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Nano-Selenium Elevating Leaf Quality and Growth Via Microbial-Regulating Nitrogen Availability Under Ammonium and Nitrate Spraying in Tea Plants

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ABSTRACT

Nano-selenium fertilizers can promote plant growth and nitrogen availability. However, little information is available on the effects of nano-selenium on tea leaf quality, soil nutrient availability and associated microbe-driven mechanisms. This study examined the effects of nano-selenium on the tea leaf quality and soil nitrogen cycling in 20-year-old tea plantations when the leaves were sprayed with ammonium or nitrate. Leaf selenium and amino acid contents increased ninefold and 9%, respectively, with nano-selenium in "Zhongcha108" and "Longjing43." Rhizosphere bacterial and fungal community compositions were more sensitive to selenium and nitrogen applications in "Longjing43" than in "Zhongcha108." "Zhongcha108" enriched more taxa related to microbial growth, while more taxa related to cellular maintenance and nutrient acquisition enriched in "Longjing43." Nano-selenium application decreased the copy number of *AOA* and *AOB* genes, and *nosZ* and *nirK* genes by 59%, 53%, 37% and 46% under ammonium, and by 77%, 43%, 38% and 65%, respectively, under nitrate spraying, in "Longjing43." However, the expression of these genes increased by nano-selenium in "Zhongcha108" with ammonium spraying. It is concluded that a nano-selenium application increases tea leaf quality, and this effect on nitrogen cycling and ecological functioning largely depends on the tea cultivar-specific bacterial and fungal composition and function.

1 | Introduction

Tea (*Camellia sinensis*) is one of the most important crops in China. Since tea plants prefer ammonium (NH_4^+) relative to nitrate (NO_3^-), excessive amounts of ammonium-based fertilizers have been applied into tea soils to harvest larger leaves with elevated amino acid content (Ruan et al. 2007). However, such excessive application of nitrogen (N) fertilizers has caused soil acidification, N leaching and denitrification losses, which, in turn, has decreased nutrient availability (Ding, Tao, and Chen 2022). Previous studies have reported that nitrous oxide greenhouse gas emissions from tea plantations are higher compared to other plantations (Fan and Han 2020; Wang et al. 2020). Therefore, it is urgent to elevate N availability and reduce the application of N fertilizers via improved field management methods and the application of soil conditioners (Guardia et al. 2021).

Selenium (Se) is an essential element for animals and humans, mainly obtained from plants via the food chain (Brown and Arthur 2001). Se is beneficial also for plant growth and development due to, e.g., photosynthetic carbon and N accumulation, and it enhances plants' tolerance to abiotic and biotic stresses (Ribeiro

et al. 2016; Lei et al. 2022). The application of environmentally friendly Se fertilizers has been recently reported to elevate N uptake and availability by plants (Day and Smith 2021; Huang et al. 2022). In addition, Se application improves plant growth indirectly via the recruitment of beneficial microbiomes that elevates soil nutrient availability (Cai et al. 2019; Marco et al. 2022; Khanna et al. 2023). Se application affects the composition and function of rhizosphere microbial communities in a way that promotes plant growth and resistance to stressors (Hanson et al. 2003). For example, the application of Se fertilizers increases the diversity of rhizosphere microbial communities and benefits bacteria, such as Acidobacteriota and Actinobacteriota, which become enriched in the rhizosphere (Guo et al. 2023; Kang et al. 2024). The use of nano-Se fertilizers has been increasing due to an improved productivity and quality of crops in agricultural systems, low toxicity, high bioavailability and high degradability (Wadhwani et al. 2016; Samynathan et al. 2023). However, some studies have argued that the application of Se does not affect some microbial diversity, enzymatic activity and nutrient availability (Iqbal et al. 2015; Li et al. 2021; Liu et al. 2024).

The beneficial role of Se in regulating the diversity and functioning of microbial communities largely depends on the plant species, Se concentration and soil type, and so on (Cai et al. 2019; Yang et al. 2023). N forms affect the Se uptake, and physiological and ecological functions in plants (Ríos et al. 2010; Brengi and Abouelsaad 2019). NO_3^--N and NH_4^+-N are the greatest inorganic N sources in soils. A nitrate application promotes Se uptake as nitrate transporter NRT1.1 is involved in the Se uptake by plants (Zhang et al. 2019). Amino acids, the N assimilation products, are also involved in the Se accumulation and metabolism (Dumont, Vanhaecke, and Cornelis 2006; Mehdi et al. 2013). The leaf spraying of N fertilizers is regarded as an important supplement for N feeding in plants, as it reduces N losses via leaching and denitrification (Lei et al. 2022). Tea plants preferentially uptake NH_4^+-N via the root pathway relative to NO_3^--N. However, the effects of leaf-spraying nano-Se, combining NO_3^- and NH_4^+ spraying, on the composition and functioning of soil microbial communities under different N forms are still unclear.

The soil microbiome produces enzymes to mineralize organic matter to obtain energy for metabolism and growth (Li et al. 2024). Se application has been found to increase, decrease or to have no effects on soil enzyme activities related to organic N, phosphorus (P) and carbon mineralization, depending on plant species and other factors (Cai et al. 2019). In tea soils, Se fertilizer promotes N fixation, and upregulates the expression of N-fixation genes *nifH* (Lei et al. 2022). Soil nitrification and denitrification are also affected by the application of Se fertilizer (Lei et al. 2022). Se application can increase soil nitrification rate, the copies of genes related to nitrification (*AOA*), but decreased the copies of denitrification genes (*narG*, *nirS*) (Lei et al. 2022). These changes lead to changes in soil NO_3^-/NH_4^+, and N uptake and utilization. However, whether and how a nano-Se application can change the composition and ecological functioning of rhizosphere microbial communities related to N dynamics is still unclear.

Longjing tea is the most famous premium green tea in China, and it is renowned internationally for its high quality, long history, and rich cultural connotations [30]. "Longjing43" (LJ43), one of the famous Longjing tea types, is widely cultivated in China. "Zhongcha108" (ZC108) was generated from LJ43 through irradiation-based mutation breeding, and it is resistant to anthracnose (Wang et al. 2016; Han et al. 2024). ZC108 is also a widely cultivated and famous Longjing tea type in Zhejiang Province (Wang et al. 2016). Previous studies have revealed that the resistance against anthracnose in ZC108 is mediated via hypersensitive response and hydrogen peroxide accumulation (Wang et al. 2018). However, the taxon-specific effects on the leaf quality and on rhizosphere bacterial and fungal communities under different N forms in ZC108 and LJ43 are still unclear. In this study, we proposed the following hypotheses: (1) Se application differently affects leaf flavor and leaf Se accumulation under NO_3^- and NH_4^+ supplies. (2) The application of nano-Se promotes rhizosphere microbial N fixation and nitrification potential but decreases denitrification, and the effects are affected by the tea plant cultivars and N forms.

2 | Materials and Methods

2.1 | Site Description and Sampling

The experiment was performed at the Shengzhou integrated experimental station, Zhejiang Province, China (120°48′ E, 29°75′ N). The study site belongs to the subtropical monsoon climate with the following conditions: 16.0–17.5°C annual mean temperature, 1100–2200 h annual sunshine time and 900–1500 mm annual precipitation. Two tea cultivars (LJ43 and ZC108) were planted in 2003 with the same management practices (Zhang et al. 2022). The soil of the tea garden contains 17 g kg^{-1} organic matter, 8.9 g kg^{-1} available P, 40.8 mg kg^{-1} available sulfur and 1.23 mg kg^{-1} total Se. We randomly chose 4 sampling plots with the size of 20 cm × 20 cm per tea cultivar for 2 Se (+nano-Se, -nano-Se) × 2 N (NO_3^-, NH_4^+) treatments. On each plot, we chose 4 tea plants as one replicate, thus a total of 32 samples (2 tea cultivars × 2 nano-Se treatments × 2 N form treatments × 4 replicates) were included. The buffer zones between treatments were at least 4 m. The nano-Se and N treatments were conducted as foliar sprays. The synthesis of the nano-Se fertilizer was according to previously described methods (Wang et al. 2018). The final Se concentration was 10 mg L^{-1} and the final NO_3^--N or NH_4^+-N concentration was 2 mM. The leaf spray was conducted before 10:00 in the morning and the spraying was conducted twice with the interval of 7 days.

Soil samples were collected on the sites of both tea cultivars. Rhizosphere soil samples were detached from the fine roots, and the leaf biomass was separately harvested and dried to a constant weight. Rhizosphere soil samples were separated into three parts. One part of the soil samples was air-dried at room temperature and used to measure physiochemical properties. Another part of the soil samples was used to measure enzyme activities, and the residual soil samples were stored at −80°C until analysis.

