

Fertilization Regimes Shape Soil Bacterial Community Assembly and Tea Quality in Yellow Tea Plantations via Differential Modulation of Soil Nutrients and Enzyme Activities

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Abstract

Background and aims: Fertilization regulates tea quality, but how fertilizer type shapes soil microbial communities and yellow tea quality remains unclear.

Methods: We compared unfertilized control (YTCK), conventional fertilization (YTCF; urea + compound fertilizer), and organic–inorganic combined fertilization (YTOF; urea + compound fertilizer + rapeseed cake manure, 30% nitrogen from organic sources) in a Jinyun yellow tea plantation. Soil physicochemical properties, enzyme activities, bacterial 16S rRNA sequencing, functional metabolic profiles, and tea quality were analyzed using Spearman correlation with FDR correction.

Results: YTCF primarily increased available phosphorus and potassium, whereas YTOF distinctly enhanced urease, catalase, and sucrase activities. Both treatments similarly increased total flavonoids, soluble sugars, tea polyphenols, and water extract. YTCF promoted free amino acids, while YTOF reduced caffeine and the phenol/ammonia ratio. Fertilization drove directional bacterial community assembly and functional differentiation: YTCF enriched *Sphingomonas* and favored labile organic matter turnover, whereas YTOF enriched *Albibacterium*/Flavobacterium and activated aromatic-degrading and phosphorus-cycling functions. FDR-corrected correlations ($q < 0.05$) identified seven genus-quality links, including positive associations of *Gemmatimonas* with soluble sugars and free amino acids, and *Candidatus Koribacter* with phenol/ammonia ratio but negative with water extract.

Conclusions: Conventional fertilization favored amino acid accumulation, whereas organic–inorganic combined fertilization reduced caffeine and the phenol/ammonia ratio, improving flavor balance. These strategies differentially affect yellow tea quality by shaping distinct topsoil bacterial community assembly and functional pathways, providing a basis for optimizing fertilization to meet specific quality targets.

Introduction

As the center of origin for *Camellia sinensis*, China remains the world's largest tea producer and consumer. The tea industry plays an irreplaceable role in rural revitalization and enhancing farmers' incomes (Yildiz et al., 2026). Among the six major tea categories—green, black, white, yellow, oolong, and dark tea—yellow tea distinguishes itself through its unique "yellowing" (menhuang) process, during which moderate degradation of chlorophyll and non-enzymatic oxidation of polyphenols occur under controlled temperature and moisture conditions, yielding the characteristic "yellow liquor and yellow leaves" visual profile and a mellow, sweet taste signature (Fan et al., 2022). Compared with green tea, yellow tea exhibits markedly reduced astringency and diminished irritancy, while amino acids, soluble sugars, and characteristic aroma compounds (e.g., linalool and its oxides) serve as core quality markers that collectively confer the fresh, sweet, and mellow flavor profile of yellow tea (Bai et al., 2023; Xu et al., 2026). Jinyun yellow tea, as a rare and premium variety within the yellow tea category, is cultivated in mountainous tea gardens at elevations exceeding 500 m above sea level. The distinctive natural ecological conditions of this region endow the tea with exceptional quality attributes, rendering it of considerable economic value and germplasm research significance (Shi et al., 2024).

While the intrinsic quality of Jinyun yellow tea is largely shaped by the inherent ecological conditions of the producing region, fertilization remains the most critical agronomic intervention for modulating the material foundation of tea quality. In recent years, substantial progress has been made in understanding the effects of fertilization on soil microbial communities and tea quality in plantation systems. At the macroscopic level, Zhu et al. conducted a comprehensive review of four management practices—planting patterns, irrigation, fertilization regimes, and weed control—on soil microbial communities in tea plantations, demonstrating that scientifically informed management can reshape microbial composition and structure, improve soil fertility, and exert positive effects on tea yield and quality (Zhu et al., 2023). Regarding fertilizer type comparisons, Lei compared the effects of chemical and organic fertilizers in two tea plantation types, revealing that chemical fertilizers directly increased soil mineral element contents, whereas organic fertilizers significantly enhanced tea polyphenol accumulation while reducing caffeine content, with both fertilization regimes exerting significant effects on microbial beta diversity (Lei et al., 2024). With respect to the optimal substitution ratio of organic for chemical fertilizer, a meta-analysis indicated that organic substitution at 25%–75% maximally improved tea quality, with the effect diminishing beyond 75% substitution; field trials in Meitan, Guizhou, further demonstrated that substituting 20% of chemical nitrogen with organic nitrogen enhanced both tea yield and quality, while concurrently increasing soil pH, organic matter, and nitrogen content (Ren et al., 2024). Regarding combined organic-inorganic fertilization, a study published in *Scientia Horticulturae* in 2024 reported that after consecutive years of application of high-carbon inorganic fertilizer and low-carbon organic fertilizer, the organic fertilizer treatment exhibited significantly higher abundances of biomarker taxa for both Gram-positive and Gram-negative bacteria in soil, with phytosterols and carbohydrates identified as core components of the metabolic processes, and the organic-amended soils demonstrated greater potential for humus formation, biochemical stability maintenance, and soil-borne disease suppression (Jiang et al., 2024). Complementary studies in Fujian tea plantations further confirmed that integrated organic-inorganic fertilization can optimize microbial community structure, balance functional expression, and mitigate the negative effects associated with chemical fertilizer alone (Miao et al., 2024). In addition, Zhou et al. employed metagenomic sequencing to elucidate the seasonal succession patterns of soil microbial communities under different fertilization regimes (Zhou et al., 2024), while Yan et al. investigated changes in bacterial community structure and functional groups in tea plantation soils after six years of contrasting fertilization treatments (Yan et al., 2024). At the enzymatic level, combined organic-inorganic fertilization resulted in peak activities of polyphenol oxidase and urease, significantly improving the efficiency of soil microbial carbon source utilization (Miao et al., 2024). At the mechanistic level, fertilization not only directly influences tea plant growth by altering soil nutrient availability but also indirectly affects tea quality by modulating the assembly processes of soil microbial communities—accumulating evidence has confirmed that fertilization-induced differences can reshape the diversity and functionality of soil microbial communities, which in turn exert cascading effects on tea plant performance and quality formation (Lu et al., 2026).

However, several critical knowledge gaps remain in the existing literature. First, the research subjects have been predominantly confined to green tea and oolong tea cultivation systems, whereas the response patterns of soil microbial communities to fertilization regimes in yellow tea—a specialty category with distinct quality requirements—and the underlying mechanisms governing their contributions to quality formation remain largely unexplored (Chen et al., 2023;

Zheng et al., 2026). Second, most previous studies have focused on single fertilization types or simple comparisons between chemical and organic fertilizers, with limited in-depth elucidation of the causal linkages between microbial community assembly processes and tea quality formation under combined organic-inorganic fertilization conditions(Zhang et al., 2025). Third, the optimization of fertilization strategies to synergistically regulate soil fertility and the rhizosphere microecological environment for the targeted improvement of tea quality remains a frontier challenge that urgently requires breakthrough in the fields of tea plant cultivation and soil health research(Huang et al., 2026; Lei et al., 2024).

Material and methods

Study Site and Experimental Design

The field experiment was conducted at the core tea planting area of Rongda Ecological Agriculture Co., Ltd., in Jinyun County, Lishui City, Zhejiang Province, China (28°39'30.35" N, 120°17'04.89" E). The experimental site is privately owned and managed by Rongda Ecological Agriculture Co., Ltd., and permission to collect soil and tea leaf samples was obtained from the landowner/management company prior to sampling. The region has a mid-subtropical monsoon climate, with a mean annual temperature of 16.9°C, mean annual precipitation of 1450.9 mm, and a frost-free period of 245 days. The soil type was red soil (or yellow-red soil). The tea plants used in this study were a locally cultivated yellow tea cultivar (*Camellia sinensis*), not a wild or protected species. Because the harvested leaves were processed into yellow tea for quality analysis, conventional herbarium voucher specimens could not be prepared. To document the identity and origin of the plant material, geo-referenced sampling records, field photographs, cultivar records, and processed tea samples have been retained at the College of Tea Science and Tea Culture, Zhejiang A & F University. The cultivar identity was confirmed by Jiawei Ma, College of Tea Science and Tea Culture, Zhejiang A & F University.

