10.05, and 15.0 kg ha⁻¹, respectively) were supplemented with inorganic phosphorus and potassium fertilizers so that the total P₂O₅ and K₂O inputs were held constant at 105 kg ha⁻¹ across all treatments.

The organic fertilizer was purchased from Beijing Zhonglvkang Biotechnology Co., Ltd., (Beijing, China) with nutrient content of total nitrogen 3.5%, P₂O₅ 3%, K₂O 3%, organic matter content ≥45%, and the field application rate was 0.47 kg·m⁻¹. Compound fertilizer was sourced from Anhui Hongjingtian Agricultural Technology Co., Ltd., (Anhui, China) and was a balanced 15-15-15 ternary compound fertilizer, with N, P, and K each at 15%. The application amounts of organic and compound fertilizers in each treatment were adjusted to maintain equal total N input and are listed in Table 1. Base fertilizer was uniformly applied via single-sided trenches on October 13 each year, and the application trenches were 20 cm wide, 25 cm deep, and located 30 cm from the main trunk of the tea plants. On April 25 of the following year, sample collection was uniformly completed during the vigorous growth period of the spring tea leaves. Field management practices—including weeding, irrigation, and integrated green pest and disease control—were fully standardized across all treatments throughout the entire growing season.

2.2. Sample Collection

2.2.1. Tea Leaf Samples

Eight fixed sample frames (10 dm² each) were randomly placed in each plot to count bud density; the eight frames within each plot were treated as subsamples and averaged to obtain one plot-level value. Mature functional leaves were randomly selected, and SPAD values were measured using a TYS-N chlorophyll meter, with eight measurements per plot (averaged to the plot level). One hundred standard “one bud, two leaves” tea buds were randomly picked and weighed to calculate the weight per 100 buds, with four subsamples per plot (averaged to the plot level). All tea-growth variables were thus aggregated to the plot level ($n = 3$ per treatment) before statistical analysis.

2.2.2. Rhizosphere Soil Samples

From each plot, three tea plants with uniform growth and free of pests and diseases were randomly selected. The root systems were completely excavated, and large clumps of soil were gently shaken off. The rhizosphere soil adhering to the surface of the roots was gently brushed off using a sterile brush. Equal volumes of soil from the root systems of the three tea plants were mixed to form one biological replicate. Because the three plants were pooled, each biological replicate is a composite sample representing the plot; accordingly, three composite biological replicates ($n = 3$) were analyzed per treatment, and within-plot subsamples were not treated as independent replicates in any statistical analysis. The soil sample was passed through a 2-mm sterile sieve to remove fine roots, gravel, and impurities, then divided into two portions. One portion was placed in a well-ventilated area to air-dry at room temperature, ground, and sieved through 2-mm and 0.149-mm sieves for the determination of soil physicochemical properties. A separate unground air-dried subsample of the bulk soil (taken before 2-mm sieving) was retained for the aggregate size fractionation, so that the intact >2-mm fraction could be quantified. The other portion was rapidly frozen in liquid nitrogen and stored in a -80°C ultra-low temperature freezer for the determination of microbial biomass, extracellular enzymes, and BIOLOG carbon source cultivation.

2.3. Soil Physicochemical Property Determination

Determinations of total nutrients, available nutrients, and pH were performed in accordance with standard methods for soil agrochemical analysis [20]. Soil pH was determined using the potentiometric method. Soil organic matter (OM) was determined using the potassium dichromate oxidation–external heating method; total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were determined using a fully automated elemental analyzer following high-temperature digestion with concentrated sulfuric acid. Alkali-digestible nitrogen (AN) was determined using the alkali diffusion method. Available phosphorus (AP) was determined using sodium bicarbonate extraction followed by the molybdenum-antimony colorimetric method. Determination of available potassium (AK) using the ammonium acetate extraction–flame photometry method; each parameter was determined with three technical replicates per composite sample.

2.4. Soil Aggregate Grading and Stability Calculations

Aggregate-size fractionation was performed on the unground air-dried subsample described in Section 2.2.2. Soil aggregate size fractionation was performed using the dry sieving method. Air-dried soil was placed in a set of sieves (2 mm, 1 mm, 0.5 mm, 0.25 mm), shaken horizontally for 10 min, and the soil collected from each sieve layer was weighed to calculate the mass percentage of each size fraction. The dry-sieving protocol assesses the current aggregate-size distribution and its mechanical stability; water-stable aggregate

content was not measured in this study. Accordingly, the results are interpreted in terms of aggregate-size distribution and mechanical stability indices rather than aggregate formation processes. Formulas for calculating core stability parameters:

① $R_{0.25}$: Percentage of aggregate mass in the >0.25 mm size fraction relative to the total soil sample;

② MWD (Mean Weight Diameter) = $\sum(\text{average diameter of the particle size fraction} \times \text{mass fraction of soil in that fraction})$;

③ GMD (Geometric Mean Diameter) = $\exp [\sum(\ln \text{average diameter of the particle size fraction} \times \text{mass fraction of soil in that fraction})]$.

2.5. Soil Microbial Biomass Carbon, Nitrogen, and Phosphorus

The chloroform fumigation extraction method was used to determine microbial biomass indices [20]. Briefly, 10 g of fresh soil was fumigated with alcohol-free chloroform vapor for 24 h at 25 °C in the dark; after removal of the fumigant, the soils were extracted at a 1:4 soil-to-extractant ratio. MBC and MBN were calculated from the differences between fumigated and unfumigated extracts using conversion factors $k_{EC} = 0.45$ and $k_{EN} = 0.54$, respectively, and MBP using $k_{EP} = 0.40$. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were extracted using a $0.5 \text{ mol} \cdot \text{L}^{-1} \text{ K}_2\text{SO}_4$ solution. Microbial biomass phosphorus (MBP) was extracted using a $0.5 \text{ mol} \cdot \text{L}^{-1} \text{ NaHCO}_3$ solution. Microbial biomass content was calculated by multiplying the difference between the extracted solutions from fumigated and unfumigated soils by the corresponding conversion factor. Each sample was analyzed with three technical replicates per composite sample.

2.6. Determination of Extracellular Enzyme Activities and Ecoenzymatic Stoichiometry

Four key extracellular enzymes for nutrient uptake such as β -glucosidase (BG), N-acetyl- β -D-glucosidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (ACP), all were determined using kits purchased from Shanghai Youxuan Biotechnology Co., Ltd. (Shanghai, China) Activities were measured colorimetrically using the chromogenic substrates supplied in the kits, following the manufacturer's protocols; one unit (U) of enzyme activity was defined as the amount of enzyme releasing $1 \mu\text{mol}$ of chromophore per minute per gram of dry soil, and activities are expressed as U g^{-1} dry soil. BG and ACP belonged to the carbon-acquiring enzyme and phosphorus-acquiring enzyme, respectively. NAG and LAP belonged to nitrogen-acquiring enzymes, and total nitrogen-acquiring enzyme activity = NAG activity + LAP activity. Each enzyme activity was determined with three technical replicates per composite sample, and all statistical analyses were performed at the plot level ($n = 3$).

The natural logarithms of the enzyme activity values were calculated to compute the two sets of ecological enzyme metric ratios, $\ln \text{BG} / \ln \text{ACP}$ and $\ln \text{BG} / \ln (\text{NAG} + \text{LAP})$. Following the ecoenzymatic stoichiometry framework [21], all enzyme activities were natural-log-transformed before computing the ratios. The length (Vector L) and angle (Vector A) of the microbial nutrient-limitation vector were calculated using the classic Sinsabaugh algorithm:

$$\text{Vector L} = \sqrt{[(\ln \text{BG} / \ln \text{ACP})^2 + (\ln \text{BG} / \ln (\text{NAG} + \text{LAP}))^2]}$$

$$\text{Vector A} = \text{Degrees}[\text{ATAN2}(\ln \text{BG} / \ln \text{ACP}, \ln \text{BG} / \ln (\text{NAG} + \text{LAP}))]$$

According to this definition, Vector A $> 45^\circ$ indicates relatively stronger phosphorus limitation, whereas Vector A $< 45^\circ$ indicates relatively stronger nitrogen limitation. Linear regression was used to examine the relationship between vector length and vector angle [21].