2.2 | Measurements of Free Amino Acids and Total Selenium Content in Leaves

The free amino acids in leaves were extracted with 50% ethanol and centrifuged at 4°C for 10 min. The clear supernatant was then added into 0.5 mL phosphate buffer and 0.5 mL 2%

ninhydrin, and the free amino acids were determined at 570 nm (Lohaus and Moellers 2000). To measure the leaf total Se content, tea leaves were digested with HNO_3 and $HClO_4$ (4:1, v/v) at 180°C for 45 min. The total Se content was determined with the 2, 3-diaminonaphthalene fluorometric method. For inorganic Se measurements, the leaves were extracted with distilled water and HCl to reduce Se^{6+} to Se^{4+} (Hu, Pan, and Zhu 2002). The Se content in the solution was in an inorganic form. The leaf organic Se content was calculated by total Se subtracting inorganic Se. In this study, we give the leaf Se content in an organic form since the leaf inorganic Se content is below the determining value.

2.3 | Soil Physiological Traits and Enzyme Activity Measurements

Soil samples were mixed with deionized water (1:5, v:v) and pH was measured with a PHBJ-260 pH meter (Shanghai Leici, China) after stabilization for 30 min (Fu et al. 2016). The soil available P content was measured using the molybdenum blue method (Watanabe and Olsen 1965). Soil total C and N were determined using C and N analyser (Multi C/N 3100; Jena Analytics, Jena, Germany). Soil NH_4^+ and NO_3^- contents were determined using a flow analytical system (SEAL Analytical Auto Analyzer 3) after soil samples were extracted with 1 M KCl (Sahrawat and Prasad 1975; Sims and Jackson 1971). The soil cation exchange capacity was determined with the ammonium acetate method (Kitsopoulos 1999).

The soil protease activity was measured using a previously described method (Ladd and Butler 1972). Briefly, 2 g soil samples were extracted with 2.5 mL of 0.2 M Tris buffer (pH 8.0) and 2.5 ml of Na-caseinate solution (2%) and incubated at 50°C for 2 h. The incubated solution was combined with 5 mL of trichloroacetic acid (10%) and centrifuged for 10 min. The protease activity was measured at 680 nm after mixing with 0.75 mL of Na_2CO_3 and 0.25 ml of the Folin-Ciocalteu reagent. The protease activity was expressed as the reduced NH_4^+ mg g^{-1} d^{-1}. The soil glycosaminidase activity was measured with the method of Frankenberger and Tabatabai (Frankenberger and Tabatabai 1991). An amount of 1 g soil samples was extracted with 0.2 mL toluene and 9 mL Tris buffer. After adding 1 mL of 0.5 M glutamine solution, the mixture was incubated at 50°C for 2 h. The mixture was combined with 50 ml KCl-Ag_2SO_2 and the NH_4^+ content was determined. The soil chitinase activity was measured with a previously described method (Rodriguez-Kabana et al. 1983). Soil samples were mixed with 1.5 mL toluene. After 15 min, the solution was mixed with 10 mL of 1% chitin suspension and incubated at 37°C for 18 h. The produced N-acetyl-glucosamine in the clear supernatant was determined with the method of Aminoff, Morgan and Watkins (1952). The chitinase activity was expressed as the produced N-acetyl-glucosamine content per g soil per h. The soil acid phosphatase activity was determined with the method of Tabatabai and Bremner (Tabatabai and Bremner 1969). Briefly, 2 g soil samples were mixed with the extraction solution (150 mM p-nitrophenyl phosphate solution, 0.5 mL methylbenzene, 200 mM CH_3COONa buffer, pH 5.2). The mixed solution was incubated for 24 h at 30°C and the acid phosphatase activity was determined at 400 nm. The acid phosphatase activity was expressed as the reduced p-nitrophenyl phosphate content.

2.4 | Sequencing and Bioinformatic Analysis

In the analysis, 0.5 g soil samples were extracted with PowerSoilTM DNA Isolation Kit (MO BIO Laboratories Inc, Carlsbad, CA, USA). Universal primers were used to amplify the V3–V4 region of 16S ribosomal RNA bacterial genes (338 F: 5′-ACTCCTACGGGAGGCAGCAG-3′ and 806 R: 5′-GGACTACHVGGGTWTCTAAT-3′) (Mori et al. 2014), and the internal transcribed spacer (ITS2) region of fungal genes (5′-GCATCGATGAAGAACGCAGC-3′ and ITS4R: 5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). PCR products were pooled and purified with the QIAquick gel extraction kit (Qiagen) and sequenced using the Illumina MiSeq instrument (Illumina, San Diego, California, USA) (sequencing length of PE300). For each sample, sequencing reads were quality-trimmed with QIIME (version 1.8.0) and paired-end reads were merged with FLASH (version 1.12). The chimeric sequences were removed and the bacterial and fungal OTUs were assigned with the Greengenes ribosomal database (V1308) and the Ribosomal Database Product Classifier (V 2.2), respectively. Raw DNA sequence files and associated metadata were deposited in the NCBI data bank with the accession number PRJNA1164332.

2.5 | Real-Time Quantitative PCR

Soil samples (about 500 mg) were used to extract DNA with PowerSoilTM DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The real-time quantitative PCR was performed by real-time q-PCR (fluorescence quantitative PCR instrument, ABI7300, Applied Biosystems, American) with ChamQ SYBR Color qPCR Master Mix (2X) kits (Vazyme Biotech Co Ltd, Nanjing, China). The primers are listed in Supporting Information S1: Table 1. The PCR reaction system contained 2 μL of template DNA, 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 10 μL of 2× ChamQ SYBR Color qPCR Master Mix, 0.4 μL 50 X ROX Reference Dye and 6 μL of ddH_2O with the following conditions: 95°C initial denaturation for 5 min, melting for 5 s at 95°C, annealing for 30 s at 58°C, and extension for 1 min at 72°C, a total of 40 cycles. The plasmid (pMD18-T, 2692 bp) standards were constructed with the targeted primers and thus used to prepare a standard curve with a series of concentration gradients. The gene copy number was calculated per ng of DNA per gram of soil.

2.6 | Statistical Analysis

For the tea leaf quality, soil enzyme activities and gene copy numbers, we performed statistical analyses with the SPSS software (version 22) followed by Duncan's test (version 22.0) with $p < 0.05$. The Chao index (α-diversity index) was calculated with R (V3.5.3) and visualized with the "ggplot2" package. The beta diversity of bacterial and fungal communities was assessed by Bray-Curtis dissimilarity distance matrices and then ordinated using a nonmetric multidimensional scaling (NMDS) (McMurdie and Holmes 2013). The linear discriminant analysis (LDA) effect size (LEfSe) algorithm was used to find potential biomarkers within rhizosphere soil microbiomes (from phylum

to genus levels) between different treatments. The online Galaxy application (version 1.0) was performed with a threshold of 4.0 (http://huttenhower.sph.harvard.edu/galaxy/). The network analysis of bacterial and fungal communities was performed with Cytoscape v.3.5 (Shannon et al. 2003) and visualized in Gephi (Bastian, Heymann, and Jacomy 2009). The Spearman correlation scores $r > 0.6$ and $p < 0.05$ were kept. The network properties were analyzed as described in a previous study (Wagg et al. 2019), and hub nodes were defined as the nodes with high degree and closeness centrality values (Van Der Heijden and Hartmann 2016). Pearson's correlations and Mantel tests were used to analyze the correlations between the composition of rhizosphere microbial communities and physiochemical factors, and the results were visualized by the *"ggcor"* package in R (Huang et al. 2021). Bacterial function predictions were performed using the reconstruction of unobserved states (PICRUSt2) software (https://picrust.github.io/picrust/) (Langille et al. 2013). The fungal function prediction was performed by the Fungi Functional Guild (FUNGuild) v.1.0 (https://github.com/UMNFuN/FUNGuild).

3 | Results

3.1 | Leaf Total Se and Amino Acid Content

Foliar nano-Se application elevated the leaf total Se and amino acid contents in both LJ43 and ZC108 (Figure 1). Less leaf inorganic Se was detected after a nano-Se application. Thus, the increased leaf total Se content can be considered as the total organic Se content. Foliar nano-Se application increased leaf total Se by 10-fold and ninefold in LJ43 and by ninefold and sevenfold in ZC108, under NH_4^+ and NO_3^- supply, respectively (Figure 1a; Supporting Information S1: Table 2). ZC108 had a greater accumulation of leaf amino acids than LJ43 under a nano-Se application (Figure 1b). Leaf polyphenols are important markers of tea quality due to their sensory and health-promoting properties. Nano-Se increased the leaf polyphenol content in LJ43 with NH_4^+ and in ZC108 with NO_3^- but did not affect that in LJ43 with NO_3^- or in ZC108 with NH_4^+ supply (Supporting Information S1: Figure 1). Chlorophyll is an important pigment and can determine the final color and quality of a green tea infusion. We found that chlorophyll contents were similarly elevated by nano-Se in both tea plant cultivars but did not affect the chlorophyll a/b (Supporting Information S1: Figure 2).