Three fertilization treatments were established: (1) no fertilizer control (YTCK); (2) conventional fertilization (YTCF), with urea at 600 kg·hm⁻² and compound fertilizer (N–P₂O₅–K₂O: 22–5–13) at 2250 kg·hm⁻²; and (3) combined application of chemical fertilizer and rapeseed cake manure (YTOF), with urea at 420 kg·hm⁻², compound fertilizer at 1575 kg·hm⁻², and rapeseed cake manure at 4800 kg·hm⁻² (where 30% of the total nitrogen was supplied by rapeseed cake manure, replacing an equivalent amount of chemical nitrogen). Each plot was approximately 100 m², and all fertilizers were applied as a single basal dressing. Each treatment was replicated three times.

Except for the fertilization regimes, all other agronomic practices, including irrigation, weeding, and pest and disease control, were kept consistent across all plots.

Soil and Tea Sample Collection

Soil samples were collected during the spring tea season. Within each plot, five subsamples were collected from the 0–20 cm layer following a five-point sampling method and pooled into one composite sample weighing no less than 1 kg. Surface litter, gravel, and other debris were removed prior to sampling. The collected soil samples were immediately placed into sterile zipper bags, stored in a portable cooler at 4°C, and transported to the laboratory on the same day. Each composite sample was divided into three portions: one portion was stored at -80°C for soil microbial total DNA extraction; the second portion was passed through a 2-mm sieve and stored at 4°C for soil enzyme activity assays; the third portion was air-dried at room temperature for 7–10 days for routine physicochemical property analysis. Tea leaf samples were harvested according to the standard of “one bud with two leaves”, and were immediately subjected to fixation (steam or pan-firing) and drying processes for subsequent quality parameter determination.

Soil Physicochemical Property Analysis

Soil pH was determined potentiometrically at a soil-to-water ratio of 1:2.5. Soil organic matter content was analyzed by the potassium dichromate oxidation method. Alkaline-hydrolyzable nitrogen was measured using the alkaline diffusion method. Available phosphorus was determined by the sodium bicarbonate extraction-molybdenum antimony colorimetric method. Available potassium was quantified by the flame photometry method. All determinations were performed following the standard protocols described by Bao(Bao, 2000).

Soil Enzyme Activity Assays

Soil enzyme activities were determined by colorimetric methods. Urease activity was assayed using the indophenol blue colorimetric method. Catalase activity was determined by potassium permanganate titration. Acid phosphatase activity was measured by the disodium phenyl phosphate colorimetric method. Sucrase activity was assayed using the 3,5-dinitrosalicylic acid colorimetric method.

Tea Quality Parameter Determination

Tea quality parameters of Jinyun yellow tea included total flavonoids, soluble sugars, tea polyphenols, caffeine, free amino acids, soluble protein, phenol-to-amino acid ratio, and water extract. Total flavonoid content was determined by the aluminum chloride colorimetric method. Soluble sugar content was measured by the anthrone colorimetric method. Tea polyphenol content was determined by the ferrous tartrate colorimetric method (referenced to GB/T 8313). Caffeine content was measured by ultraviolet spectrophotometry (referenced to GB/T 8312). Free amino acid content was determined by the ninhydrin colorimetric method (referenced to GB/T 8314). Soluble protein content was measured by the Coomassie Brilliant Blue method. The phenol-to-amino acid ratio was calculated as the ratio of tea polyphenol content to free amino acid content. Water extract content was determined by the gravimetric method (referenced to GB/T 8305).

DNA Extraction, PCR Amplification, and High-Throughput Sequencing

Total microbial genomic DNA was extracted from 0.5 g of each soil sample using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. DNA quality and concentration were assessed by 1.0% agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Liu et al., 2016). PCR products were examined by 2% agarose gel electrophoresis, purified using the PCR Clean-Up Kit (YuHua, Shanghai, China), and quantified with Qubit 4.0 (Thermo Fisher Scientific, USA). Purified amplicons were pooled in equimolar amounts and sequenced on the Illumina NextSeq 2000 platform (Illumina, San Diego, USA) using paired-end 2 × 300 bp reads at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw reads were quality-filtered and merged using fastp (Chen et al., 2018) (<https://github.com/OpenGene/fastp>, version 0.19.6) and FLASH (v1.2.11) (Magoč & Salzberg, 2011). Optimized sequences were clustered into operational taxonomic units at 97% similarity using UPARSE v7.1 (Edgar, 2013; Stackebrandt & Goebel, 1994), and chimeras were removed. Taxonomic assignment was performed against the Silva 138 database using the RDP Classifier v2.11 (Wang et al., 2007) with a confidence threshold of 70%. To minimize the effects of sequencing depth, all samples were rarefied to the same sequence number. Functional prediction was conducted using PICRUSt2 v2.2.0 (Douglas et al., 2020) based on 16S rRNA gene sequences.

Bioinformatics and Statistical Analysis

Raw sequencing reads were quality-filtered, merged, and clustered into amplicon sequence variants (ASVs) at 97% sequence similarity, with taxonomic assignment performed against the Silva database. Alpha diversity (Chao1, Shannon), beta diversity (PCoA based on Bray-Curtis, ANOSIM), taxonomic composition (phylum/genus), PICRUSt2 functional prediction, redundancy analysis, and Spearman correlation between bacterial genera and tea quality indicators were performed based on ASV abundance data. Tea quality parameters, soil physicochemical properties, and enzyme activities were analyzed using one-way analysis of variance in SPSS, with graphical visualization performed in RStudio.

Results

Fertilization-Driven Changes in Soil Physicochemical Properties and Enzyme Activities

Soil Physicochemical Properties

One-way ANOVA results (Fig. 1) showed that soil pH and organic matter content did not differ significantly among treatments. However, significant differences were observed in soil available potassium (AK) and available phosphorus (AP) across fertilization treatments ($P < 0.05$), with both nutrients being highest in the conventional fertilization treatment and significantly higher than in the organic-inorganic combined fertilization and control treatments, indicating that conventional fertilization provided the most immediate nutrient availability. For alkali-hydrolyzable nitrogen (AN), no significant difference was found between the two fertilization treatments (YTCF and YTOF), but both were significantly higher than the control, suggesting that both conventional fertilization and organic-inorganic combined fertilization significantly increased soil AN content, with comparable promoting effects.

Soil Enzyme Activities

The four soil enzymes assayed exhibited distinct response patterns to fertilization treatments (Fig. 2). Urease and catalase activities followed identical trends: YTOF exhibited the highest activities, followed by YTCF, with YTCK showing the lowest values, and all inter-group differences reached statistical significance. This indicates that organic–inorganic combined fertilization most effectively promoted nitrogenous organic matter decomposition and soil redox metabolism. Acid phosphatase activity showed a different pattern: both fertilized treatments (YTCF and YTOF) significantly exceeded the control, with no significant difference between the two fertilized groups, suggesting that both conventional and organic-amended fertilization effectively promoted organic phosphorus mineralization. Sucrase activity was highest in YTOF, significantly surpassing both YTCF and YTCK, indicating a stronger activation effect of organic amendment on carbon-cycling enzymes.

Overall, fertilization treatments significantly influenced soil enzyme activities. The organic–inorganic combined treatment exhibited superior performance in urease, catalase, and sucrase activities, while conventional fertilization and organic amendment were equally effective in enhancing acid phosphatase. Considering all four enzymes collectively, organic–inorganic combined fertilization appears most favorable for comprehensively enhancing soil biochemical activities related to carbon, nitrogen, and phosphorus cycling.

Fertilization-Driven Assembly of Bacterial Communities

Alpha Diversity Responses to Fertilization

One-way ANOVA was employed to compare microbial diversity indices across treatments. In the Jinyun yellow tea soils, bacterial alpha diversity exhibited differential responses to fertilization regimes. Specifically, the Chao1 richness index was significantly higher in the YTOF treatment than in YTCF and YTCK ($P < 0.05$, Fig. 3A), indicating that organic amendment markedly enhanced bacterial species richness. In contrast, the Simpson evenness index did not differ significantly among treatments ($P > 0.05$, Fig. 3B), suggesting that the increase in species richness induced by organic fertilization was not accompanied by a substantial change in community evenness. Collectively, these findings imply that the beneficial effect of organic–inorganic combined fertilization on bacterial diversity was primarily driven by an increase in species richness, whereas evenness remained largely unaltered. The introduction of organic matter

likely created novel ecological niches that facilitated the proliferation of diverse taxa, yet the dominance structure of the resident microbial community was not significantly perturbed.