2.7. Determination of Carbon Source Utilization Characteristics by BIOLOG-ECO

In total, 10 g of rhizosphere soil was weighed, to which 90 mL of 0.85% sterile sodium chloride solution was added. The mixture was shaken at $120 \text{ r} \cdot \text{min}^{-1}$ for 10 min and allowed to stand, followed by serial dilution to prepare a 1000-fold soil suspension. A volume of 150 μL of the soil suspension was added to each microwell, and incubation was performed at 28°C in constant darkness for 7 d. Absorbance was measured at a wavelength of 590 nm every 24 h, and the OD value obtained at 72 h was selected for subsequent calculations [22]. The 72-h time point corresponds to the exponential phase of microbial substrate utilization, in which differences among treatments were most pronounced in preliminary assays. The inoculum was prepared from the same soil mass and the same dilution for all treatments; the BIOLOG profiles therefore reflect the carbon-source utilization potential per unit soil mass, and the large differences in microbial biomass among treatments should be considered when interpreting the profiles.

Formulas for core functional indicators:

$$\text{AWCD} = \sum (\text{Substrate OD} - \text{Control OD}) / 31;$$

Richness: Number of carbon sources with $\text{OD} > 0.2$;

$$\text{Shannon } H' = -\sum p_i \ln p_i; \text{ Evenness } E = \text{Shannon } H' / \ln (\text{Rich}); \text{ Levins } B = 1 / \sum p_i^2;$$

The 31 carbon sources (Table S1) were classified into six major categories: sugars, amino acids, carboxylic acids, amines, polymers, and phenolic acids. Principal Coordinate Analysis (PCoA) and a random forest model were used to analyze carbon utilization differences across treatments and to identify characteristic differential carbon sources. AWCD was calculated after subtracting the control-well OD, and negative corrected values were set to zero. Richness was defined as the number of carbon sources with corrected $\text{OD} > 0.2$, a threshold commonly used in BIOLOG-ECO studies to distinguish active substrate utilization [22].

2.8. Statistical Analysis of Data

SPSS 26.0 software was used to perform one-way analysis of variance (ANOVA), with the LSD method for multiple comparisons, and the significance level was set at $p < 0.05$. Prior to ANOVA, normality (Shapiro–Wilk test) and homogeneity of variance (Levene’s test) were checked; when these assumptions were violated, data were log-transformed or, if the violations persisted, analyzed with the non-parametric Kruskal–Wallis test. The LSD test was chosen because the number of treatments was limited to five and the main interest was pairwise comparisons among a small set of a priori-defined treatments. R 4.2 software was used to plot box plots, heat maps, and rank plots. Redundancy analysis (RDA), the Mantel test, and PERMANOVA were performed using R version 3.5.1 with the vegan package (version 2.5.6) on z-score-standardized data. The random forest analysis was performed using R version 4.2.0 with the varSelRF package (version 0.7.8); the linear regression, the Kruskal–Wallis test, and the Pearson/Spearman correlation analyses were performed using R version 4.2.0 (the correlation matrices were visualized with the ggcor package, version 0.9.8.1); and the principal coordinate analysis (PCoA) in Section 3.5.2 was performed using R version 4.2.0 with the phyloseq package (version 1.40.0). PERMANOVA (adonis2, Bray–Curtis dissimilarity, 999 permutations) was used to test for differences in microbial carbon utilization among groups. The Mantel test was performed with 999 permutations using Pearson’s method. Pearson correlation was applied after confirming normality; Spearman’s rank correlation was used when the assumption was not met. Linear regression was used to fit the relationship between nutrient vectors. Random forest analysis was implemented with 1000 trees ($\text{ntree} = 1000$), and characteristic carbon sources were identified based on

the mean decrease in accuracy and the Gini coefficient; model discrimination performance was evaluated by 10-fold cross-validation (out-of-bag error rate).

3. Results

3.1. Effects of Different Fertilization Methods on Tea Plant Growth

The proportion of organic fertilizer substitution had significant differential regulatory effects on agronomic traits such as chlorophyll content and 100-buds weight in tea plants (Figure 1). Chlorophyll content was highest in the OF67 treatment, followed by the OF33 treatment, and both were significantly higher than those in the OF50, CF, and OF100 treatments ($p < 0.05$), while the OF50 treatment was significantly higher than the CF and OF100 treatments ($p < 0.05$). The 100-buds weight was highest in the OF50 treatment, followed by OF33; both were significantly higher than the CF treatment ($p < 0.05$), whereas 100-buds weight in treatments with high organic substitution ratios (OF67 and OF100) was significantly lower than that in the CF treatment ($p < 0.05$). Shoot density was highest in the CF treatment and lowest in the OF33 treatment and there were no significant differences in shoot density among the OF50, OF67, and OF100 treatments. Overall, the highest chlorophyll content and 100-bud weight were associated with moderate organic substitution (33–50%), whereas high substitution rates (67% and 100%) were associated with a lower 100-bud weight than CF. It should be noted that fresh shoot yield was not directly measured in this study; the growth responses reported here are based on chlorophyll content, 100-bud weight, and shoot density.

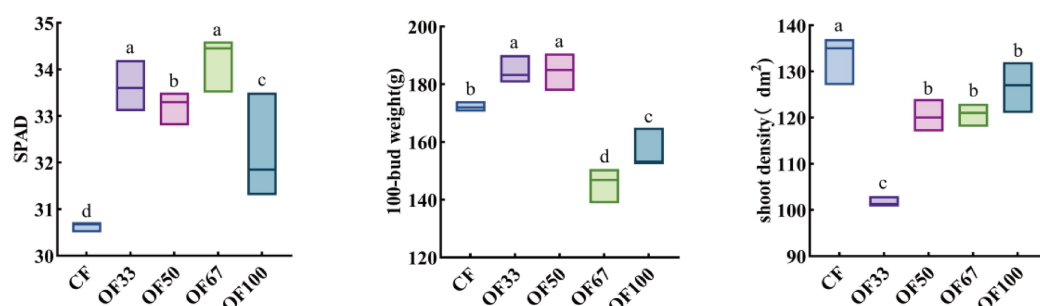


Figure 1. Growth indices of Huangguanyin tea plants under different organic fertilizer substitution treatments. CF: chemical fertilizer; OF33, OF50, OF67, and OF100 represent treatments in which 33%, 50%, 67%, and 100% of chemical fertilizer was replaced with organic fertilizer, respectively. Different lowercase letters indicate significant differences among treatments ($p < 0.05$, LSD test).

3.2. Effects of Different Fertilization Methods on the Physicochemical Properties of Soil in the Root Zone of Tea Plants

Soil physicochemical properties showed a marked response to fertilization (Figure 2). Soil pH of all organic substitution treatments was significantly higher than that of the pure chemical fertilizer control ($p < 0.05$), with the following order of OF33 > OF67 > OF100 > OF50 > CF. Among these, OF33 and OF67 were most effective in ameliorating the acidification of tea plantation soils, with pH increases ranging from 0.46 to 0.60 units. Soil organic matter (OM) content showed a steady upward trend as the organic substitution ratio increased, with significant differences among treatments ($p < 0.05$). The CF treatment had the lowest OM content at only 2.44%, while the OF100 treatment had the highest at 3.31%, representing a 35.2% increase compared to CF. The response of total nitrogen (TN) content to organic substitution followed a nonlinear pattern, with extremely significant differences among treatments ($p < 0.05$). The OF33 treatment had the highest total nitrogen content, showing a substantial increase of 57.7% compared to CF. The OF100 and OF50 treatments followed, showing increases of 39.9% and 35.8%, respectively, compared to CF.

In contrast, the OF67 treatment had the lowest total nitrogen content, which decreased by 14.3% compared to CF. Total potassium (TK) content generally decreased under organic substitution treatments, with significant differences among treatments ($p < 0.05$). The total potassium (TK) content was highest in the CF treatment, at 8.95 g/kg, and the OF67 treatment had the lowest content, which was 8.1% lower than that of CF. The total potassium contents in the OF33, OF50, and OF100 treatments were 8.37 g/kg, 8.54 g/kg, and 8.30 g/kg, respectively, with decreases ranging from 4.6% to 7.3%. Total phosphorus (TP) content was significantly reduced under all organic alternative treatments, with extremely significant differences among treatments ($p < 0.05$). Because the total P inputs were approximately balanced across treatments (Table 1), the marked reductions in soil TP under organic substitution are unlikely to result from differential P application rates; possible causes include the slower release of organic P forms, enhanced plant and microbial P uptake, and pH-related changes in P sorption equilibria. The CF treatment had the highest total phosphorus content at 1.11 g/kg, while the OF50 treatment had the lowest, representing a 72.8% reduction compared to CF. The total phosphorus contents in the OF33, OF67, and OF100 treatments were 0.51 g/kg, 0.61 g/kg, and 0.66 g/kg, respectively, representing reductions of 54.4%, 44.6%, and 40.3% compared to the CF treatment. Overall, organic substitution significantly reduced total phosphorus reserves in the soil, while its impact on total potassium was relatively minor.