3.2 | Bacterial and Fungal Community Diversity and Composition

Plants' responses to environmental conditions affected the rhizosphere microbial taxa recruitment and microbial ecological functions. To characterize the changes in the microbial community composition, we analyzed the 16 S sequencing data from the rhizosphere of two tea plant cultivars with different N and nano-Se treatments. Foliar nano-Se and inorganic N spraying did not affect the α-diversity (Shannon and Chao index) of bacterial and fungal communities in the rhizosphere of LJ43 and ZC108 (Supporting Information S1: Figure 3). However, LJ43 showed higher Chao1 and Shannon indexes in the bacterial community compared to ZC108. The NMDS analyses showed that the β-diversity of the bacterial and fungal communities was more significantly affected by nano-Se and N forms (Figure 2a). The clusters of the bacterial and fungal communities were significantly different between Se and non-Se samples in LJ43, and such difference was greater under the NH_4^+ supply than NO_3^- supply (Figure 2a,b). PERMANOVAs were performed to analyze the contribution of different factors to the microbial community composition. PERMANOVA results showed that the tea plant cultivar had the largest contribution to both the bacterial and fungal community composition (40% and 29%, respectively) (Supporting Information S1: Tables 3, 4). Leaf Se spraying explained a considerable part of the bacterial and fungal community composition (21% and 21%, respectively).

The LEfSe analysis can help to identify differential microbial taxa between the two tea cultivars based on their relative abundance (Figure 2b). LEfSe results showed that the rhizosphere of LJ43 was mainly enriched by the bacterial

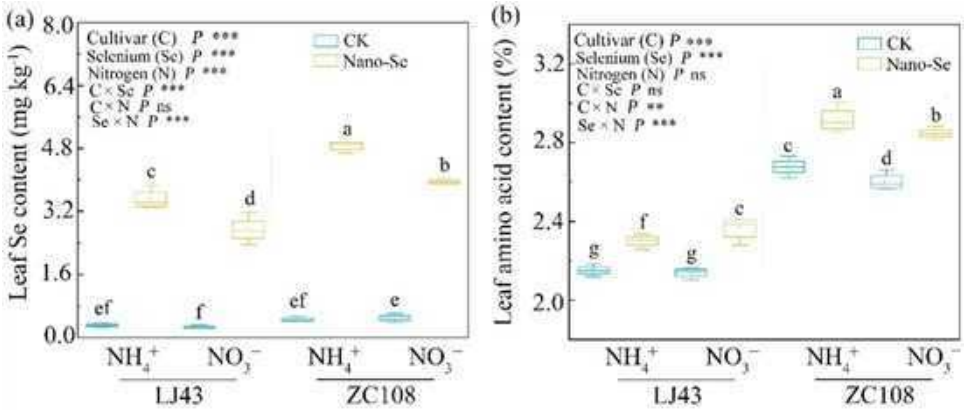

FIGURE 1 | Leaf selenium (Se) (a) and amino acid contents (b) of "Longjing 43" (LJ43) and "Zhongcha 108" (ZC108) with or without leaf sprayed nano-Se (Se, 10 mg L^{-1}) under 2 mM ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) for 14 days. NH_4^+-N or NO_3^--N was also leaf sprayed. Lowercase letters on the columns represent significant differences at the $p \leq 0.05$ level according to Duncan's test. Data are presented as means ± SE ($n = 4$). "Cultivar (C)" represents tea cultivar main effect; "Se" represents Se treatment main effect; "N" represents N treatment main effect; C×Se represents the interaction of tea cultivar and Se; C×N represents the interaction of tea cultivar and N; Se×N represents the interaction of Se and N. ns, not significant, * $p \leq 0.05$, ** $0.001 < p \leq 0.01$, and *** $p \leq 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

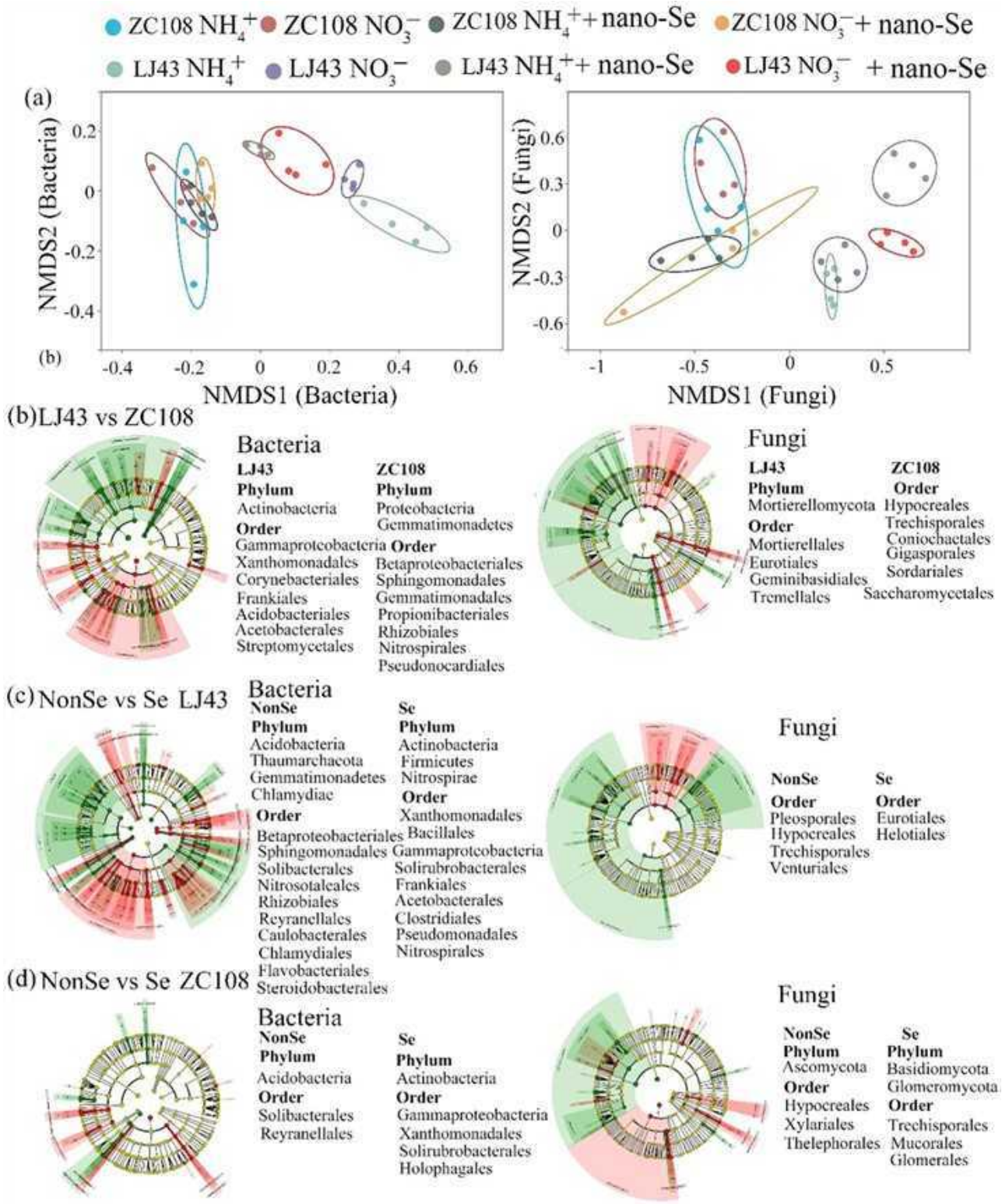

FIGURE 2 | Taxonomic diversity and difference analysis in the rhizosphere of "Longjing 43" (LJ43) and "Zhongcha 108" (ZC108) with or without leaf sprayed nano-selenium (Se, 10 mg L^{-1}) under 2 mM ammonium nitrogen (NH_4^+) or nitrate nitrogen (NO_3^-) for 14 days. NH_4^+-N or NO_3^--N was also leaf sprayed. (a) The nonmetric multidimensional scaling (NMDS) of bacterial and fungal community compositions. The linear discriminant analysis effect size was used to identify differential bacterial and fungal taxa between tea cultivars (b) and between non-nano-Se and nano-Se treatments in the rhizosphere of LJ43 (c) and ZC108 (d). [Color figure can be viewed at wileyonlinelibrary.com]

Actinobacteria phylum, Gammaproteobacteria, Xanthomonadales, Corynebacteriales, Frankiales, Acidobacteriales, Acetobacterales, and Streptomycetales orders (Figure 2b). ZC108 had higher abundances of the bacterial Proteobacteria and Gemmatimonadetes phyla, as well as Betaproteobacteriales, Sphingomonadeles, Gemmatimonadales, Propionibacteriales, Rhizobiales, Nitrospirales and Pseudonocardiales orders when compared to the rhizosphere of LJ43 (Figure 2b). For fungal taxa, LJ43 had higher abundances of the phyla Mortierellomycota, the orders of Mortierellales, Eurotiales, Geminibasidiales and Tremellales in the rhizosphere when compared to ZC108 (Figure 2b). The orders of Hypocreales, Trechisporales, Sordariales, Coniochaetales, Gigasporales and Saccharomycetales showed greater abundances in the rhizosphere of ZC108 than in LJ43 (Figure 2b).