Beta Diversity Responses to Fertilization

To reveal the structural differentiation characteristics of soil bacterial communities under different fertilization regimes, principal coordinates analysis (PCoA) was employed to visualize the community composition in a reduced-dimensional space (Fig. 3C). The results showed that the first two principal coordinate axes collectively explained 78.95% of the total variation in community structure, indicating that the ordination model exhibited high representativeness. In the ordination space, samples from the YTCK, YTCF, and YTOF treatments were clearly separated along the axes, forming non-overlapping, distinct clusters. This observation was further corroborated by ANOSIM ($R = 1.000$, $P = 0.005$), confirming that fertilization served as the dominant factor driving the directional differentiation of bacterial communities in the tea plantation soils, and that the inputs of chemical and organic fertilizers exerted fundamentally divergent effects on community assembly.

Community Composition at Phylum and Genus Levels

At the phylum level (Fig. 4A), Pseudomonadota, Acidobacteriota, and Bacteroidota were the absolute dominant phyla across all three treatments. To elucidate the differences in soil bacterial community composition at the phylum level among treatments, the top 10 bacterial phyla with the highest average relative abundances across all samples were selected, and Kruskal–Wallis H test was performed to assess significant differences among the YTCK, YTCF, and YTOF groups, with results visualized as a bar chart (Fig. 4B). The results revealed that the average relative abundances of Chloroflexota, Actinomycetota, Gemmatimonadota, and Nitrospirota were significantly higher in the YTCK treatment than in the other two groups. Verrucomicrobiota exhibited the highest relative abundance in the YTCF group. Additionally, low-abundance phyla such as Latescibacterota, Thermodesulfobacteriota, and FCPU426 were predominantly enriched in the YTOF treatment.

At the genus level (Fig. 4C), *Rhodanobacter* was identified as the core genus shared among all three treatment groups, while each treatment also harbored its own unique dominant genera. Specifically, the YTCK group was characterized by the presence of *Chujaibacter* and *Bryobacter* as dominant genera, whereas the YTCF and YTOF groups shared similar dominant genus compositions, predominantly comprising *Albibacterium* and *Sphingomonas*. To further elucidate the differentiation characteristics of soil bacterial communities at the genus level driven by different fertilization regimes, the top 10 bacterial genera with the highest average relative abundances across all samples were selected, and the Kruskal–Wallis H test was performed to assess the significance of abundance differences among the YTCK, YTCF, and YTOF groups, with results visualized as a bar chart (Fig. 4D). The results revealed that the average relative abundances of *Rhodanobacter* and *Bryobacter* in the YTCK treatment were significantly higher than those in the other two groups; *Albibacterium* and *Lysobacter* exhibited the highest abundances in the YTOF treatment; and *Sphingomonas* showed higher relative abundance in the YTCF group.

Collectively, the phylum and genus-level results indicated that fertilization significantly altered soil bacterial community composition, with distinct response magnitudes across taxonomic levels. At the phylum level, the dominant phyla (Pseudomonadota, Acidobacteriota, and Bacteroidota) remained prevalent across all treatments, whereas the relative abundances of Chloroflexota and Actinomycetota decreased substantially following fertilization, and certain low-abundance phyla such as Latescibacterota were predominantly enriched in the organic-amended treatment. At the genus level, community differentiation was more pronounced: *Rhodanobacter* was the core genus shared among all treatments yet exhibited the highest abundance in the control, whereas *Albibacterium*, *Lysobacter*, and *Sphingomonas* showed specific enrichments in different fertilization regimes. Overall, genus-level taxa responded more sensitively to fertilization than phylum-level taxa, and compositional reorganization was more conspicuous at finer taxonomic resolutions.

Functional Metabolic Profiling of Soil Microbial Communities

Using the non-fertilized control (YTCK) as reference, differentially abundant enzymes were identified with thresholds of $|\log_2 FC| > 1.5$ and mean relative abundance > 50 . The top 15 enzymes showing the largest total variation were selected for visualization (Fig. 5). Functional annotation revealed that fertilization differentially reprogrammed pathways related to carbon/nitrogen metabolism, antioxidant defense, and aromatic compound degradation. Specifically, both treatments consistently activated carbohydrate-active enzymes (cellobiose phosphorylase EC 2.4.1.20, sucrose phosphorylase EC 2.4.1.7) and antioxidant enzymes (L-ascorbate oxidase EC 1.10.3.3, arachidonate 15-lipoxygenase EC 1.13.11.33), while significantly suppressing xenobiotic-degrading enzymes (cis-2,3-dihydrobiphenyl-2,3-diol dehydrogenase EC 1.3.1.56, (R)-benzylsuccinyl-CoA dehydrogenase EC 1.3.8.3) and an anaerobic respiratory enzyme (fumarate reductase EC 1.3.4.1) that were enriched in the control. Conventional fertilization (YTCF) specifically enhanced organic matter decomposition (Aspergillus nuclease S1 EC 3.1.30.1, saccharopine dehydrogenase EC 1.5.1.10), whereas inorganic combined fertilization (YTOF) uniquely upregulated aromatic degradation (4-hydroxymandelate synthase EC 1.13.11.46) and phosphorus cycling (phosphatidylinositol diacylglycerol-lyase EC 4.6.1.13, 3-phytase EC 3.1.3.8). Collectively, both fertilization strategies alleviate C/N stress, yet YTCF favors labile organic matter turnover while YTOF drives aromatic compound degradation and P mobilization, providing functional evidence for optimizing fertilization practices.

Differential responses of tea quality indicators under different fertilization regimes.

One-way ANOVA revealed that fertilization treatments significantly affected the main quality parameters of Jinyun yellow tea (Fig. 6). The contents of total flavonoids, soluble sugars, tea polyphenols, and water extract were all significantly higher in both conventional fertilization and organic–inorganic combined fertilization treatments than in the unfertilized control, with no significant differences between the two fertilized treatments. This pattern indicates that fertilization per se was the key driver for improving these basic quality components, whereas fertilizer type exerted limited effects. Notably, water extract content exhibited the largest increase, rising from 17.83% in the control to above 32% in the fertilized groups. In contrast, caffeine and free amino acid contents displayed divergent responses to fertilizer type. Conventional fertilization significantly promoted free amino acid accumulation, yielding the highest content among the three groups, while organic–inorganic combined fertilization specifically reduced caffeine content and concurrently lowered the phenol-to-ammonia ratio; the latter two parameters showed no difference between the unfertilized control and conventional fertilization. Soluble protein content remained unchanged across treatments. Collectively, conventional fertilization excelled in enhancing amino acid content, whereas organic–inorganic

combined fertilization demonstrated unique advantages in reducing caffeine and the phenol-to-ammonia ratio, thereby improving tea infusion flavor balance and decreasing stimulant components. Both fertilization regimes were equally effective in elevating polyphenols, soluble sugars, and water extract content.

Linking soil physicochemical properties, rhizobacterial community assembly, and tea quality traits.

To disentangle the environmental drivers of bacterial community differentiation and their potential links to tea quality, we first performed redundancy analysis (RDA) based on genus-level relative abundances and soil physicochemical parameters (Table 1, Fig. 7). The RDA ordination clearly separated the three treatment groups (YTCK, YTCF, and YTOF), confirming that fertilization regime was the overriding force shaping community composition. Environmental fitting indicated that pH was the predominant factor associated with the unfertilized control; available nitrogen (AN) dominated the organic–inorganic combined treatment (YTOF); whereas available phosphorus (AP), available potassium (AK), and soil organic matter (SOM) collectively regulated the conventional fertilization group (YTCF). These results demonstrate that different fertilization strategies restructure bacterial communities by altering distinct sets of soil nutrient factors.

Table 1
Envfit analysis results of environmental factors on soil bacterial community structure in Jinyun Yellow Tea plantations

Environmental factor	RDA1	RDA2	r ²	P-value
pH	0.95907	-0.28317	0.4495	0.167
AK	-0.93424	-0.35664	0.95302	0.003
AP	-0.89989	-0.43611	0.92856	0.003
AN	-0.95072	0.31005	0.99383	0.013
SOM	-0.41967	-0.90768	0.33738	0.318
Note: RDA1 and RDA2 represent the correlation coefficients between environmental factors and ordination axes; r ² indicates the explanatory power of environmental factors for community structure variation.				