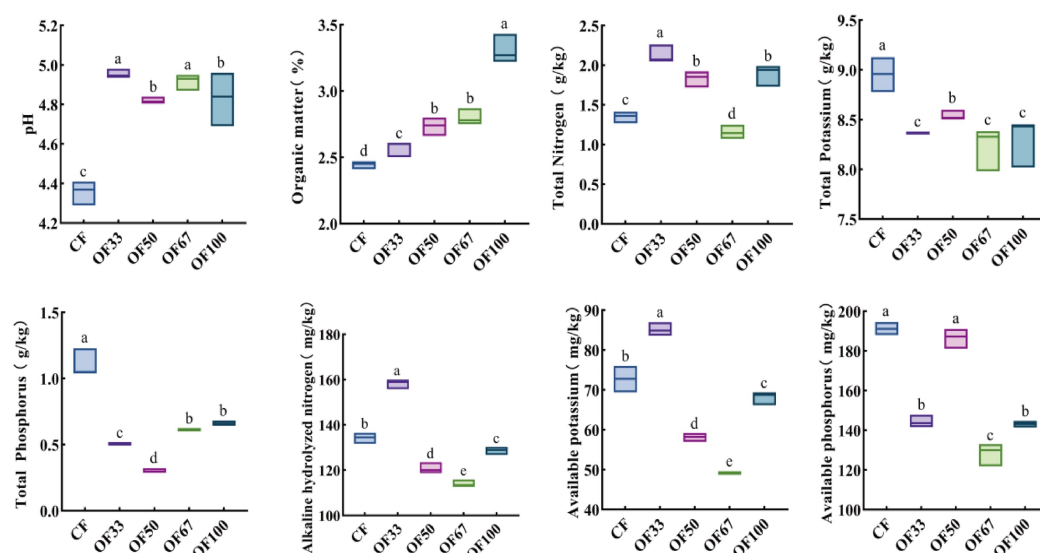


Figure 2. Physicochemical indices of soil in the rhizosphere of tea plants under different fertilization treatments. CF: Chemical fertilizer; OF33, OF50, OF67, and OF100 represent treatments in which 33%, 50%, 67%, and 100% of the chemical fertilizer was replaced with organic fertilizer, respectively. Different lowercase letters indicate significant differences among treatments ($p < 0.05$, LSD test).

The response patterns of readily available nutrients differed from those of total nutrients. Alkaline-hydrolyzable nitrogen (AN) content was highest in the OF33 treatment, showing a significant increase of 17.9% compared to the CF control ($p < 0.05$). The OF67 treatment had the lowest AN content at 113.97 mg/kg, representing a 15.1% decrease compared to the CF control; the OF50 and OF100 treatments were 10.0% and 4.2% lower than CF, respectively. There were significant differences in available potassium (AK) content among the treatments ($p < 0.05$). The OF33 treatment had the highest available potassium content, reaching 85.16 mg/kg, which was 17.1% higher than CF. The OF67 treatment had the lowest content, at only 49.14 mg/kg, representing a 32.4% decrease compared to CF. The OF50 and OF100 treatments showed decreases of 20.1% and 6.4%, respectively, compared to CF. The available phosphorus (AP) content was highest in the CF treatment and lowest in the OF67

treatment, which was 32.9% lower than CF ($p < 0.05$). Notably, the available phosphorus content in the OF50 treatment did not differ significantly from that of the CF treatment, while the OF33 and OF100 treatments showed reductions of 24.5% and 25.2%, respectively, compared to CF. In summary, the 33% organic substitution treatment performed best in increasing soil available nitrogen and available potassium, whereas the 67% substitution rate resulted in a comprehensive decrease in all available nutrient indicators.

3.3. Effects of Different Fertilization Methods on Soil Enzymes and Their Stoichiometric Ratios in Tea Plants

Extracellular enzymes involved in carbon, nitrogen, and phosphorus acquisition exhibited distinct responses to organic substitute gradients (Figure 3). β -glucosidase (BG, a carbon-acquiring enzyme) activity ranged from 11.5 U/g (OF67) to 31.7 U/g (OF100), enzyme activity increased significantly overall following the application of organic fertilizer, with the order OF100 > OF33 > OF50 > CF > OF67. Among these, the carbon-degrading enzyme activity in OF100 and OF33 was significantly higher than that in CF and OF67 ($p < 0.05$). Acid phosphatase (ACP, phosphorus-acquiring enzyme) activity was significantly higher in all organic fertilizer application treatments than in the CF treatment ($p < 0.05$), with OF33 showing the highest activity, followed by OF67, and there was no significant difference between the two, but both were significantly higher than OF50 and OF100 ($p < 0.05$). This result is also consistent with the conclusions of related studies. Under the treatment of applying 6000 kg/hm² of refined organic fertilizer, the phosphorus content, phosphorus uptake, and phosphorus fertilizer utilization rate of maize plants all increased significantly, with the phosphorus fertilizer utilization rate reaching as high as 67.0%—a 38.5% increase compared to the application of chemical fertilizer alone—indirectly confirming the positive effect of increased acid phosphatase activity on crop phosphorus uptake. Nitrogen acquisition enzyme (NAG + LAP) activity was highest in the CF treatment and significantly higher than in the OF100 treatment, which had the lowest activity ($p < 0.05$). Overall, the CF treatment—which received only chemical fertilizer—exhibited the highest total nitrogen acquisition enzyme activity in the soil. As the organic fertilizer substitution rate increased, soil N-acquiring enzyme activity showed an overall downward trend.

The enzymatic stoichiometric ratio provided an intuitive reflection of microbial nutrient limitation characteristics (Figure 3E,F). The $\ln\text{BG}/\ln\text{ACP}$ ratio ranged from 1.153 (OF67) to 1.818 (OF100), with OF100 exhibiting the highest value. Vector length (Vector L) characterized the degree of microbial carbon limitation, and the vector length for OF100 was significantly higher than that of other treatments ($p < 0.05$), indicating that microbial carbon limitation was most severe under the 100% organic fertilizer application; the OF67 treatment showed the lowest vector length, which was significantly lower than that of the CF treatment (Figure 3E). The vector angle (Vector A) ranged from 62.62° (CF) to 66.23° (OF100). According to the classic Sinsabaugh vector interpretation, Vector A > 45° indicates relatively stronger phosphorus limitation. All treatments exceeded 45°, and the vector angle differed significantly among treatments ($p < 0.05$), with the highest values in the OF100 and OF33 treatments and the lowest in the CF treatment (Figure 3F). Therefore, OF100, with the highest vector angle, had the strongest relative phosphorus limitation, whereas CF had the lowest vector angle among treatments, indicating a relatively weaker phosphorus limitation or a stronger relative nitrogen limitation compared with OF100. Linear regression results showed a significant positive correlation between vector length and vector angle ($R^2 = 0.45$, $p = 0.006$; $n = 15$ individual observations, i.e., three replicates $\times$ five treatments), indicating that as the organic substitution rate increased, microbial carbon and phosphorus limitations intensified simultaneously.