The LEfSe analysis also identified differential microbial taxa between Se and non-Se treatments in the rhizosphere of two tea cultivars (Figure 2c,d). The differential bacterial taxa between control and nano-Se treatment were more frequent in LJ43 (2.71% relative to total taxa, 162/5979) than in ZC108 (0.59% relative to total taxa, 35/5965). Leaf nano-Se spraying increased the abundance of bacterial Actinobacteria, Firmicutes and Nitrospirae phyla and some bacterial orders, such as Xanthomonadales, Bacillales, Gammaproteobacteria, Solirubrobacterales, and Frankiales in LJ43 (Figure 2c). In ZC108, the nano-Se spraying promoted the dominance of bacterial Actinobacteria phyla, as well as the orders of Gammaproteobacteria, Xanthomonadales, Solirubrobacterales and Holophagales (Figure 2d). The fungal orders Eurotiales and Helotiales were predominant in the rhizosphere of LJ43, while the phyla of Basidiomycota and Glomeromycota, as well as the orders of Trechisporales, Mucorales and Glomerales were enriched in the rhizosphere of ZC108 (Figure 2c,d). The effects of nano-Se on microbial abundances depended also on the N forms.

N forms affected the differential enrichment of the bacterial and fungal taxa in the two tea cultivars. In LJ43, nano-Se specifically enriched the bacterial Actinobacteria phylum and Solirubrobacterales order under the NO_3^- supply, as well as the bacterial Firmicutes phylum and Bacillales order under the NH_4^+ supply (Supporting Information S1: Figure 4a). In ZC108, nano-Se specifically increased the abundance of the Actinobacteria phylum and Propionibacteriales order under the NO_3^- supply, and the Firmicutes phylum, and Bacillales and Solirubrobacterales orders under the NH_4^+ supply (Supporting Information S1: Figure 4a). In fungi, nano-Se spraying increased the abundance of the Mortierellaceae phylum and Mortierellales order under both N treatments in the rhizosphere of LJ43. In the rhizosphere of ZC108, nano-Se promoted the fungal Basidiomycota and Mucoromycota phyla, and Mucorales and Trechisporales orders under the NO_3^- supply, and the Eurotiales order under the NH_4^+ supply (Supporting Information S1: Figure 4b).

3.3 | Microbial Functional Groups in the Rhizosphere Soil Aggregates

The PICRUSt analysis was performed to predict the metagenome gene functions of the bacterial community, and the Fungi Functional Guild analysis was conducted to predict the fungal gene functions. The PICRUSt analysis predicted that genes related to glycan biosynthesis, transcription, terpenoids, cofactors metabolism and cell growth in the rhizosphere of ZC108 had higher expressions under the NO_3^- supply regardless of the Se application (Figure 3a). By contrast, LJ43 had higher expressions of microbial genes related to transport, lipid metabolism, xenobiotic metabolism, carbohydrate metabolism, amino acid metabolism and membrane transport in the rhizosphere of LJ43, and these effects were greater under the combination of Se and NH_4^+.

The FUNGuild database prediction suggested that the rhizospheres of LJ43 and ZC108 were mainly colonized by endophyte (24%), litter saprotroph (20%) and soil saprotroph fungi (16%) (Figure 3b). The trophic fungal distribution was more affected by the application of nano-Se in the rhizosphere of ZC108 than in LJ43. The Se application increased the proportion of fungal taxa related to dung saprotroph, wood saprotroph and plant pathogen fungi, but decreased the fungal taxa related to fungal pathogens, insect parasites and algal parasites in the rhizosphere of ZC108 under the NH_4^+ supply. Under the NO_3^- supply, the proportions of fungal taxa related to soil saprotroph, wood saprotroph and litter saprotroph fungi increased and the proportions of insect parasites, lichen parasites and plant pathogens decreased by the application of nano-Se in the rhizosphere of ZC108 (Figure 3b). The nano-Se application caused a slight decrease in fungal pathogens and plant pathogens under the NH_4^+ supply in the rhizosphere of LJ43.

3.4 | Microbial Co-Occurrence Networks

The co-occurrence patterns of bacterial and fungal communities were assessed to characterize the host, Se and N forms of the tea plant microbiomes. Our results suggested that the effects of tea cultivars on the microbial network complexity (a higher average degree meaning a greater network complexity) and connectiveness (network betweenness) differed between Se and N form treatments. LJ43 had a higher bacterial network degree and lower betweenness than ZC108 under NO_3^- spraying, regardless of the Se application; a contrary trend was detected under NH_4^+ spraying (Figure 4a). The Se application reduced the bacterial network degree of LJ43 and ZC108 under NO_3^- spraying, but it did not affect them under other treatments. The fungal network degree was lower than those of bacterial communities (Figure 4b). The network degree and betweenness of fungal communities were not affected by the tea cultivar, Se and N forms.

3.5 | Soil Enzyme Activities Related to Organic N and P Mineralization

Elevated soil N availability in tea plantations can promote the growth and quality without ecosystem-wide negative effects. The protease, glucosaminidase and chitinase enzymes are involved in soil organic N mineralization and promote available N release. The phosphatase enzymes promote organic P mineralization and soil P availability. Soil enzyme activities related to organic N and P mineralization were affected by the application of nano-Se and N forms, and this effect was greater in the rhizosphere of LJ43 than in ZC108 (Figure 5a–d). The nano-Se