Having established that fertilization drives community assembly via differential nutrient filtering, we next asked whether the shifted community composition, in turn, correlates with the quality parameters of yellow tea. Spearman correlation analysis with Benjamini–Hochberg false discovery rate (FDR) correction ($q < 0.05$) revealed seven statistically significant genus–quality associations (Fig. 8). For positive associations, *Gemmatimonas* showed significant positive correlations with soluble sugars (SS, $r = 0.917$, $q = 0.031$) and free amino acids (FAA, $r = 0.933$, $q = 0.025$), suggesting a potential role in enhancing both sweet and umami taste components. Additionally, *Albibacterium* ($r = 0.967$, $q = 0.020$) and *Flavobacterium* ($r = 0.933$, $q = 0.025$) were positively correlated with water extract (WE), implying their involvement in improving tea broth thickness and soluble content extractability. For negative associations, *Chujaibacter* was significantly negatively correlated with SS ($r = -0.917$, $q = 0.031$), indicating a possible suppressive effect on sugar accumulation. Notably, *Candidatus Koribacter* exhibited a dual association pattern—positively correlated with the phenol-to-amino acid ratio (PAR, $r = 0.950$, $q = 0.020$) but negatively with WE ($r = -0.950$, $q = 0.020$). Given that PAR is a key indicator of bitterness and astringency, this pattern suggests that *Candidatus Koribacter* may simultaneously elevate astringent potential and reduce infusion fullness, thereby exerting a complex influence on taste balance.

Collectively, these findings link fertilization-driven changes in soil nutrients and bacterial community composition to specific quality outcomes, providing a mechanistic framework for understanding how management practices ultimately shape yellow tea flavor profiles.

Discussion

Differential regulation of soil physicochemical properties and enzyme activities by fertilization regimes

This study revealed a clear divergence in how fertilization regimes regulated soil available nutrients and enzyme activities. Conventional fertilization (YTCF) significantly increased available potassium and phosphorus, directly attributable to the rapid dissolution of mineral nutrients from the compound fertilizer, consistent with the immediate nutrient-supplying effects of chemical fertilizers reported in tea plantations(Lei et al., 2024). In contrast, the organic–inorganic combined treatment (YTOF) exhibited lower available nutrient levels than YTCF, as rapeseed cake manure releases nutrients slowly through microbial mineralization rather than providing readily available forms. Notably, YTOF showed significantly higher urease, catalase, and sucrase activities than the other treatments, indicating that organic amendment confers unique advantages in activating soil biochemical processes related to carbon and nitrogen cycling.

Fertilization drives the directional assembly of soil bacterial communities via environmental filtering

The shaping effect of fertilization on soil bacterial communities was particularly evident at the β -diversity level—the complete separation of the three treatment groups in PCoA ordination (ANOSIM: $R = 1.000$, $P = 0.005$) indicates that fertilization was the overriding driver of community differentiation, surpassing even intrinsic soil heterogeneity in magnitude. The overriding influence of fertilization on bacterial community differentiation observed here underscores that nutrient management serves as the primary deterministic filter shaping soil microbiome assembly in agricultural systems. Regarding assembly mechanisms, the additional ecological niches created by organic matter inputs—including novel carbon sources and increased microhabitat heterogeneity—likely underpin the significantly higher Chao1 richness in YTOF. However, the unchanged Simpson evenness across treatments suggests that

the dominance structure of the resident community was not disrupted, a pattern consistent with observations by Miao et al. (2024) in organic-amended Fujian tea plantations.

Reprogramming of bacterial community functional metabolic profiles by fertilization exhibits strategic divergence

Functional prediction via PICRUSt2 revealed a marked divergence in microbial metabolic "investment strategies" between the two fertilization regimes—a finding that extends our understanding beyond taxonomic shifts. Both treatments consistently upregulated carbohydrate-active enzymes and antioxidant enzymes, suggesting that fertilization alleviated carbon–nitrogen stress and enhanced microbial capacity for carbon acquisition and oxidative defense, in agreement with the metagenomic observations of Shao et al. (Shao et al., 2024) in tea plantations. However, functional divergence was equally pronounced: YTCF specifically enhanced nucleic acid-degrading and lysine-metabolizing enzymes, implying that under conventional fertilization, microbes prioritize the turnover of labile organic matter for rapid proliferation. In contrast, YTOF uniquely upregulated aromatic-degrading and phosphorus-cycling enzymes (phytase and phospholipase). Rapeseed cake manure is rich in aromatic compounds (e.g., lignin derivatives) and phytate-bound organic phosphorus, and this functional adaptation likely represents a precise microbial response to substrate chemistry. Notably, the elevated phytase potential in YTOF suggests an enhanced capacity to mobilize recalcitrant organic phosphorus—a mechanism also reported by Song et al. in organic-manured tea soils, though the enrichment of phytase-related functions in our study was more pronounced, possibly attributable to the specific phosphorus composition of rapeseed cake (Song et al., 2023).

Correlation between dominant bacterial genera and yellow tea quality indicators

In this study, Spearman correlation analysis with FDR correction identified seven statistically significant associations between bacterial genera and quality indicators, providing new insights into the microbial mechanisms underlying quality differentiation in yellow tea processing.

For positive associations, *Gemmatimonas* showed significant positive correlations with soluble sugars (SS) and free amino acids (FAA). Members of *Gemmatimonas* (phylum Gemmatimonadota) are widely distributed in soil and rhizosphere environments, with reported capabilities in polysaccharide degradation and nitrogen metabolism (Mujakic et al., 2022; Zheng et al., 2022). During the yellow tea pile-fermentation process, enrichment of this genus may promote the breakdown of cellulose and hemicellulose, releasing soluble sugars, while simultaneously enhancing amino acid accumulation through proteolysis or amino acid synthesis pathways. This dual function positions *Gemmatimonas* as a potential key contributor to the sweet and umami taste characteristics of yellow tea. Notably, KEGG orthology analysis of the type strain *Gemmatimonas aurantiaca* T-27 further revealed the presence of a complete urease gene cluster (*ureC*, *ureB*, and *ureA*, corresponding to K01428–K01430), which encodes the enzymatic capacity to hydrolyze urea into ammonium—a direct nitrogen source for tea plant assimilation and amino acid biosynthesis. This genetic evidence is substantiated by the soil urease activity data, which showed a significant increase under YTOF treatment (Fig. 2A). Although bulk soil urease activity reflects community-level functional output rather than the contribution of a single genus, the urease-encoding potential of *Gemmatimonas* provides a genetic basis for its involvement in rhizosphere urea hydrolysis. The ammonium generated through this process may serve as a substrate for tea plant nitrogen assimilation, thereby supporting amino acid accumulation. Collectively, these lines of evidence—ranging from functional gene annotation (KEGG) to phenotypic enzyme activity (soil urease) and ultimately to tea quality traits (FAA)—support the hypothesis that *Gemmatimonas* contributes to the umami quality of yellow tea through urease-mediated nitrogen mineralization in the soil.

Additionally, *Albibacterium* and *Flavobacterium* were significantly positively correlated with water extract (WE), a comprehensive indicator of tea broth thickness and soluble content richness. Some *Flavobacterium* strains have been reported to secrete various extracellular enzymes (e.g., proteases, pectinases) (Kolton et al., 2013; Kraut-Cohen et al., 2021), which may disrupt tea leaf cell wall structures and promote soluble component extraction. Although *Albibacterium* has been rarely reported in tea microbiome studies, its strong correlation with WE ($r = 0.967$, the highest in this study) suggests that this genus may play a non-negligible role in enhancing soluble content yield, warranting further investigation.

For negative associations, *Chujaibacter* was significantly negatively correlated with SS content. This genus, belonging to the phylum *Acidobacteria*, has limited functional reports in tea fermentation. Its negative correlation with soluble sugars suggests that *Chujaibacter* proliferation may be associated with sugar consumption or indirect inhibition of glycoside hydrolase activity, thereby reducing sugar release and accumulation.

Notably, *Candidatus Koribacter* exhibited a distinctive dual association pattern, showing positive correlation with the phenol/ammonia ratio (PAR) but negative correlation with WE. PAR, the ratio of tea polyphenols to free amino acids, is a core indicator of tea bitterness, astringency, and taste balance. Elevated PAR typically indicates excessive polyphenol accumulation or insufficient amino acids, leading to increased bitterness and astringency (Li et al., 2016), while reduced WE implies diminished soluble solids and a "thinner" tea infusion. *Candidatus Koribacter* belongs to the *Acidobacteria*, some members of which are adapted to oligotrophic environments and exhibit high activity under acidic conditions (Kalam et al., 2020). In the YTOF treatment, enrichment of this genus may accompany organic acid secretion, exacerbating localized acidification, which in turn promotes polyphenol oxidation/polymerization or inhibits protein hydrolysis, ultimately manifesting as elevated PAR and reduced WE.