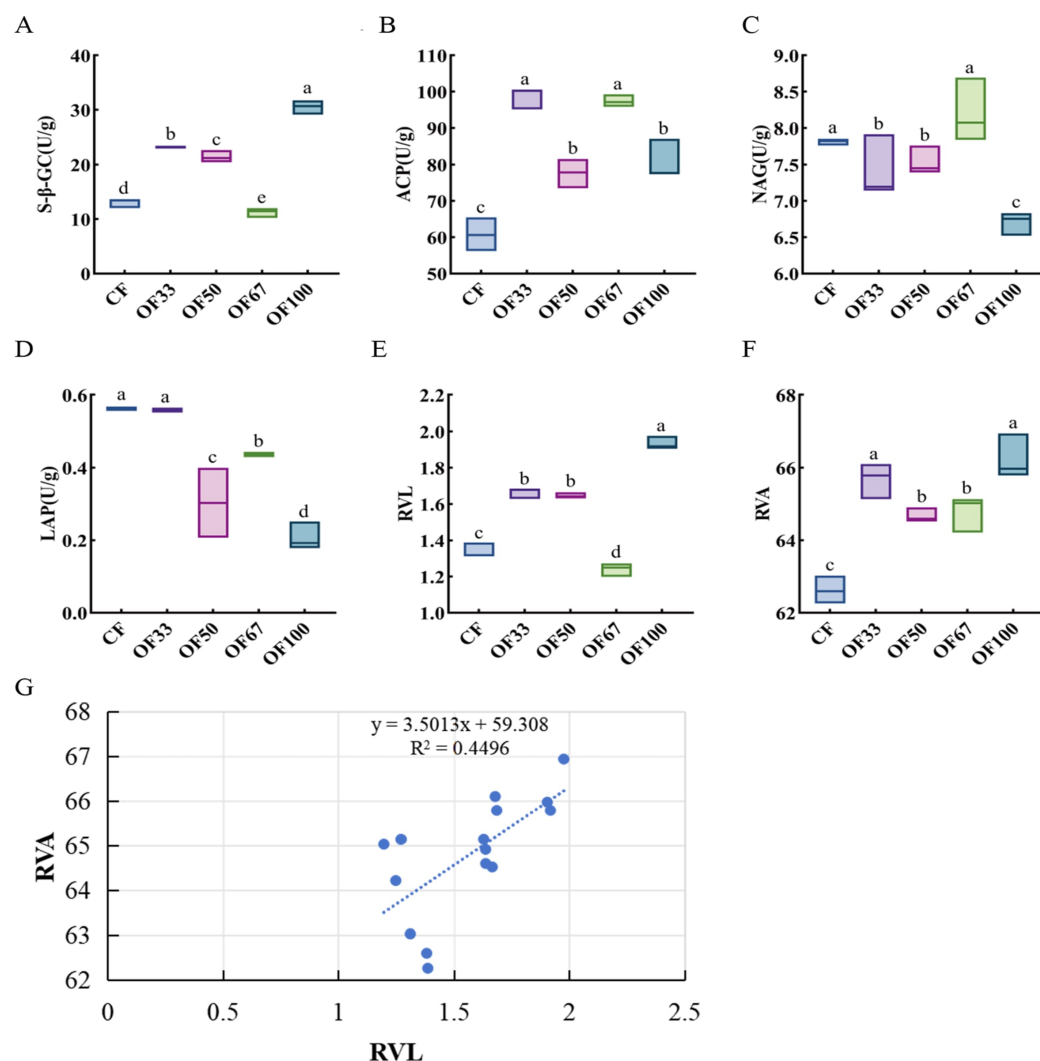


Figure 3. Enzyme activity and stoichiometric ratios in the rhizosphere soil of tea plants under different fertilization treatments. (A): β -glucosidase; (B): acid phosphatase; (C): N-acetyl- β -D-glucosaminidase; (D): leucine aminopeptidase; (E): Vector L; (F): Vector A; (G): linear regression of Vector L and Vector A; CF: Chemical fertilizer; OF33, OF50, OF67, and OF100 represent organic fertilizers replacing 33%, 50%, 67%, and 100% of chemical fertilizer, respectively. Different lowercase letters indicate significant differences among treatments ($p < 0.05$, LSD test).

3.4. Chemometric Characteristics of C, N, and P in Soil Microbial Biomass in the Rhizosphere of Tea Plants Under Different Fertilization Methods

The application of organic fertilizers significantly altered the soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) reserves and their mass balance relationships (Figure 4). MBC increased continuously as the organic fertilizer application rate rose. The MBC content in the OF100 treatment was significantly higher than that in all other treatments ($p < 0.05$) and was 3.5 times that of the CF treatment. The MBC in the OF50 treatment was significantly higher than that in the OF33 and OF67 treatments ($p < 0.05$). MBN was highest in the CF treatment and decreased significantly after the application of organic fertilizer, and the OF33 treatment had the lowest MBN. MBP was highest in the OF33 treatment, significantly higher than in other treatments ($p < 0.05$), followed by OF100, OF67, and OF50, with CF having the lowest MBP.

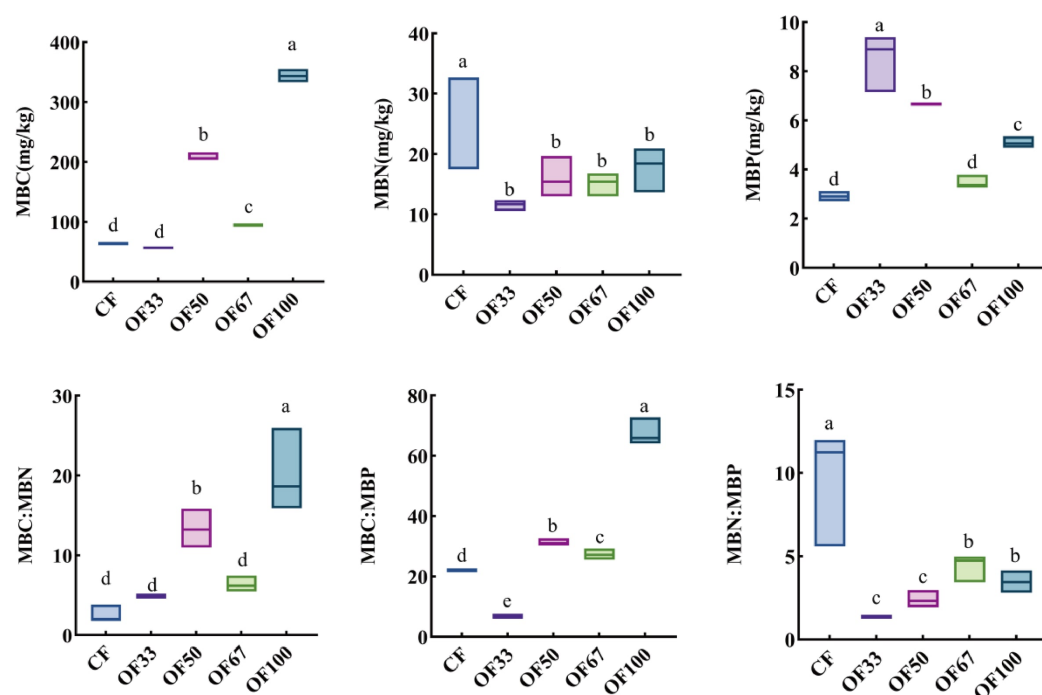


Figure 4. Microbial biomass C, N, and P and their stoichiometric ratios in the rhizosphere soil of tea plants under different fertilization treatments. MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBP: microbial biomass phosphorus; MBC:MBN: microbial biomass carbon-to-nitrogen ratio; MBC:MBP: microbial biomass carbon-to-phosphorus ratio; MBN:MBP: microbial biomass nitrogen-to-phosphorus ratio; CF: Chemical fertilizer; OF33, OF50, OF67, and OF100 represent treatments in which 33%, 50%, 67%, and 100% of chemical fertilizer was replaced with organic fertilizer, respectively. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

The (MBC:MBN) increased continuously as the organic substitution rate increased, with the highest ratio observed in the OF100 treatment. The trend in the microbial biomass carbon-to-phosphorus ratio (MBC:MBP) was identical with the above microbial biomass carbon-to-nitrogen ratio, indicating that organic fertilizer input primarily expands the microbial carbon pool, while the increase in the phosphorus pool lags behind. In contrast, the microbial biomass nitrogen-to-phosphorus ratio (MBN:MBP) in the pure chemical fertilizer treatment was significantly higher than in all organic fertilizer co-application treatments, and the sole application of chemical fertilizer causes an imbalance in the distribution of nitrogen and phosphorus within microorganisms, whereas organic substitution altered the stoichiometric balance of microbial biomass nutrients; whether this redistribution optimizes microbial nutrient allocation cannot be determined from biomass ratios alone.

3.5. BIOLOG Analysis of Soil in the Rhizosphere of Tea Plants Under Different Fertilization Methods

3.5.1. Functional Diversity in Carbon Source Utilization

BIOLOG-ECO reflects the metabolic potential of the culturable, actively respiring microbial community under standardized laboratory conditions and should not be equated with the overall functional diversity of the total soil microbial community. The average color change rate (AWCD), carbon source abundance, Shannon diversity, evenness, and Levins niche width all reflect the regulatory effects of organic substitution on microbial carbon utilization capacity (Figure 5). Following the experimental design of relevant organic fertilizer substitution in tea plantations, this study established treatments with different organic substitution ratios. The results showed that AWCD, which characterizes

overall metabolic activity, was highest in the OF50 and OF100 treatments and lowest in the OF67 treatment. Carbon source richness (the number of carbon sources with OD > 0.2) was significantly highest in the OF50 treatment, indicating the broadest range of utilizable carbon substrates. Shannon diversity and evenness peaked in the OF33, OF50, and OF100 treatments, while OF67 exhibited the lowest diversity and evenness among all groups. The Levins niche width results confirmed that moderate levels of organic substitution (OF33, OF50) significantly broadened the ecological niche of soil microorganisms for carbon source utilization compared to pure chemical fertilizer.