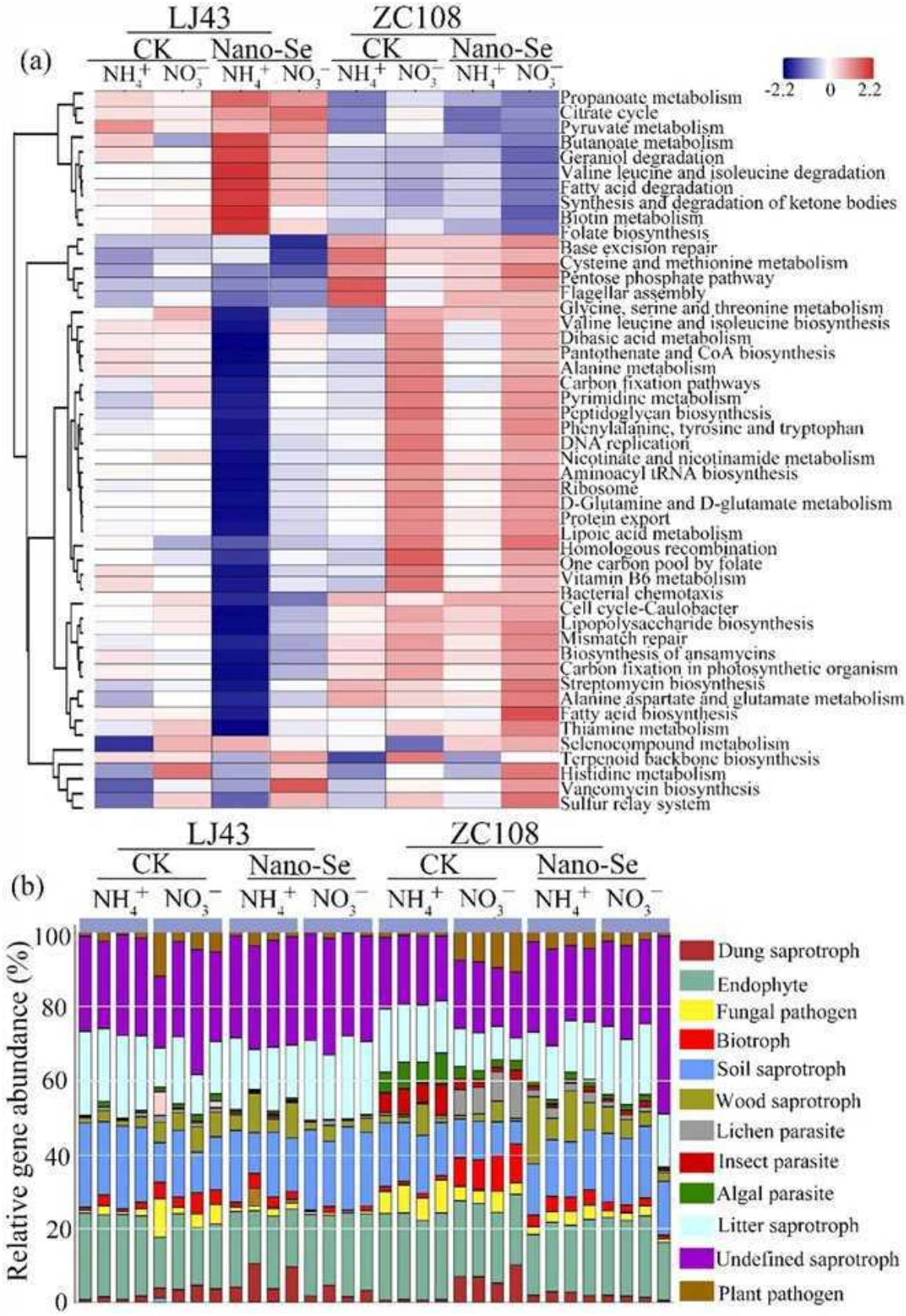

FIGURE 3 | The predicted bacterial (a) and fungal (b) metabolism profiles based on PICRUSs and Fungi Functional Guild, respectively, in the rhizosphere of "Longjing 43" (LJ43) and "Zhongcha 108" (ZC108) with or without leaf sprayed nano-selenium (Se, 10 mg L^{-1}) under 2 mM ammonium nitrogen (NH_4^+) or nitrate nitrogen (NO_3^-) for 14 days. Red blocks indicate functional groups with higher abundances, and blue ones indicate functional groups with lower abundances in corresponding treatments. NH_4^+-N or NO_3^--N was also leaf sprayed. [Color figure can be viewed at wileyonlinelibrary.com]

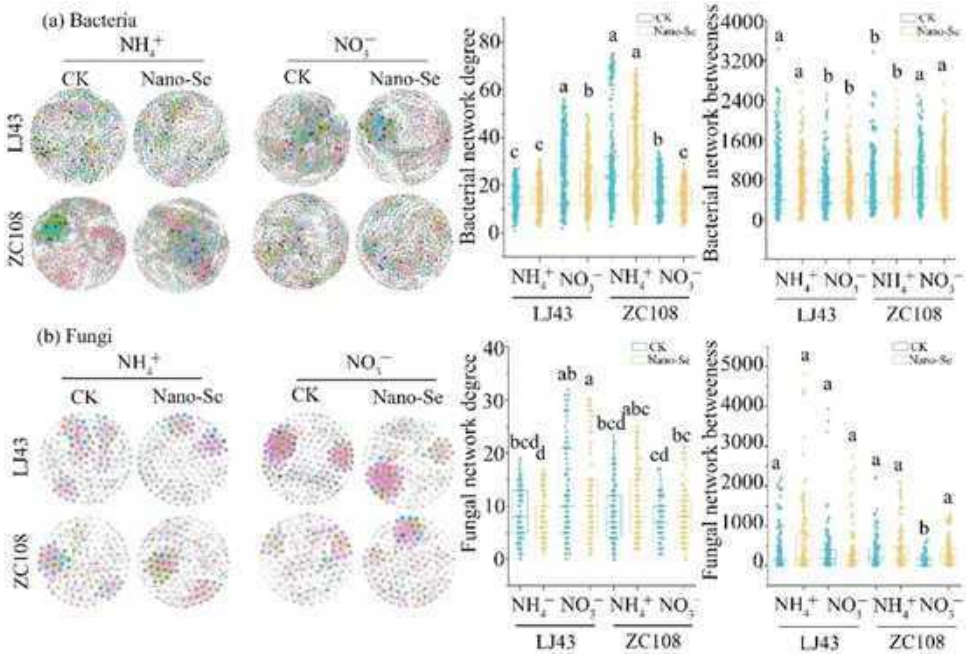

FIGURE 4 | Co-occurrence networks of bacterial (a) and fungal communities (b) and network properties in the rhizosphere of "Longjing 43" (LJ43) and "Zhongcha 108" (ZC108) with or without leaf sprayed nano-selenium (Se, 10 mg l^{-1}) under 2 mM ammonium nitrogen (NH_4^+) or nitrate nitrogen (NO_3^-) for 14 days. Network nodes represent individual ASVs, and their sizes are positively related to the network degree, and network centrality is positively related to the betweenness. Nodes represent individual ASVs; edges represent significant Pearson's correlations ($r > 0.90$ and $p < 0.05$), whereas red lines show positive correlations, and green lines show negative correlations. Different letters above the columns indicate statistically significant differences ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

application enhanced protease, glucosaminidase, chitinase and acidic phosphatase activities in LJ43 under both N forms. The protease activity did not differ between LJ43 and ZC108 under the two N forms, and the application of nano-Se did not affect this enzyme activity (Figure 5a). The glucosaminidase activity was elevated by nano-Se in ZC108 under the NH_4^+ supply and NO_3^- supply, and the NO_3^- supply caused a greater enzyme activity than the NH_4^+ supply in both tea cultivars (Figure 5b). ZC108 had a higher chitinase activity under the NH_4^+ supply than NO_3^- supply, while the increase in the chitinase activity was more significant under the NO_3^- supply in LJ 43 (Figure 5c). The acidic phosphatase activity increased by the application of nano-Se in ZC108, and this effect was greater under the NH_4^+ supply (Figure 5d). LJ43 had a greater increase in the acidic phosphatase activity under the NO_3^- supply than NH_4^+ supply.

3.6 | Expression of N- and P-Related Microbial Genes

We performed the absolute quantification of gene expression with the real-time q-PCR analysis. The soil inorganic N cycling was strongly affected by the tea cultivar and the nano-Se application, and the effect of nano-Se on inorganic N cycling was greater in the rhizosphere of LJ43 (Figure 6). The application of nano-Se decreased the expression of *AOA*, *AOB*, *nirK*, *nosZ* and *nifH* genes by 59%, 53%, 37%, 46% and 49% under the NO_3^- supply, and by 77%, 43%, 38%, 65% and 40% under the NH_4^+ supply in the rhizosphere of LJ43 (Figure 6a–e). ZC108 had a 61%, 47% and 25% lower expression of *AOA*, *nirK* and *nifH* genes after the application of nano-Se, but a similar expression level of *AOB* and *nosZ* genes in control and nano-Se-treated soils under the NO_3^- application (Figure 6a–e). However, under the NH_4^+ supply, the application of nano-Se increased the expression of *AOA*, *AOB*, *nirK*, *nosZ* and *nifH* genes by 100%, 88%, 41%, 203% and 75% in the rhizosphere of ZC108. The expression of the *phoC* gene increased by 52% by the nano-Se spraying in the rhizosphere of ZC108, but nano-Se did not affect LJ43 under either N form (Figure 6f).

3.7 | Correlations between Bacterial and Fungal Communities and Soil Properties

We used the partial Mantel test to reveal drivers for the relationships between microbial community compositions and environmental factors (Figure 7a,b). The relationships between the microbial composition and environmental factors were stronger in the rhizosphere of LJ43 than in ZC108. The environmental factors explained more about the fungal community composition than about the bacterial composition in both tea cultivars. In LJ43, nearly all environmental factors were responsible for the rhizosphere fungal community composition, whereas soil NO_3^-, leaf amino acids, leaf Se, soil *nirK* gene, *nosZ* gene and *phoC* gene were responsible for the rhizosphere bacterial community composition (Figure 7a). In the rhizosphere of ZC108, the leaf Se content was responsible for the bacterial community composition, while the fungal community composition was largely explained by soil NO_3^-, leaf amino acids, leaf Se, soil *AOB* and *nirK* genes (Figure 7b).