Collectively, the identified association patterns can be categorized into two functional groups: positive regulators (e.g., *Gemmatimonas*, *Albibacterium*, *Flavobacterium*) that tend to enhance sweetness, umami, and fullness, and negative/dual-role groups (e.g., *Chujaibacter*, *Candidatus Koribacter*) that may drive bitterness/astringency and reduce broth thickness. This functional differentiation likely stems from niche competition and distinct metabolic pathways among genera, ultimately shaping the differentiated quality phenotypes across processing regimes.

It should be noted that correlation analysis does not establish causation, and the limited sample size ($n = 9$) may have reduced statistical power to detect additional associations. Future studies using targeted isolation, inoculation experiments, or metatranscriptomics are warranted to elucidate the precise

functional mechanisms of these bacterial genera in yellow tea quality formation.

Conclusion

Conventional fertilization (YTCF) rapidly supplies available phosphorus and potassium and effectively elevates free amino acid content in tea leaves. In contrast, organic–inorganic combined fertilization (YTOF) significantly increases measured urease, catalase, and sucrase activities, indicating a broader activation of carbon and nitrogen cycling, while its enrichment of aromatic-degrading and phosphorus-mobilizing functional groups remains a prediction from metagenomic inference and requires further validation. YTOF also reduces caffeine and the phenol/ammonia ratio, thereby improving flavour balance, whereas both regimes equally enhance total flavonoids, soluble sugars, tea polyphenols, and water extract. Fertilization exerts a strong deterministic effect on bacterial community assembly, with YTOF increasing Chao1 richness without altering evenness, and predicted functional profiles suggest complementary metabolic adaptations between the two strategies. Collectively, these findings provide a scientific basis for selecting or combining fertilization regimes according to specific quality objectives, although the predicted functional traits should be corroborated by targeted metagenomic or transcriptomic analyses.

Declarations

Funding information

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

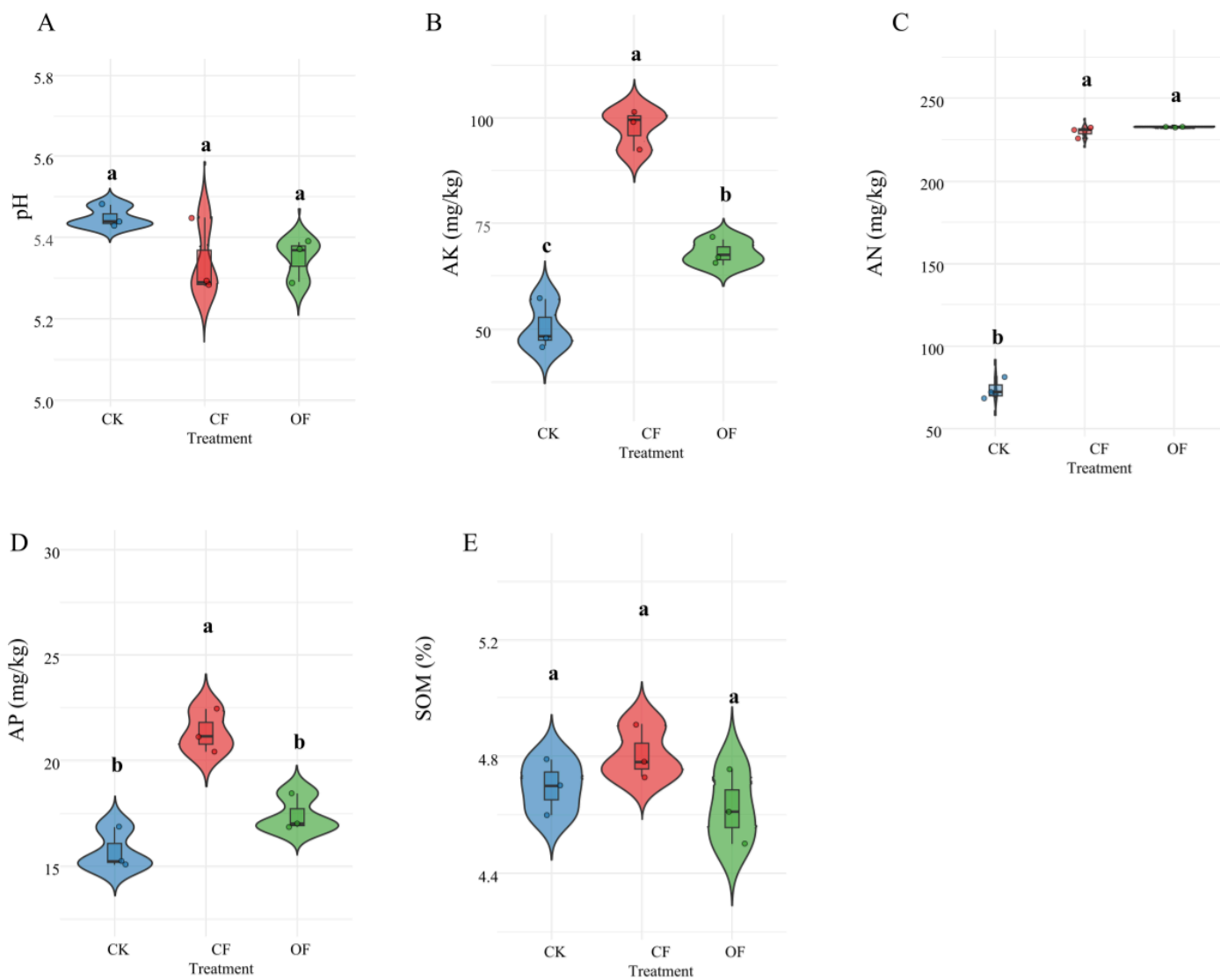


Figure 1

Violin plots of soil properties under fertilization in Jinyun tea plantations.

Note:(A) Soil pH; (B) Available potassium (AK); (C) Alkali-hydrolyzable nitrogen (AN); (D) Available phosphorus (AP); (E) Soil organic matter (SOM).