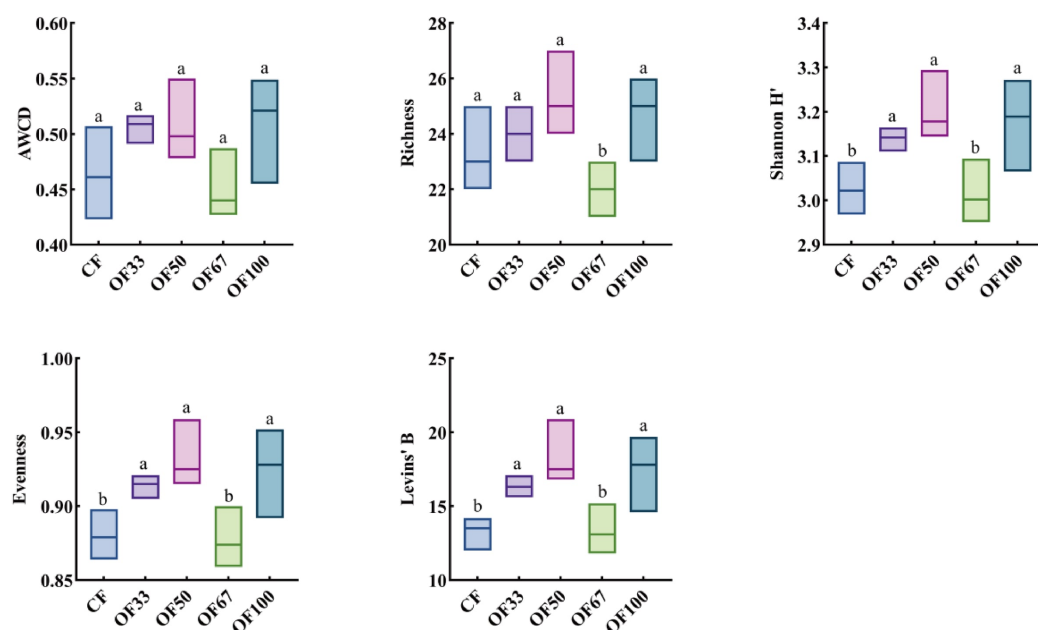


Figure 5. Functional diversity of carbon source utilization in the rhizosphere soil of tea plants under different fertilization treatments. AWCD: Average color change rate; Richness: Carbon source richness; Shannon H': Shannon diversity index; Evenness: Evenness; Levins B: Levins niche width. CF: Chemical fertilizer; OF33, OF50, OF67, and OF100 represent treatments in which 33%, 50%, 67%, and 100% of chemical fertilizer was replaced with organic fertilizer, respectively. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.5.2. Key Carbon Sources Distinguishing Fertilization Treatments

Principal Coordinate Analysis (PCoA) based on carbon source utilization patterns separated samples from each fertilization treatment, and PERMANOVA confirmed that the carbon-source utilization profiles differed significantly among treatments ($p < 0.05$); PCoA1 and PCoA2 explained 53.53% and 14.55% of the total functional variation, respectively (Figure 6A). Signature carbon substrates that distinguish between treatments were identified using the mean decrease in accuracy and Gini coefficient from random forest analysis (Figure 6B), including amino acids (L-asparagine, L-arginine), carboxylic acids (D-malic acid, γ -hydroxybutyric acid), polymers (Tween 40, Tween 80), and sugars (D-mannitol, N-acetyl-D-glucosamine), were the core carbon sources responsible for functional differences between groups. These patterns should be regarded as descriptive differences in carbon-source utilization profiles identified from ordination and variable-importance analyses. Soil microorganisms in the moderate organic fertilizer substitution treatments (OF33, OF50) exhibited a stronger ability to utilize amino acids and low-molecular-weight organic acids, and microorganisms in the OF100 treatment preferred polysaccharide substrates, while microorganisms in the pure chemical fertilizer (CF) treatment primarily utilized simple sugars and phenolic acids as carbon sources.

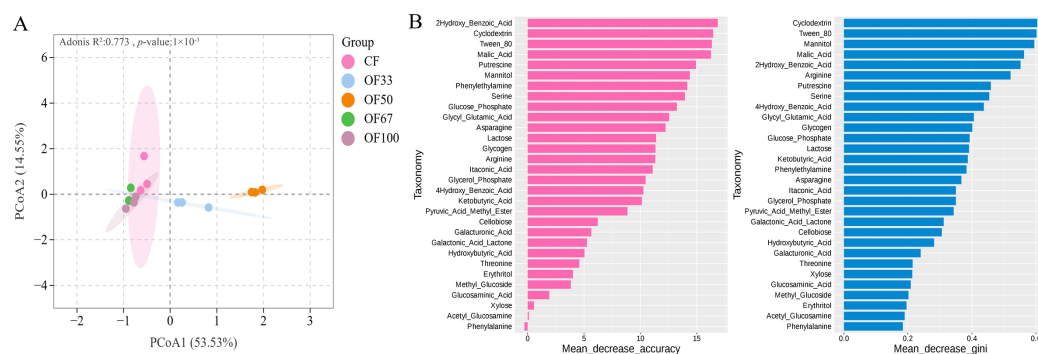


Figure 6. Differences in carbon source utilization by soil microorganisms in the rhizosphere of tea plants under different fertilization treatments, and screening of differentially utilized carbon sources. (A): Principal coordinate Analysis (PCoA) score plot for carbon source utilization; (B): Screening of key differentially utilized carbon sources using the average precision reduction coefficient of the random forest algorithm.

3.6. Mechanical Stability of Aggregates

Organic fertilizer substitution reshaped the particle size distribution and structural stability of soil aggregates (Figure 7). In terms of particle size composition, the mass fraction of large aggregates (>2 mm) was higher in the OF33 and OF100 treatments than in the CF treatment. Intermediate aggregates (1–2 mm) were highest in the OF50 treatment, followed by the OF33 treatment. The mean proportion of microaggregates (<0.25 mm) was lower in all organic amendment treatments than in the pure chemical fertilizer treatment. The proportion of stable aggregates larger than 0.25 mm ($R_{0.25}$) was highest in the OF33 treatment, followed by the OF50 treatment, with the ranking OF33 > OF50 > OF100 > OF67 > CF, indicating that a 33% organic fertilizer application is most conducive to the formation of large aggregates. The ranking of the two stability indices—mean weight diameter (MWD) and geometric mean diameter (GMD)—was consistently OF33 > OF50 > OF100 > OF67 > CF. The results indicate that the application of a low proportion of organic fertilizer (33%) is most effective in promoting the development of large aggregates and enhancing soil mechanical stability, followed by the 50% treatment; a high organic fertilizer proportion (100%) did not further enhance aggregate stability and was less effective than the 33% and 50% treatments, while the 67% treatment showed the smallest improvement. Because aggregate-size distribution was determined by dry sieving, these results reflect the current aggregate-size distribution and its mechanical stability rather than the process of aggregate formation.

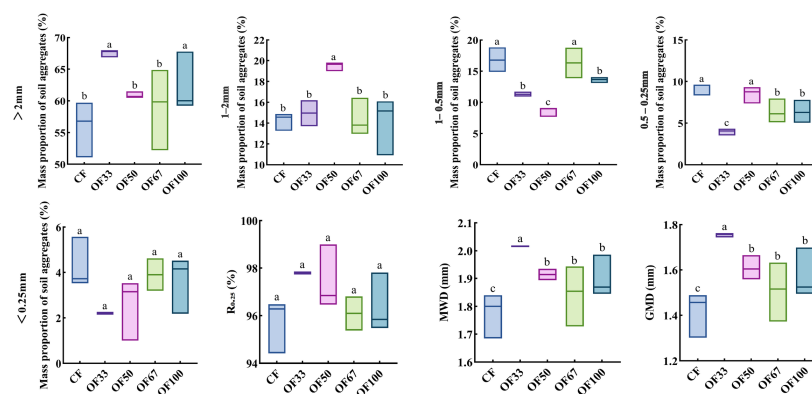


Figure 7. Soil aggregates in the rhizosphere of tea plants under different fertilization methods. $R_{0.25}$: The proportion of stable aggregates larger than 0.25 mm; MWD: Mean weight diameter; GMD: geometric mean diameter. Data are presented as mean $\pm$ standard deviation ($n = 3$). Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.7. Correlation Analysis and Redundancy Analysis

The Mantel test and Pearson correlation matrix revealed close associations among tea plant growth, soil physicochemical properties, soil aggregates, and microbial carbon utilization traits (Figure 8). Tea shoot growth indices were significantly positively correlated with MBP, TN, TP, AN, AK, BG, ACP, NAG, and LAP ($p < 0.05$). Aggregate stability indices ($R_{0.25}$, MWD, GMD) were significantly positively correlated with MBP, AN, AK, BG, and NAG ($p < 0.05$).