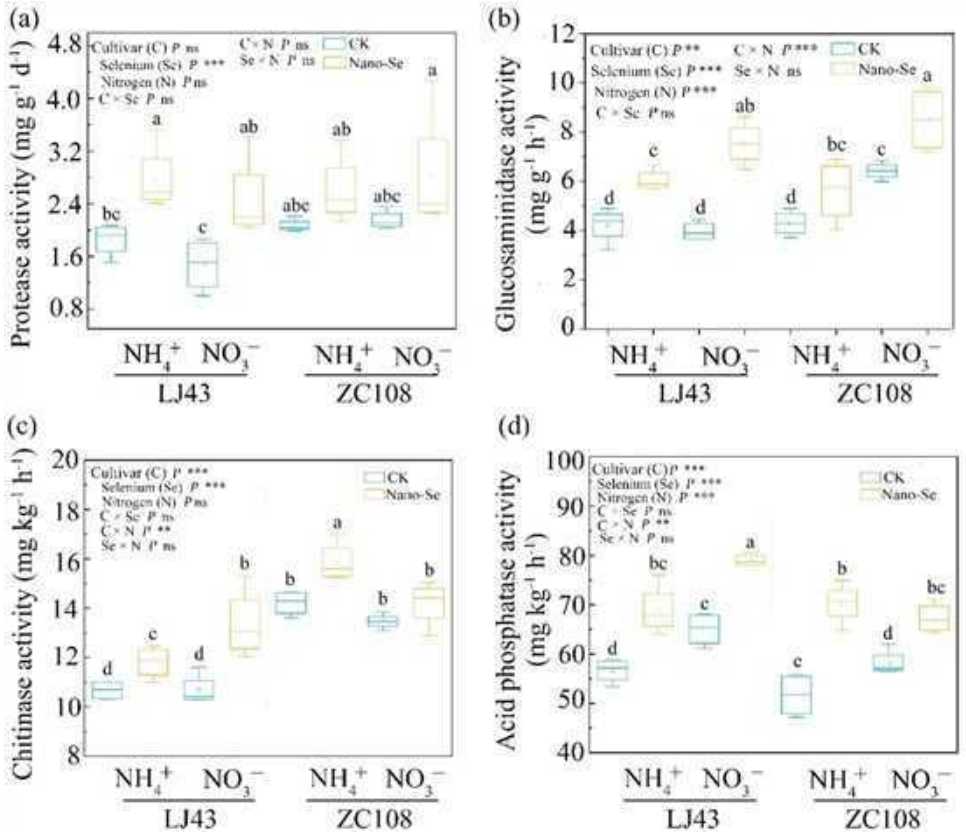

FIGURE 5 | Protease (a), glucosaminidase (b), chitinase (c) and acid phosphatase activities (d) in the rhizosphere of "Longjing 43" (LJ43) and "Zhongcha" 108 (ZC108) with or without leaf sprayed nano-selenium (Se, 10 mg L^{-1}) under ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) for 14 days. NH_4^+-N or NO_3^--N was also leaf sprayed. Lowercase letters on the columns represent significant differences at the $p \leq 0.05$ level according to Duncan's test. Data are presented as means ± SE ($n = 4$). "Cultivar (C)" represents tea cultivar main effect; "Se" represents Se treatment main effect; "N" represents N treatment main effect; C×Se represents the interaction of tea cultivar and Se; C×N represents the interaction of tea cultivar and N; Se×N represents the interaction of Se and N. ns, not significant, * $p \leq 0.05$, ** $0.001 < p \leq 0.01$, and *** $p \leq 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

4 | Discussion

4.1 | ZC108 Plants Have Higher Leaf Amino Acid and Se Contents Than LJ43 Under the Application of Nano-Se and N Forms

Nano-Se is broadly used to promote plant growth, organic Se accumulation, nutrient availability, and tolerance to abiotic and biotic stresses due to its reduced toxicity, substantial surface-to-volume quotient, solubility, and multifunctionality (Khurana et al. 2019; Li et al. 2022). In this study, the application of nano-Se elevated leaf Se and amino acid accumulation in both tea plants (Figure 1), which was consistent with previous studies (Huang et al. 2023). Moreover, ZC108 had a higher Se and amino acid accumulation with nano-Se than LJ43 (Figure 1). The effects of nano-Se on bioactive Se and amino acid accumulation vary among plant species, and even among plant genotypes (Samynathan et al. 2023). Plant species with great N metabolism and amino acid accumulation generally increase Se accumulation and its chelation with amino acids (Schiavon and Pilon-Smits 2017). ZC108, generated from LJ43 via irradiation, has a higher N metabolism rate and leaf amino acid content than LJ43 (Wang et al. 2016). Elevated amino acid levels may be associated with increased Se accumulation and transport, which are controlled by the sulfate transport family (El Mehdawi et al. 2018; Liao and Zhu 2022). The increased amino acid accumulation in plants provides more precursors for producing Se-cysteine and Se-methine in the primary Se metabolism and in the secondary metabolism of selenodiglutathione and selenopersulfide (Dumont, Vanhaecke, and Cornelis 2006; Mehdi et al. 2013).

ZC108 tea plants enhance the glutamate synthetase enzyme activity and promote the amino acid synthesis relative to LJ43 (Zhang et al. 2018). The increased amino acids may promote Se chelation with amino acids and also Se accumulation in ZC108 (Figure 1). Se is transported to the plant cell via the sulfate transporter, silicon influx transporter and nitrate uptake transporter, as no specific Se transporter has been identified (Liang et al. 2019; Zhao et al. 2020). ZC108 has a higher N transport ability and NH_4^+ uptake preference than LJ43 (Zhang et al. 2023), which may also promote Se accumulation in the leaves. Consistently, LJ43 and ZC108 had a higher leaf Se accumulation under the NH_4^+ supply than NO_3^- supply. In turn, the application of Se promoted amino acid accumulation in the leaves of ZC108 and LJ43 plants, and this promoting effect was greater in ZC108 under the NH_4^+ supply but there was no difference between the two tea plant cultivars under the NO_3^- supply. Tea plants prefer the NH_4^+ uptake, which may facilitate Se accumulation via the increased amino acid

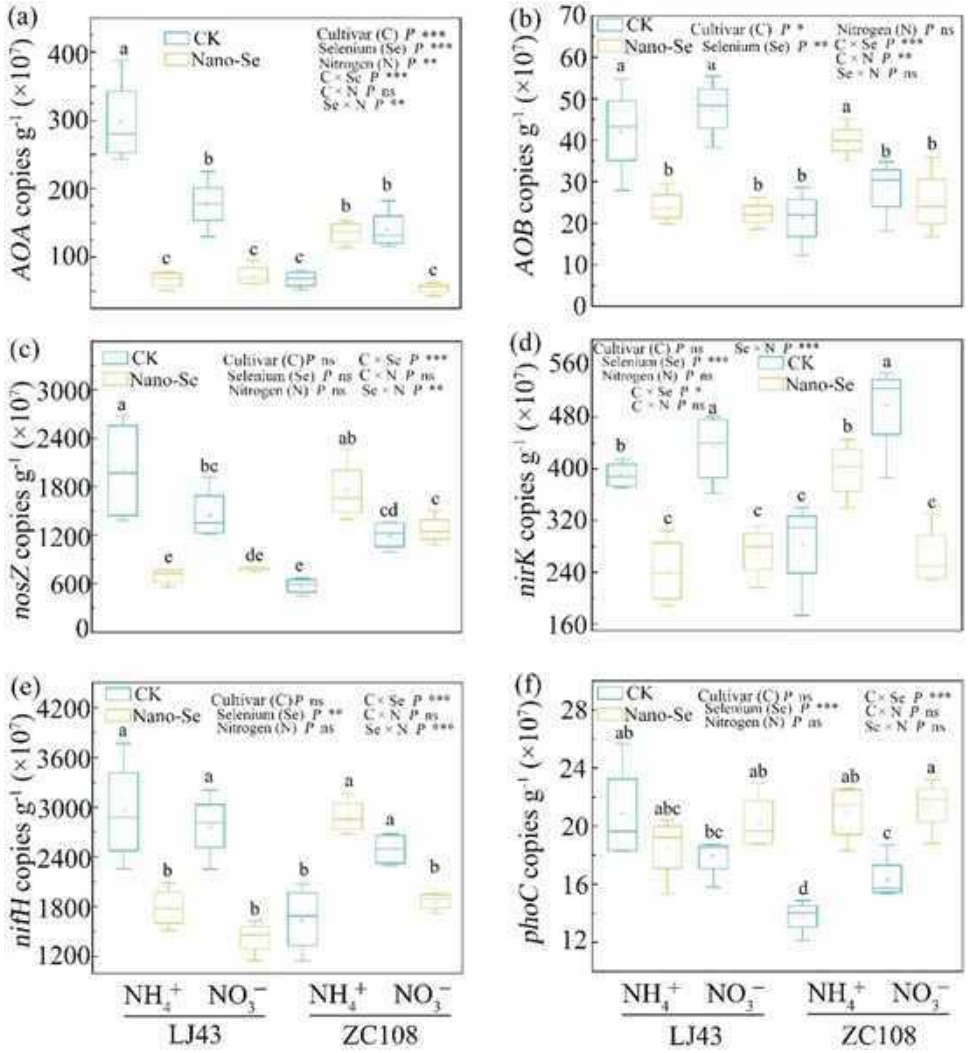

FIGURE 6 | Copy number of genes related to soil nitrogen cycling and phosphorus mineralization in the rhizosphere of "Longjing 43" (LJ43) and "Zhongcha 108" (ZC108) with or without leaf sprayed nano-selenium (Se, 10 mg L^{-1}) under 2 mM ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) for 14 days. NH_4^+-N or NO_3^--N was also leaf sprayed. The genes are related to nitrification (*AOA* (a) and *AOB* (b)), denitrification (*nosZ* (c) and *nirK* (d)), nitrogen fixation (*nifH*) (e), and phosphorus mineralization (*phoC*) (f). The absolute quantification of gene copy numbers was performed by real-time q-PCR. The constructed plasmid (pMD18-T, 2692 bp) standards were used to prepare a standard curve with a series of concentration gradients. The gene copy number was calculated per ng of DNA per gram of soil. Lowercase letters on the columns represent significant differences at the $p \leq 0.05$ level according to Duncan's test. Data are presented as means ± SE ($n = 4$). "Cultivar (C)" represents tea cultivar main effect; "Se" represents Se treatment main effect; "N" represents N treatment main effect; C×Se represents the interaction of tea cultivar and Se; C×N represents the interaction of tea cultivar and N; Se×N represents the interaction of Se and N. ns, not significant, * $p \leq 0.05$, ** $0.001 < p \leq 0.01$, and *** $p \leq 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

accumulation (Liao and Zhu 2022). ZC108 accumulated greater leaf Se levels, which may explain the greater accumulation of leaf amino acids relative to LJ43.