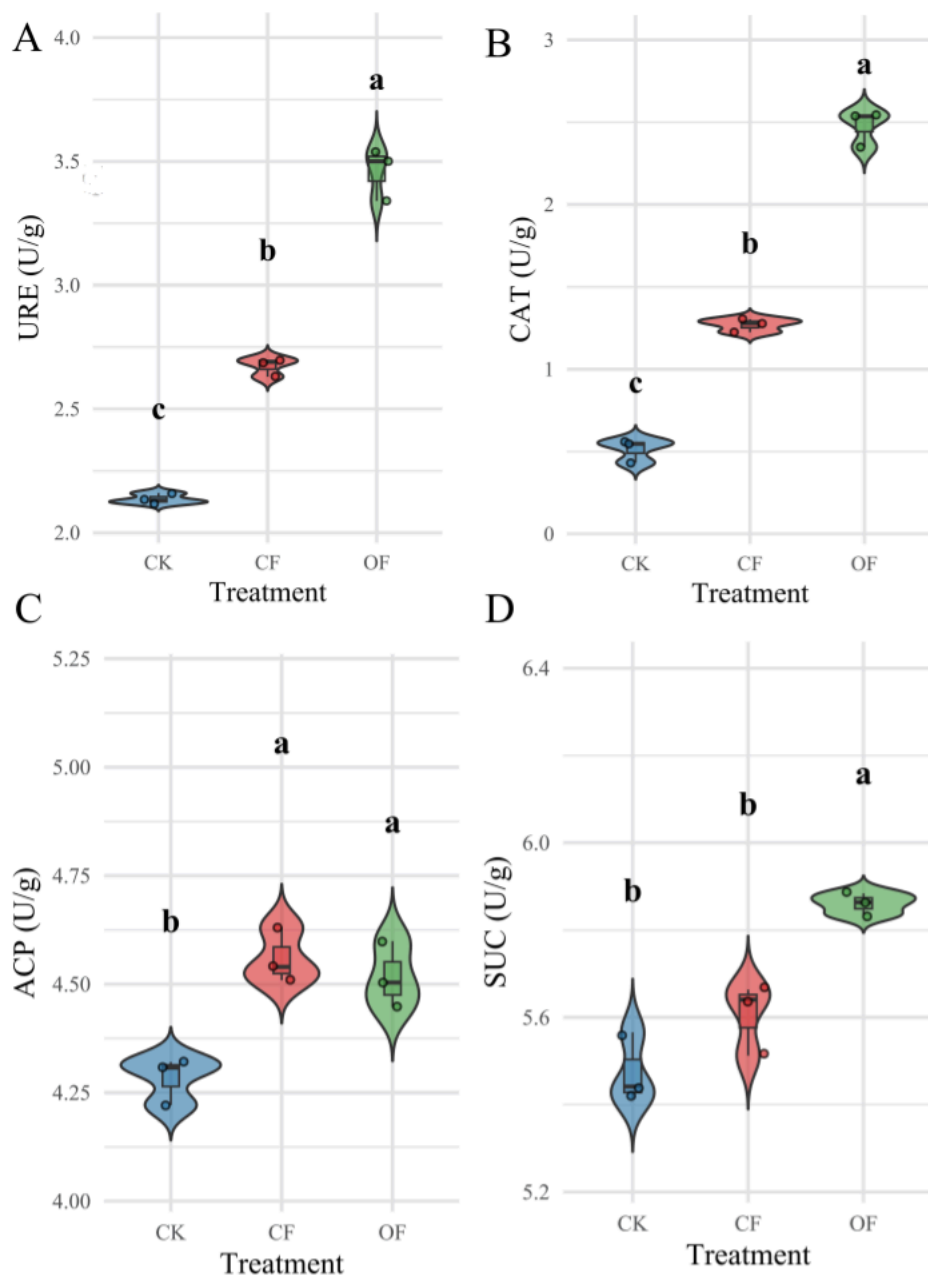


Figure 2

Violin plots of four soil enzyme activities under different fertilization treatments.

Note:(A) Urease; (B) Catalase; (C) Acid phosphatase; (D) Sucrase.

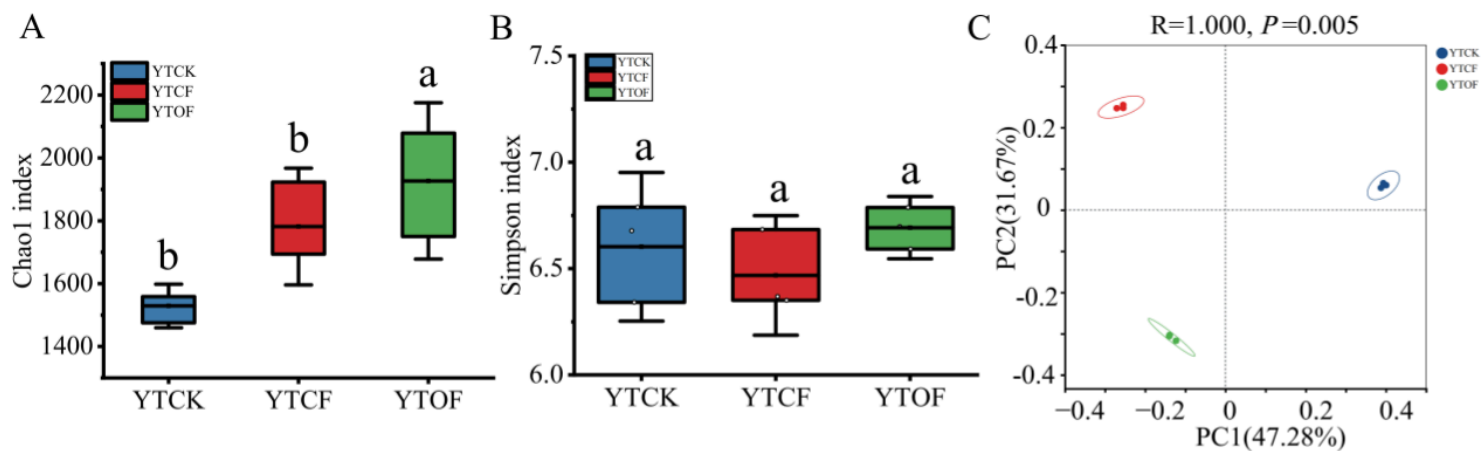


Figure 3

Alpha diversity indices and principal coordinate analysis of soil microbial community structure under different fertilization treatments.

Note:(A) Chao1 richness index; (B) Simpson evenness index. (C) Principal coordinate analysis (PCoA) based on Bray-Curtis distance. Percentages in parentheses for PC1 and PC2 indicate the proportion of variation explained by each axis. Ellipses represent 95% confidence intervals for each treatment group.

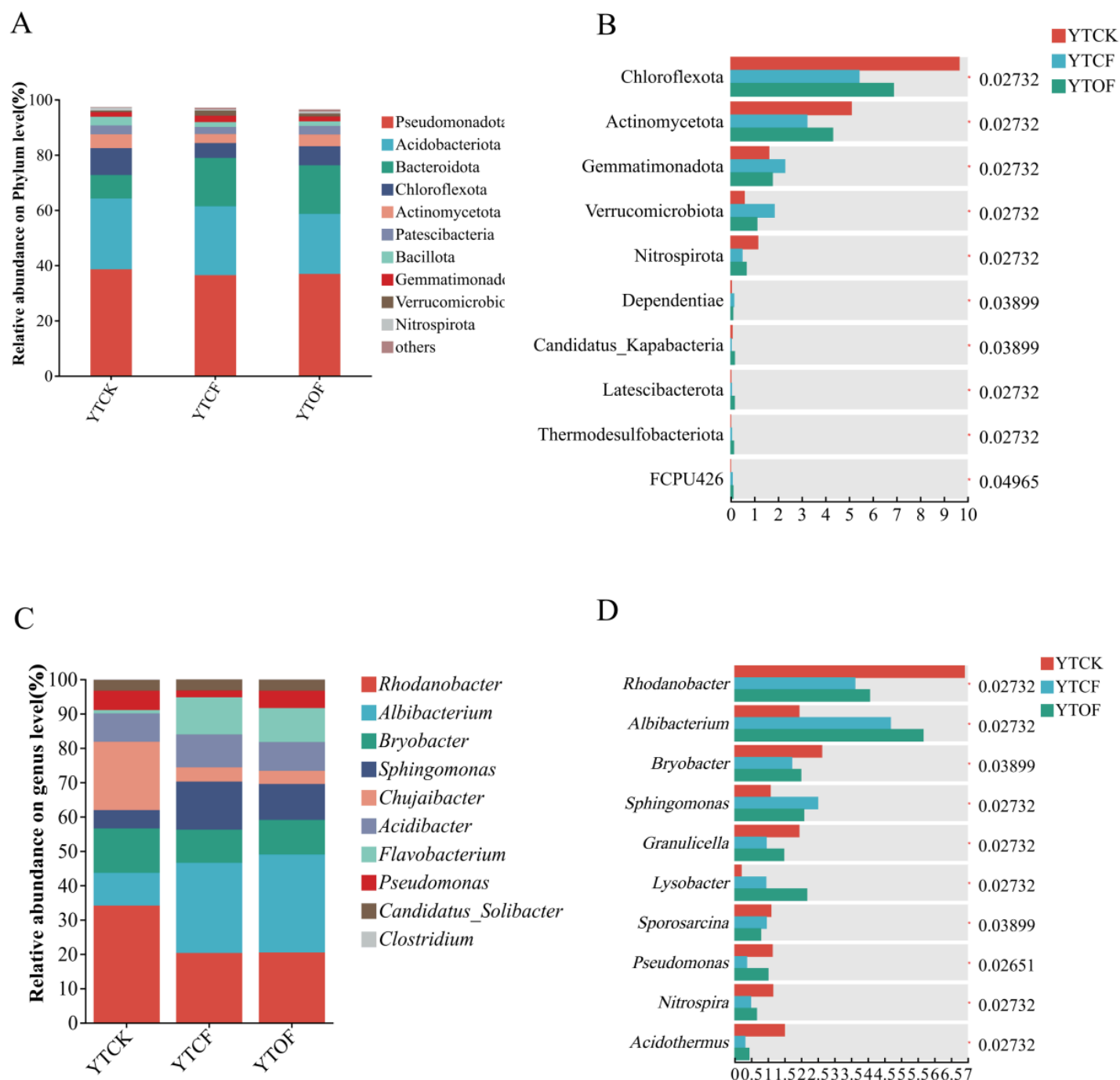


Figure 4
Phylum-level (A, B) and genus-level (C, D) community composition and differential abundance of soil bacteria under different fertilization treatments.