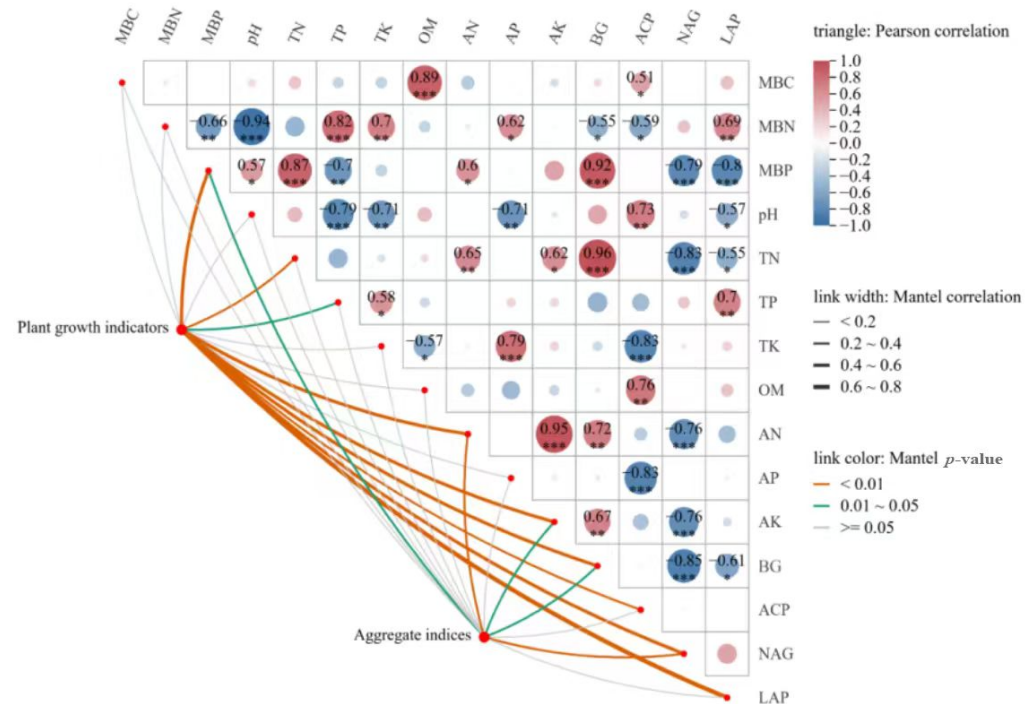


Figure 8. Correlation analysis of physicochemical indices, soil enzymes, and soil microbial biomass in the rhizosphere of tea plants under different fertilization treatments with tea plant growth indices and soil aggregates. TP: total phosphorus; TK: total potassium; TN: total nitrogen; OM: organic matter; AP: available phosphorus; AK: available potassium; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBP: microbial biomass phosphorus; LAP: leucine aminopeptidase; BC: β -glucosidase; NAG: N-acetylglucosaminidase; ACP: acid phosphatase. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

This study used redundancy analysis (RDA) to identify the environmental variables associated with microbial carbon source utilization patterns (Figure 9). When tea plant growth indices were used as constraint variables (Figure 9A), the first two axes explained 84.29% of the total variance (RDA1: 58.48%; RDA2: 25.81%); specifically, SPAD (chlorophyll content) was positively correlated with OF33, HBW (weight of 100 buds) was positively correlated with OF50, and SD (shoot density) was positively correlated with CF. When soil physicochemical properties and extracellular enzyme activities were used as constraint variables (Figure 9B), the first two axes explained 51.15% of the total variation (RDA1: 33.89%; RDA2: 17.26%). TK, AP, MBP, BG, and TN were positively correlated with OF50; ACP, OM, MBC, and LAP were positively correlated with OF100; TP, MBN, and NAG were positively correlated with OF67; and AN was positively correlated with OF33 and CF. When aggregate stability indices were used as constraint variables (Figure 9C), the first two axes explained 82.14% of the total variation (RDA1: 61.65%; RDA2: 20.49%), and $R_{0.25}$ and GMD were positively correlated with OF100, and MWD was positively correlated with OF67. Permutation tests (999 permutations) were used to assess the significance of the

RDA axes and explanatory variables; the explained variances and significance levels are reported in Figure 9. These associations are correlative rather than causal.

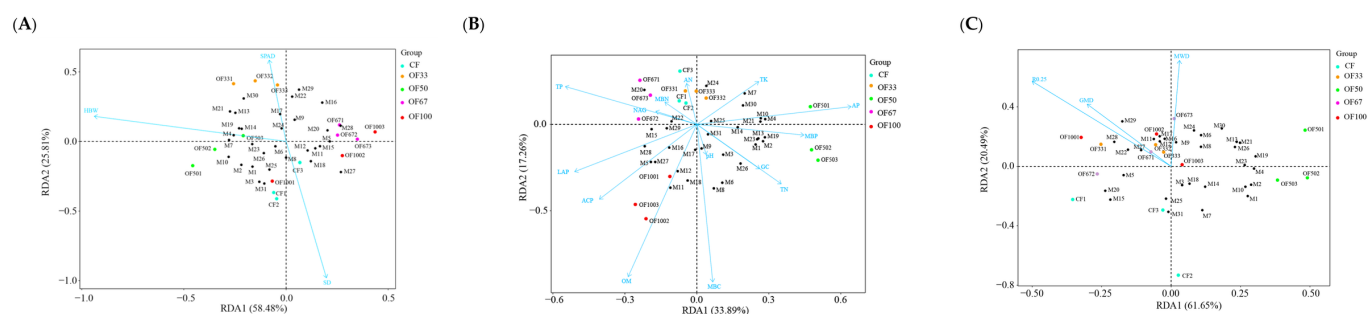


Figure 9. RDA analysis of soil in the rhizosphere of tea plants under different fertilization methods. (A): RDA analysis of microbial carbon source utilization in the rhizosphere soil of tea plants under different fertilization treatments versus tea plant growth indices; (B): RDA analysis of microbial carbon source utilization in the rhizosphere soil of tea plants under different fertilization treatments versus soil physicochemical properties, enzyme activity and soil microbial biomass carbon, nitrogen, and phosphorus; (C): RDA analysis of microbial carbon source utilization in the rhizosphere soil of tea plants under different fertilization treatments versus soil aggregates. SPAD: chlorophyll content; HBW: weight of 100 buds; SD: shoot density; TP: total phosphorus; TK: total potassium; TN: total nitrogen; OM: organic matter; AP: available phosphorus; AK: available potassium; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBP: microbial biomass phosphorus; LAP: leucine aminopeptidase; BC: β -glucosidase; NAG: N-acetylglucosaminidase; ACP: acid phosphatase.

4. Discussion

4.1. The Physicochemical Filtration Factors Reshaped by Organic Fertilizer Substitution in the Rhizosphere of Tea Plantations to Establish a Differentiated Microbial Resource Matrix

Long-term sole application of chemical fertilizers (CF) has continuously driven acidification and nutrient imbalance in subtropical red soil tea plantations [23]. This strongly acidic environment released large amounts of active Al^{3+} , which not only inhibited the development of lateral roots and the nutrient transport system in tea plants but also immobilized active phosphorus in the soil through metal ion coprecipitation, thereby forming an extreme resource matrix characterized by low pH, readily available nitrogen, and low active carbon [2,24]. Consistent with previous long-term studies in acidified tea plantations [23,25], the graded application of organic fertilizers provided acid-base buffering through humic functional groups; the soil pH in the OF33, OF67, and OF100 treatments increased by 0.46–0.60 units compared to the CF control. Meanwhile, soil organic matter (OM) content increased linearly with the proportion of organic substitution, reaching 3.31% in the OF100 treatment—a 35.2% increase compared to the chemical fertilizer treatment. The organic fertilizer used in this study had an organic matter content $\geq 45\%$; its complex constituents (e.g., polysaccharides and proteins) may undergo humification to form stable, mineral-bound organic carbon, may prolong the soil carbon turnover cycle and providing the microbial community with a more heterogeneous resource matrix [4]; direct measurements of carbon turnover would be required to confirm this effect.