4.2 | Effects of Se on Bacterial and Fungal Community Composition and Functions Are Dependent on Tea Cultivars and N Forms

Different plant species or cultivars can select for different soil microbial communities (Burns et al. 2015; Lan et al. 2024; Liu et al. 2021, 2024, 2025), which can promote plant growth, metabolism and health (Guo et al. 2024; Liu et al. 2024). These selective pressures are especially strong in the rhizosphere, the area around the roots that is directly influenced by root processes and is the home of the rhizosphere microbiome (de Vries et al. 2020; Okutani et al. 2020; Zhao et al. 2023). ZC108 and LJ43 have differences in nutrient uptake, N metabolism and responses to abiotic and biotic stressors (Wang et al. 2018), which may be cascaded into the rhizosphere microbial structure and function (Zhang et al. 2022; Liu et al. 2025). ZC108 has a higher diversity and stability in the rhizosphere bacterial and fungal communities, as evidenced by the greater α-diversity index, lower community network nodes and increased modularity compared to LJ43 (Figures 2 and 4). A higher microbial diversity can reduce the impact of disturbance events, as indicated by the higher stability of the ZC108 bacterial and fungal network (Figure 4). We speculated that a sharp reduction in the

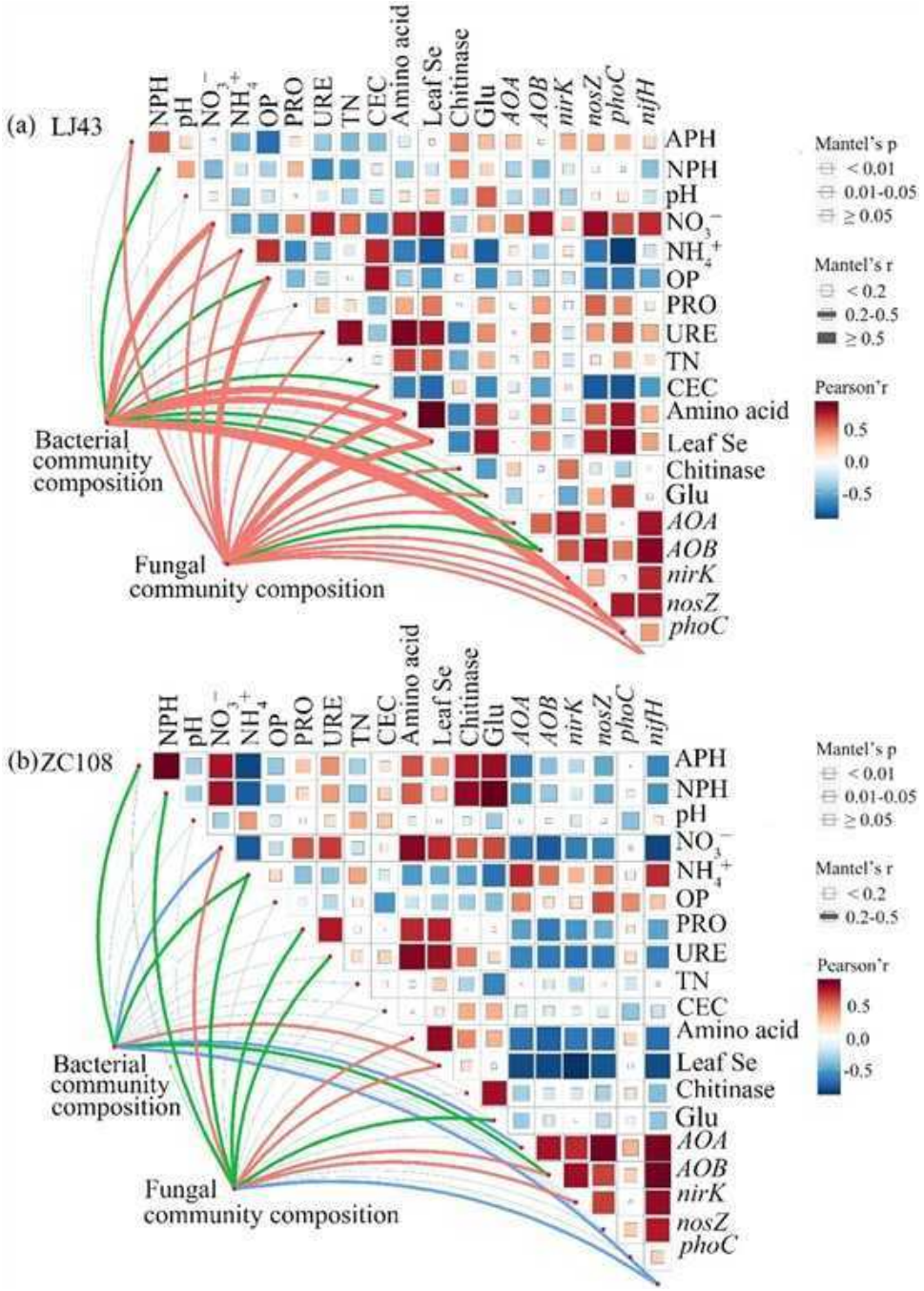

FIGURE 7 | Correlations of soil physiochemical properties and rhizosphere bacterial and fungal community compositions in the rhizosphere of "Longjing 43" (LJ43) (a) and "Zhongcha" 108 (ZC108) (b) with or without leaf sprayed nano-selenium (Se, 10 mg L^{-1}) under 2 mM ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) for 14 days. NH_4^+-N or NO_3^--N was also leaf sprayed. The microbial community composition was calculated based on the dissimilarity matrix with the Bray-Curties distance, while environmental factors were calculated based on the dissimilarity matrix with the Euclidean distance. The line width indicates the Mantel's r value and line color indicates the corresponding *P* value. The blue squares mean a positive relationship and the red squares mean a negative correlation. APH, acidic phosphatase; NPH, neutral phosphatase; OP, organic P; PRO, Protease; URE, urease; TN, total nitrogen; CEC, cation exchange capacity; amino acid; leaf amino acid; Glu, glucosaminidase; *AOA* and *AOB*, genes related to nitrification; *nirK* and *nosZ*, genes related to denitrification; *nifH*, gene related to nitrogen fixation; *phoC*, gene related to acidic phosphatase activity. [Color figure can be viewed at wileyonlinelibrary.com]

fungal diversity of LJ43 would make the fungal community more vulnerable to metabolites and the ecosystem more fragile, thus becoming susceptible to diseases (Wang et al. 2018).

The reactivity of communities to disturbances is considered being more important than stability (Yang et al. 2023). Here, the composition of rhizosphere bacterial and fungal communities intensively responded to nano-Se and N forms and more so in LJ43 than in ZC108. Microbes are predicted to have a tradeoff between cellular growth and metabolic activities in response to stressors (Carlson and Taffs 2010; Malik et al. 2019). We found that LJ43 preferred to recruit some bacterial taxa related to

organic carbon, amino acids and lipid metabolism, thus promoting the availability of organic nutrients, which was enhanced by the nano-Se application (Figure 3). The Gammaproteobacteria is the predominant class in chitin-enriched soils (Das et al. 2010). LJ43 enriched Gammaproteobacteria in the rhizosphere, which may promote the organic matter cycling (Figures 2 and 3). In contrast, ZC108 had more microbial taxa with greater cell growth and energy metabolism, and with less dependence on soil nutrients, as shown by lower correlations with soil nutrients (Figures 3 and 6). More resource allocation into metabolic activities compensates for the energy investment via the increased availability and uptake of soil nutrients (Carlson and Taffs 2010). Most maintenance and resource acquisition strategists belong to the phylum Actinobacteria, while most growth strategists belong to the phylum Proteobacteria (Ramin and Allison 2019). The predominant enrichment of Actinobacteria in the rhizosphere of LJ43 and Proteobacteria in ZC108 further reflected the microbial niche differentiation in different tea cultivars.