Note: (A) Species composition bar chart showing the relative abundances of dominant bacterial phyla (top 10 in average relative abundance) across treatments; (B) Differential abundance bar chart displaying the relative abundances of bacterial phyla with significant differences among treatments based on the Kruskal–Wallis H test ($P < 0.05$); (C) Species composition bar chart showing the relative abundances of dominant bacterial genera (top 10 in average relative abundance) across treatments; (D) Differential abundance bar chart displaying the relative abundances of bacterial genera with significant differences among treatments based on the Kruskal–Wallis H test ($P < 0.05$).

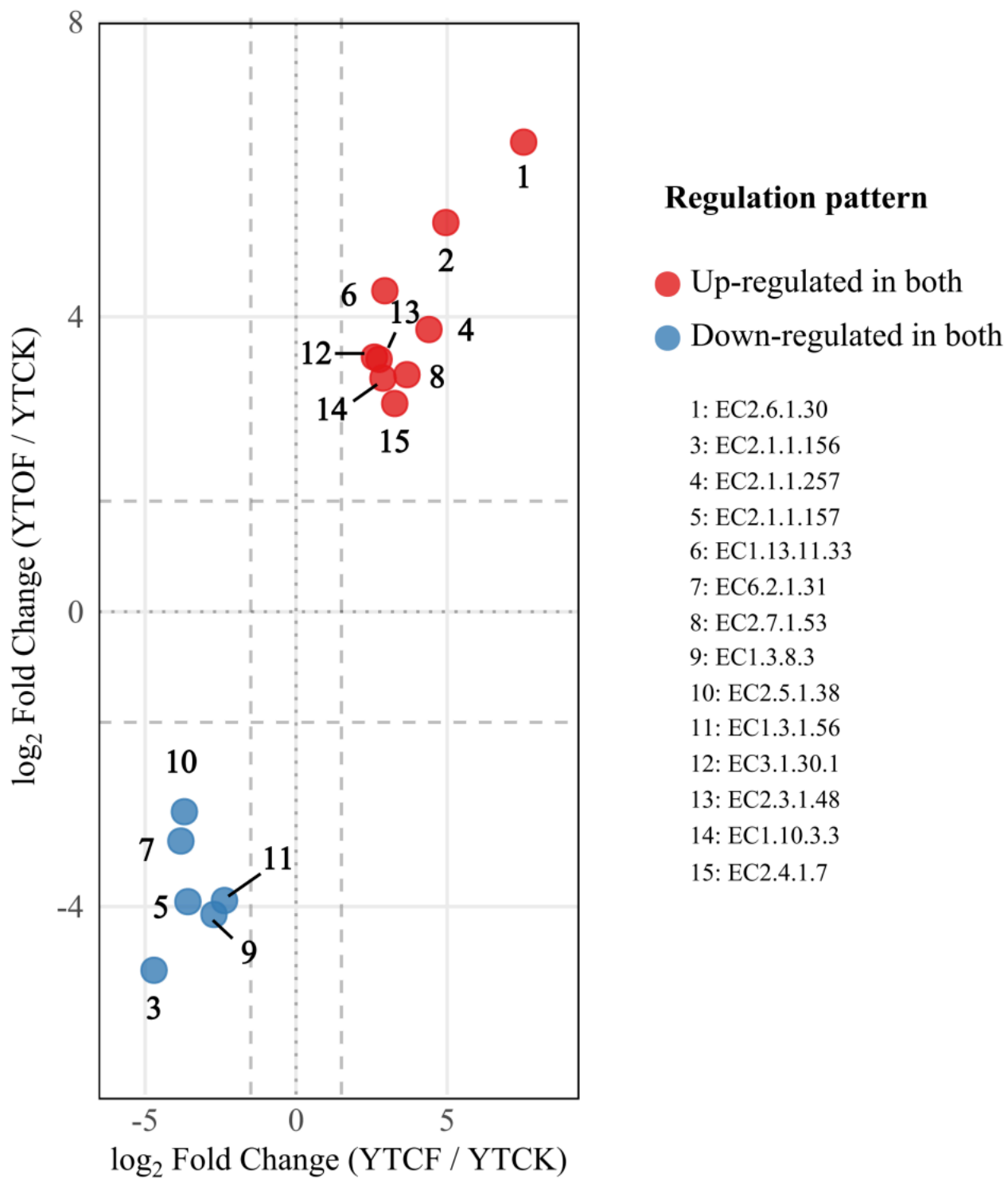


Figure 5

Expression patterns of key soil microbial enzymes under two fertilization regimes.

Note: Fold changes were calculated relative to the non-fertilized control (YTCK). The x- and y-axes show log₂ fold changes of YTCF/YTCK and YTOF/YTCK, respectively. Dashed lines mark the |log₂FC| = 1.5 threshold. Points are coloured by regulation pattern: red, up-regulated in both treatments; blue, down-regulated in both; green, up-regulated only in YTCF; orange, up-regulated only in YTOF. Each point is labelled with its EC number (leader lines link labels to data points). Only representative enzymes discussed in Results are displayed.

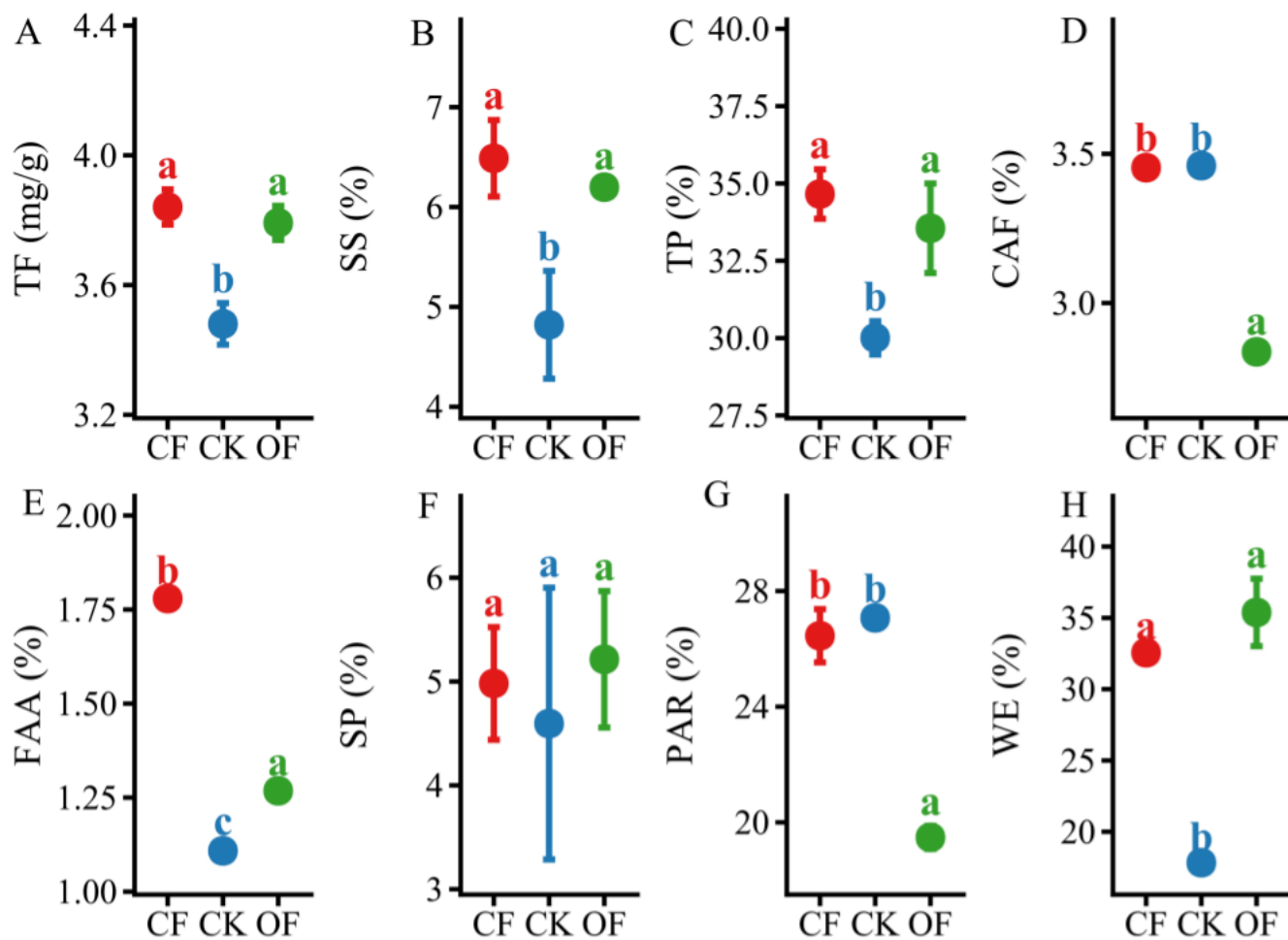


Figure 6

Effects of fertilization regimes on quality indicators of yellow tea.