Soil nutrient contents exhibited significant threshold differentiation. The 33% organic substitution (OF33) treatment showed significantly higher total nitrogen, alkaline-hydrolyzable nitrogen, and available potassium contents than the other treatment gradients. This indicated that the slow-release nitrogen and potassium from organic fertilizers formed a sequential complementary supply pattern with inorganic, readily available soil nutrients, thereby avoiding the drawbacks of pure chemical fertilizers, namely, a short-term nutrient surge followed by a later supply shortage. However, total phosphorus and available phosphorus contents continued to decline as the organic fertilizer substitution ratio increased.

Because the total P inputs were held constant at 105 kg P₂O₅ ha^{−1} across treatments (Table 1), the decline in soil total and available phosphorus is unlikely to be caused by differential P application rates; it more likely reflects the lower water-solubility and slower release of organic P forms, enhanced plant and microbial P uptake, and pH-related changes in P sorption equilibria. In addition, calcium, magnesium, and iron cations introduced by organic fertilizers may bind with soil phosphate ions to form insoluble phosphates, thereby reducing phosphorus bioavailability [2]. Similar P responses to organic fertilization have been reported in long-term tea plantation experiments [2,26]. Redundancy analysis (RDA) clearly demonstrated the differential constraining effects of various environmental factors on microbial carbon utilization functions. In the RDA model using soil physicochemical properties and extracellular enzyme activity as constraint variables, the first two axes cumulatively explained 51.15% of the total variation in microbial carbon utilization. Among these, TK, AP, MBP, BG, and TN were significantly associated with the OF50 treatment; ACP, OM, MBC, and LAP were significantly associated with the OF100 treatment; and TP, MBN, and NAG were significantly associated with the OF67 treatment, demonstrating that potassium and phosphorus availability and organic carbon substrates were the physicochemical variables most closely associated with the differentiation of microbial carbon utilization across different fertilization gradients. AN showed a positive association with the OF33 and CF treatments. Total potassium and available phosphorus were significantly associated with the microbial metabolic characteristics of the OF50 treatment, indicating that moderate organic fertilizer substitution can simultaneously activate potassium and phosphorus supply and microbial carbon metabolism [15]. In summary, the co-application of exogenous organic matter reshapes the microbial habitat by comprehensively modifying soil pH and the carbon, nitrogen, and phosphorus nutrient pools, thereby differing from the regulatory pathway of endogenous tea tree genotypes, which rely on spatial nutrient heterogeneity in the root zone to select microorganisms. It should be noted that the associations revealed by RDA are correlative rather than causal.

4.2. Nutrient Imbalances Drive Microbial Life History Trade-Offs and Reshape the Secretion Patterns of Extracellular Carbon-, Nitrogen-, and Phosphorus-Acquiring Enzymes

Ecological enzymometry is a standardized method for quantitatively analyzing microbial nutrient stress and distinguishing between resource acquisition (A) and growth-yield (Y) life history strategies [6]. In this study, the activity of the carbon-acquiring enzyme, β -glucosidase (BG) peaked under the OF100 treatment, indicating that a high proportion of organic carbon substrates may have induced microorganisms to allocate substantial metabolic resources to the degradation of macromolecular organic matter—a typical Type A mineralization strategy; direct transcriptomic evidence would be required to confirm the underlying gene-expression response [8]. The low BG activity under OF67 suggests that the carbon substrate supply in this treatment was insufficient to meet microbial demand, resulting in overall suppression of carbon metabolic pathways. Regarding phosphorus acquisition, acid phosphatase (ACP) activity in all organic co-application treatments was significantly higher than in the CF treatment. Organic fertilizers may contain organic phosphorus substrates such as phytic acid and nucleic acids, which could stimulate microorganisms to secrete large amounts of phosphatase to activate insoluble phosphorus in the soil, consistent with field studies showing that combined organic and chemical fertilization increases crop phosphorus uptake and phosphorus fertilizer utilization efficiency [16]; because the composition of the organic fertilizer used here was not specifically analyzed, this interpretation should be regarded as a plausible mechanism supported by related studies [16,27]. Among these, the OF33 and OF67 treatments exhibited the highest phosphorus mineralization potential, suggesting that moderate-to-low proportions of organic co-application can effectively alleviate the phosphorus limitation commonly found

in tea plantations [16]. This result is consistent with a field study in which application of 6000 kg ha⁻¹ refined organic fertilizer significantly increased the phosphorus content, phosphorus uptake, and phosphorus fertilizer utilization rate of maize (67.0%, a 38.5% increase over chemical fertilizer alone), indirectly supporting the role of elevated acid phosphatase activity in crop phosphorus acquisition [16].

Total nitrogen acquisition enzyme activity (NAG + LAP) showed a continuous downward trend as the proportion of organic substitution increased. The pure chemical fertilizer treatment directly supplied high concentrations of readily available inorganic nitrogen, eliminating the need for microorganisms to synthesize large amounts of proteases and chitinases to break down organic nitrogen substrates. Accordingly, the synthesis of nitrogen-acquiring enzymes decreased under organic substitution; under high organic matter co-application, soil nitrogen exists in a slowly released form bound to humus, significantly alleviating microbial nitrogen stress [1]. RDA results further corroborated the link between enzyme activity and the fertilization gradient, and the carbon-acquiring enzyme BG and the phosphorus-acquiring enzyme ACP were concentrated in areas with high organic substitution treatments, and the carbon- and phosphorus-degrading functions of these two enzyme classes were closely associated with the large amounts of organic carbon and organic phosphorus substrates supplied by organic fertilizers. In contrast, the two nitrogen-acquiring enzymes, NAG and LAP, were significantly associated only with the CF pure chemical fertilizer treatment, indicating that long-term input of a single source of readily available nitrogen continuously induces microorganisms to maintain high levels of nitrogen-acquiring enzyme secretion.

Both the metabolic ratios $\ln BG/\ln ACP$ and $\ln BG/\ln(NAG + LAP)$ and the length of the nutrient limitation vector reached their maximum values under the OF100 treatment, indicating that microorganisms faced severe carbon limitation under the fully organic fertilizer treatment, forcing the community to favor Type A mineralization strategies and accelerating the decomposition and loss of organic matter [6]. The nutrient vector angles of all treatments exceeded 45°, indicating relative phosphorus limitation throughout the fertilization gradient; the vector angle increased with organic substitution and was highest under the OF100 and OF33 treatments and lowest under CF (Figure 3F), suggesting that relative phosphorus limitation was more pronounced under high organic substitution than under sole chemical fertilization. The vector length showed a significant positive correlation with the vector ($R^2 = 0.45$, $p = 0.006$), revealing that carbon and phosphorus limitations synergistically intensified, and the degree of nutrient imbalance was closely associated with the microbial life-history orientation [9]. Based on the microbial carbon pump theory, it could be inferred that under the OF33 and OF50 treatments, nutrient supply matched microbial metabolic demands, causing the microbial community to shift toward the Y growth strategy, where assimilated carbon was predominantly converted into microbial residues to form stable organic carbon; whereas under the CF and OF100 treatments, severe metabolic imbalances existed, causing the microbial community to favor the A strategy. This inference is based on ecoenzymatic stoichiometry and the microbial carbon pump framework; direct measurements of microbial community composition, carbon use efficiency, and carbon turnover would be required to verify it [6,28,29].

4.3. Moderate Application of Organic Fertilizers Broadens the Ecological Niche for Microbial Carbon Utilization and Enhances Metabolic Functional Redundancy in Microbial Communities

Based on the BIOLOG-ECO carbon source fingerprint, it is possible to quantitatively characterize microbial carbon substrate utilization preferences, niche breadth, and community functional redundancy [30]. Redundancy analysis using tea plant growth indices as constraint variables (Figure 9A) showed that the first two axes cumulatively explained 84.29% of the total variation. Specifically, chlorophyll content was positively correlated with

OF33, and the weight of 100 buds was positively correlated with OF50, while shoot density was enriched only under the CF treatment, suggesting that moderate organic fertilization (33–50%) was associated with better tea photosynthetic and yield traits, whereas pure chemical fertilizer was associated with higher shoot density; these associations are correlative and are consistent with recent integrated fertilization studies in tea plantations [31,32]. The four indicators—AWCD, carbon source richness, Shannon diversity, and Levins niche breadth—all peaked under the OF33 and OF50 treatments, confirming that this range of organic fertilizer application could maximally broaden the microbial carbon utilization spectrum. Long-term application of chemical fertilizers alone provides only small amounts of monosaccharides and phenolic acids as readily available carbon sources, which may lead to the selective enrichment of specialized, nutrient-rich microbial communities. This results in a narrow carbon-utilization ecological niche and extremely low community functional redundancy [11]; In contrast, the simultaneous application of moderate amounts of organic fertilizer introduces diverse carbon substrates—including amino acids, low-molecular-weight carboxylic acids, and high-molecular-weight polymers—which may support the coexistence of both A- and Y-type microbial communities and enhance the community's resilience to environmental disturbances [7]; these community-level inferences are hypotheses that require direct microbial compositional data to confirm.