Foliar fertilization is considered as a supplementary fertilization strategy, which can deliver nutrients directly to the aerial plant parts (Fernández and Eichert 2009; Bindraban et al. 2015). LJ43 showed higher photosynthesis, soluble sugar polyphenol accumulation and N uptake than ZC108. In contrast, ZC108 increased hydrogen peroxide accumulation, induced a hypersensitive response and enhanced the resistance to anthracnose (Wang et al. 2018; Su et al. 2020; Tu et al. 2021). Nano-Se was directly sprayed into the leaves of tea plants, which led to cultivar-dependent physiological changes in leaves. Indeed, LJ43 and ZC108 had different responses to the spraying of leaves with nano-Se regarding leaf total Se and amino acids (Figure 1), which was related to leaf Se biosynthesis and amino acid metabolism. Diverse microbial taxa have different nutrient preferences, which depend on resource availability. Tea cultivars' responses to environmental factors may change the leaf carbon biosynthesis, and transport and carbon secretion by roots, which may provide divergent microhabitats and alter microbial community structures and functions (Wang et al. 2021; Xie et al. 2022). Thus, the internal connection between Se absorption, amino acid accumulation and microbial composition may vary significantly among different tea plant cultivars. Further work is needed to uncover how physiological changes in leaves synthetically regulate cultivar-dependent microbial structures and functions in tea plants, and whether the alteration of microbial components affects the leaf Se and amino acid metabolism.

4.3 | Roles of Nano-Se in Soil Organic N and P Mineralization Depend on Tea Cultivars and N Forms

Glucosaminidase, chitinase and protease are involved in the mineralization of organic N, while acidic phosphatase mineralizes organic P in plants (Ouyang and Norton 2020). LJ43 enhanced organic P mineralization via the elevated acidic phosphatase activity, while ZC108 had a higher organic N mineralization via the increased protease, glucosaminidase and chitinase activities in the rhizosphere (Figure 5), implying a different ecological nutrient niche. Nano-Se elevated soil N- and P-related enzyme activities in LJ43, thus reducing the difference in the ability of organic N and P mineralization between the two tea cultivars. Se-containing proteins have been reported to be involved in some oxidation-reduction reactions, including formate dehydrogenase of bacteria and glycine reductase of clostridia (Stadtman 1974). The Se application also indirectly affects enzyme activities via elevated plant growth and microbial functions (Lei et al. 2022). Notably, the roles of Se in the mineralization of rhizosphere organic N and P were greater in LJ43 than in ZC108, as all related enzymes studied here were elevated by the application of nano-Se in LJ43 (Figure 5). This may be explained by the potential microbial functions in decomposing organic matter in the rhizosphere of LJ43 (Figure 3). The roles of Se in N and P mineralization also depended on N forms in ZC108. The tea plant roots preferentially uptake NH_4^+-N relative to NO_3^--N (Ruan et al. 2007). Consistently, the elevation of soil chitinase activities was greater under the spraying of NH_4^+ relative to NO_3^- in ZC108, as well as soil acid phosphatase activity in ZC108 (Figure 5). However, the elevated glucosaminidase in both tea cultivars and chitinase activities in LJ43 were greater under the spraying of NO_3^- compared to NH_4^+ (Figure 5), suggesting that the effects of a leaf fertilizer on soil nutrient cycling may differ from the application of a soil fertilizer, which needs to be explored in the future.

4.4 | Microbial Ecology of Inorganic N Cycle and P Availability in Tea Plantations

An excessive application of N fertilizers and large leaf harvests in tea soils invariably result in soil acidification, N leaching and green gas losses (Guo et al. 2004). Denitrification has been found being involved in the catalyzation by enzymes encoded by *nirS*, *nirK* and *nosZ* genes, while nitrification is catalyzed by enzymes encoded by the ammonia-oxidizing archaea (*AOA*) and ammonia-oxidizing bacteria (*AOB*) (Teixeira and Yergeau 2012). The nano-Se application reduced rhizosphere nitrification and denitrification potentials via downregulating the expression of *AOA*, *AOB*, *nosZ* and *nirK* genes in LJ43. The nano-Se application regulates the relative abundance of bacterial taxa related to soil nitrification and denitrification and affects the N cycling process (Figure 3) (Ren et al. 2018). We discovered that Se downregulated the expression of *AOA* and *nosZ* genes in the rhizosphere of LJ43, and such effect was greater under the NH_4^+ supply (Figure 6).

In tea soils, the NH_4^+ fertilizer is excessively applied due to the preferred uptake of NH_4^+ by tea plants (Ruan et al. 2007). Overall, nano-Se fertilizers may be beneficial for reducing soil N loss, although the leaf Se accumulation and amino acid content were found to increase under a NH_4^+ supply for LJ43 (Figure 6). In ZC108, genes related to nitrification and denitrification were upregulated by Se when combined with the NH_4^+ application (Figure 6). The Se application enhances the ammonia oxidation process and promotes the conversion of NH_4^+ to NO_3^- in the N cycling (Lei et al. 2022). Some bacterial taxa related to the assimilation of nitrate pathways, such as Firmicutes and Proteobacteria, have higher abundances with the addition of Se (Ren et al. 2018; Lei et al. 2022). A NH_4^+ supply increases the substrate availability to the ammonia oxidation process and nitrification ability (Zhao, Cai, and Xu 2007), which is consistent with the observed changes in gene expressions related to nitrification (Figure 6). Tea plants have less NO_3^- absorption compared with

NH_4^+ (Ruan et al. 2007). Thus, the large NO_3^--N accumulation with the Se application possibly leads to a NO_3^- loss via denitrification and/or leaching (Ruan et al. 2007). Substantial nitrification and NO_3^- accumulation have been found in tea soils, even though nitrification is expected to be low in acidic soils (Wickramasinghe, Rodgers, and Jenkinson 1985). The pH range was 3-4 in the studied tea soils, and such low pH may promote NO_3^- loss via the denitrification in the rhizosphere of ZC108 under a NH_4^+ supply, which is consistent with the upregulated expression of *nosZ* and *nirK* genes.

The higher rhizosphere expression of *nifH* and *phoC* in LJ43 under the NH_4^+ supply may compensate for the N losses via nitrification and denitrification (Figure 6), which is consistent with previous studies (Lei et al. 2022). However, nano-Se downregulated the rhizosphere expression of *nifH* gene in ZC108 under the NO_3^- supply. Se increased or decreased the symbiotic N fixation, depending on the plant taxon. The nano-Se application downregulated the *nifH* expression in the rhizosphere of LJ43, regardless of N forms (Figure 6). All forms of mineral N fertilizers decrease the symbiotic N fixation via the rhizobia symbioses (Streeter and Wong 1988). The decreased rhizosphere expression of the *nifH* gene in LJ43 may be due to the increased N availability (Figure 6) (Akter et al. 2018). These results suggested that the effects of nano-Se on soil N cycling largely depend on the tea cultivar and N forms.

5 | Conclusions

The application of nano-Se and N forms increased the leaf amino acids and Se accumulation in LJ43 and ZC108, and the Se accumulation was greater in ZC108 than in LJ43. LJ43 and ZC108 recruited different bacterial and fungal taxa in their rhizosphere. LJ43 enriched more microbial taxa related to the maintenance and resource acquisition in the rhizosphere and had greater levels of organic N and P mineralization, enhanced by the application of Se and N forms. Moreover, the composition of rhizosphere bacterial and fungal communities showed a greater correlation with soil nutrients in LJ43 relative to ZC108. In contrast, in ZC108, Proteobacteria was predominant, and the microbial composition and diversity were less affected by nano-Se and N spraying. Soil N cycling induced by Se application was less affected by N forms in the rhizosphere of LJ43, but largely varied with N forms in ZC108. The microbial regulation of nitrification and denitrification was great in tea soils. The nano-Se application downregulated the expression of nitrification genes *AOA* and *AOB*, and denitrification genes *nosZ* and *nirK* in LJ43 under both N forms, and such downregulation was greater under NH_4^+ than NO_3^- spraying. However, the Se application upregulated the expression of *AOA*, *AOB*, *nosZ*, *nirK*, N fixation *nifH* and organic P mineralization *phoC* genes in ZC108 under the NH_4^+ supply. Thus, nano fertilizers would potentially lead to improved tea leaf quality and plant growth via promoting organic nutrient mineralization and reducing N risks in the soil of LJ43, especially under the NH_4^+ supply.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw DNA sequence files and associated metadata were deposited in the NCBI data bank with the accession number PRJNA1164332.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.