Note:(A–H) denote total flavonoids (TF), soluble sugar (SS), tea polyphenols (TP), caffeine (CAF), free amino acids (FAA), soluble protein (SP), phenol-ammonia ratio (PAR), and water extract (WE).

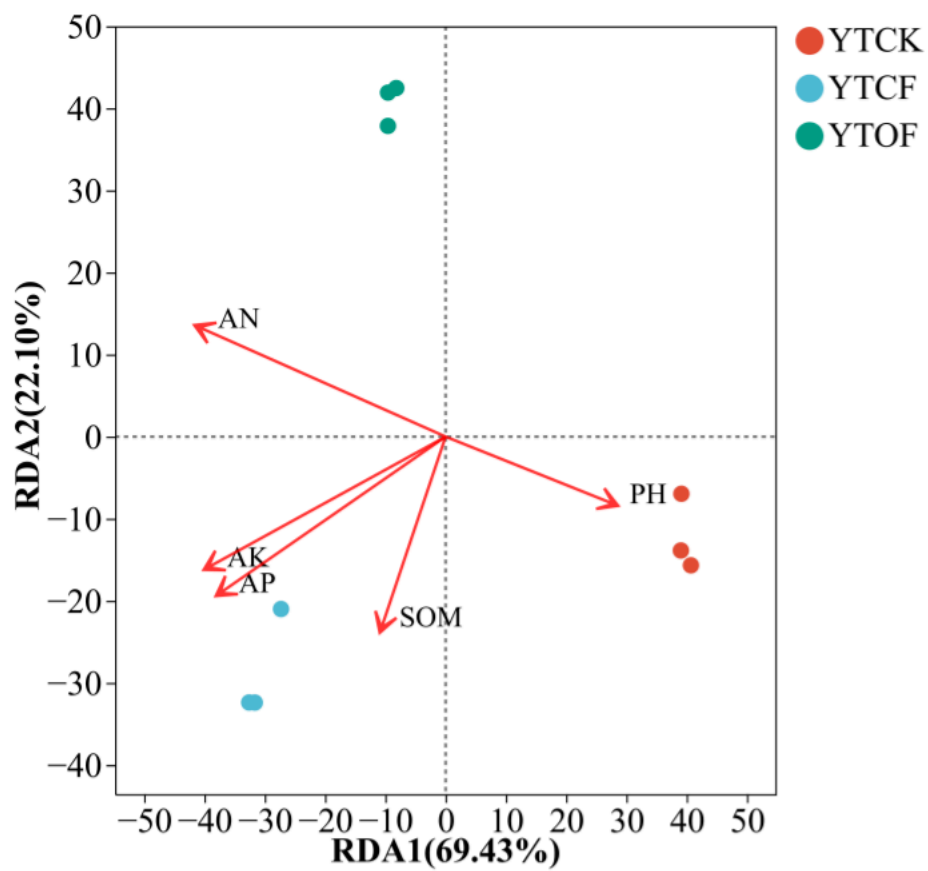


Figure 7

Analysis of the Association between Soil Microbial Communities and Environmental Factors under Different Fertilization Treatments.

Note: RDA1 and RDA2 represent the proportion of microbial community variation explained by each ordination axis, respectively; red arrows represent soil environmental factors; points represent samples.

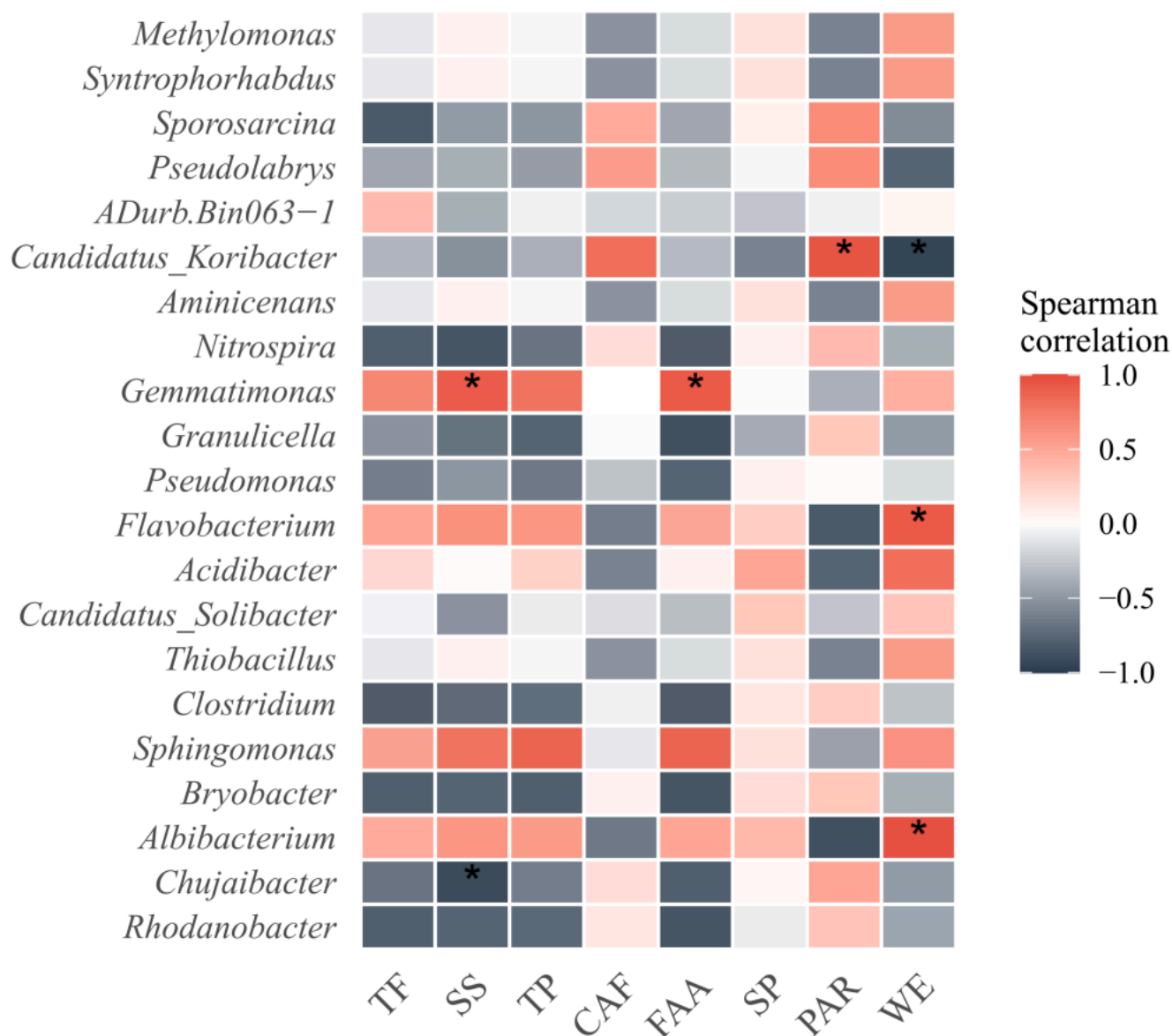


Figure 8

Spearman correlation heatmap between dominant bacterial genera and yellow tea quality indicators.

Note: The color scale represents Spearman's correlation coefficients (r), with red indicating positive correlation and gray indicating negative or weak correlation; deeper colors (red or gray) indicate stronger correlations. Asterisks indicate significance levels after Benjamini–Hochberg false discovery rate (FDR) correction for multiple testing: $*q < 0.05$. Only statistically significant associations are marked with asterisks.