Random Forest analysis further identified L-asparagine, D-malic acid, and Tween polymers as signature carbon sources distinguishing the various treatments. The OF33 and OF50 microbial communities showed relatively higher utilization of amino acids and small-molecule carboxylic acids; the OF100 microbial community showed relatively higher utilization of polysaccharide substrates; and the CF community preferentially utilized simple phenols and monosaccharides. These patterns are descriptive differences in carbon-source utilization profiles identified from ordination and variable-importance analyses; because microbial community composition was not directly measured, explanations invoking the selection of specific taxa (e.g., polysaccharide-degrading bacteria or oligotrophs) remain hypotheses. The trait-based theory of microbiology indicates that a diverse resource supply can broaden the ecological niche of microbial communities, whereas a single carbon source narrows the resource utilization range [18]. Broad-spectrum carbon-utilizing microbial communities can simultaneously activate pathways related to carbon fixation, nitrogen assimilation, and organic phosphorus mineralization, potentially alleviating multiple nutrient limitations and supporting balanced nutrient cycling “diverse carbon inputs-broadened ecological niches-balanced nutrient cycling” [14].

4.4. Organic Fertilizer Application Regulates Soil Aggregate Structure and Creates Layered, Diverse Microbial Habitats

Soil aggregates serve as physical carriers linking soil physicochemical properties, extracellular enzyme activity, and microbial carbon metabolism. It has been proposed that within aggregates of different particle sizes, aerobic and anaerobic stratified microenvironments form, regulating substrate availability, oxygen diffusion, and the efficiency of extracellular enzyme immobilization [13]; these microenvironments were not directly measured in this study, and this mechanism is inferred from the literature. An RDA model with aggregate stability indices as constraint variables showed that the first two axes cumulatively explained 82.14% of the total variation in microbial carbon utilization. Among these, the large aggregate index MWD was significantly positively correlated with OF67, while the proportion of stable aggregates ($R_{0.25}$) and the geometric mean diameter (GMD) were biased toward OF100, indicating that different organic substitution ratios exert differential regulatory effects on aggregate size structure. Consistent with previous studies reporting that partial substitution of chemical fertilizer with organic materials improves aggregate stability in tea plantations [33], the OF33 treatment exhibited the optimal values for the

proportion of large aggregates ($R_{0.25}$), MWD, and GMD for aggregates larger than 2 mm, indicating that a 33% organic substitution rate provided an appropriate amount of binding agents—such as polysaccharides and humic acids—which form stable large aggregates through particle bridging. In contrast, excessive organic fertilizer input may lead to an overabundance of binding substrates; the resulting thick particle bonding layers are prone to fragmentation, causing the proportion of microaggregates to rebound. Long-term sole application of chemical fertilizers, due to the lack of organic binding agents, may cause large aggregates to continuously disintegrate, exacerbating soil compaction, and lead to a scarcity of habitat for microorganisms [5]. These interpretations regarding aggregate formation processes are inferred from aggregate-size distribution data and require direct structural measurements to confirm.

Correlation analysis showed that aggregate stability indices were highly significantly positively correlated with OM, MBP, and ACP. The microenvironment of aggregates—with an aerobic outer layer and an anaerobic inner layer—has been proposed to allow the coexistence of both A-type and Y-type microorganisms, and the colloidal adsorption of extracellular enzymes by aggregates may reduce leaching loss, thereby sustaining phosphorus mineralization potential over the long term [17]; as noted above, these microenvironments were not directly measured here. Previous studies have confirmed that microorganisms within large aggregates exhibit a broader spectrum of carbon utilization, whereas microbial communities within small aggregates have a narrower substrate selection, and aggregate integrity is closely related to the breadth of the microbial ecological niche [12]. Based on this, a plausible regulatory pathway is proposed: the appropriate application of organic matter supplements organic cementing agents to optimize aggregate structure and construct a stratified, diverse microbial microhabitat; a diverse pore structure supports the colonization of a broad spectrum of carbon-utilizing microbial communities. And the balanced secretion of enzymes for carbon and phosphorus acquisition simultaneously enhances organic matter retention and organic phosphorus activation capabilities, ultimately working synergistically to alleviate multiple nutrient limitations in tea plantations. This pathway integrates the measured variables with literature-based mechanisms; the causal links among these components were not directly demonstrated in this study and require further experimental validation [12,33].

4.5. Suitable Substitution Range and Limitations

Several limitations of this study should be acknowledged. The experiment was conducted at a single location with a single tea cultivar (Huang Guanyin) and a single type of organic fertilizer, with three biological replicates per treatment and a single sampling period; therefore, the conclusions may not be generalizable to other tea cultivars, soil types, organic fertilizers, or climatic conditions without further validation. In addition, microbial community composition, root traits, aluminum availability, and carbon mineralization/turnover were not directly measured; consequently, the mechanisms discussed above—such as the selection of specific microbial taxa, aggregate microhabitat formation, and carbon turnover effects—are inferred from ecoenzymatic stoichiometry, BIOLOG-ECO profiles, and correlation/ordination analyses and should be regarded as hypotheses requiring direct experimental verification. Bearing these limitations in mind, the results support the following synthesized model. Gradient organic nitrogen substitution (i) increased soil pH and nutrient availability and was associated with improved aggregate mechanical stability; (ii) altered microbial biomass stoichiometry and extracellular enzyme secretion, with moderate substitution (33–50%) broadening the microbial carbon-source utilization niche and complete substitution (100%) intensifying microbial carbon limitation; and (iii) was associated with improved tea growth indices (chlorophyll content and 100-bud weight).

5. Conclusions

Based on a comprehensive analysis of soil physicochemical properties, extracellular enzyme stoichiometry, BIOLOG microbial carbon metabolism characteristics, soil aggregate composition, and multidimensional redundancy analysis, this long-term field experiment showed that organic nitrogen substitution regulates the carbon, nitrogen, and phosphorus nutrient cycles in subtropical tea plantation soils through coordinated changes in soil physicochemical properties, aggregate structure, and microbial carbon metabolism, rather than through a single pathway. An organic substitution ratio of 33–50% can be recommended as a suitable application range under the conditions of this long-term field experiment, based on the indicators measured (soil pH, available nitrogen and potassium, microbial biomass phosphorus, aggregate mechanical stability, extracellular enzyme stoichiometry, and microbial carbon-source utilization). This application regimen can produce synergistic benefits in multiple aspects. It simultaneously mitigates soil acidification in tea plantations, broadens the ecological niche for microbial carbon utilization, modifies the secretion of carbon- and phosphorus-acquiring enzymes, and improves soil aggregate structure, while also promoting chlorophyll accumulation in tea plants and higher 100-bud weight. In contrast, the use of chemical fertilizers alone was associated with a narrowing of the microbial carbon-source utilization profile, whereas 100% organic fertilizer induced severe microbial carbon limitation, as indicated by the highest vector length. Because the experiment was conducted at a single site with one tea cultivar and one organic fertilizer type, the generalizability of these findings to other tea plantation conditions requires further verification. For practical field application, organic fertilizer supplying 33–50% of the total nitrogen requirement is recommended for acidic red-soil tea plantations under conditions similar to those of this experiment; the corresponding application amount should be calculated from the nitrogen content of the organic fertilizer used. This study provides a comprehensive theoretical basis for soil microecology regarding the reduction in chemical fertilizer use and sustainable green cultivation in red soil tea plantations.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16202041/s1>, Table S1: The 31 carbon sources of BIOLOG-ECO.

Author Contributions: X.P. and Y.M. designed the experiments, collected data, and wrote the first draft of the manuscript; C.L., Y.W. and M.Z. analyzed the data and drew figures; Y.L., X.Z. and D.L. interpreted the data; X.J., H.W. and H.H. supervised the experiments and revised the manuscript. C.F. and J.Y., conceptualization, project administration, supervision, resources, funding acquisition, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the article Materials, further inquiries can be directed to the corresponding author. The plot-level dataset and the R scripts used for the multivariate analyses are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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