



OPEN ACCESS

EDITED BY

Decai Jin,
Chinese Academy of Sciences (CAS), China

REVIEWED BY

Chuan Shen,
Ankang University, China
Muhammad Zeeshan Ul Haq,
Hainan University, China

*CORRESPONDENCE

Xiliang Song
✉ sxl0424@126.com

RECEIVED 05 September 2025

REVISED 09 November 2025

ACCEPTED 10 November 2025

PUBLISHED 26 November 2025

CITATION

Song J, He Q, Cao K, Song X and Zeng Q
(2025) Effects of continuous cropping on
soil metabolomics and rhizosphere bacterial
communities in *Panax quinquefolius* L.
Front. Microbiol. 16:1698779.
doi: 10.3389/fmicb.2025.1698779

COPYRIGHT

© 2025 Song, He, Cao, Song and Zeng. This
is an open-access article distributed under
the terms of the [Creative Commons
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,
distribution or reproduction in other forums
is permitted, provided the original author(s)
and the copyright owner(s) are credited and
that the original publication in this journal is
cited, in accordance with accepted academic
practice. No use, distribution or reproduction
is permitted which does not comply with
these terms.

Effects of continuous cropping on soil metabolomics and rhizosphere bacterial communities in *Panax quinquefolius* L.

Jian Song, Qing He, Kuo Cao, Xiliang Song* and
Qiangcheng Zeng

College of Life Sciences, Dezhou University, De'zhou, China

Introduction: Continuous cropping obstacles (CCOs) due to long-term monoculture have emerged as a pervasive challenge in contemporary agriculture worldwide. The practices of CCOs are the primary causes of restraining the *Panax quinquefolius* L. (*P. quinquefolius*) growth, whereas its underlying microbial mechanisms have not been fully elucidated.

Methods: We investigated the effects of CCOs on soil physicochemical properties, enzyme activities, microbial community composition, and metabolite profiles in the rhizosphere of *P. quinquefolius* cultivated continuously for one, two, three, and four consecutive years (designated as CC1, CC2, CC3, and CC4, respectively) without crop rotation. Rhizosphere soil samples were collected from fields with different years of CCOs and analyzed for physicochemical properties and enzyme activities. Microbial community composition was assessed using Illumina high-throughput sequencing, and metabolite profiles were analyzed using non-targeted metabolomics (UPLC-MS/MS).

Results: Significant decreases were observed in soil pH (12.2–28.0%), cation exchange capacity (42.6–65.5%), organic matter (8.7–27.3%), total nitrogen (7.6–27.8%), and ammonium (NH₄⁺) content (16.9–56.6%) with an increasing number of continuous cropping years. Enzymatic activities, including urease, invertase, alkaline phosphatase, catalase, protease, and polyphenol oxidase, were also reduced. The occurrence of CCOs decreased bacterial richness and number but increased bacterial diversity. Key microbial biomarkers were shifted from Gemmatimonadota, Actinobacteriota, and Proteobacteria to Acidobacteriota, Chloroflexi, and WPS-2 with *P. quinquefolius* CCOs. Consequently, the number of beneficial microorganisms decreased, whereas the number of pathogenic microorganisms increased. Non-targeted metabolomic profiling showed significant enantioselectivity in phenylpropanoid biosynthesis and pyrimidine metabolism. Time-series analysis revealed a decrease in metabolites classified as lipids and lipid-like molecules and an increase in organic acids, derivatives, phenylpropanoids, and polyketides with continuous cropping. Partial least squares-path modeling identified reduced soil enzymatic activity due to CCOs as the primary factor regulating soil bacterial communities and metabolites.

Significance: These findings offer new insights into the microecological mechanisms of CCOs in *P. quinquefolius*, aiding in controlling pathogenic bacteria and maintaining soil health in agricultural systems.

Conclusion and prospects: *P. quinquefolius* CCOs significantly alter soil physicochemical properties, microbial community structure, and metabolite profiles, leading to reduced soil fertility and increased prevalence of soil-borne diseases. Future research should focus on exploring sustainable agricultural practices, such as crop rotation and soil amendments, to mitigate these adverse effects and improve the long-term viability of *P. quinquefolius* cultivation.

KEYWORDS

continuous cropping obstacle, soil health, microbial ecology, metabolite profiling, *Panax quinquefolius* L.

1 Introduction

Continuous cropping (CC) refers to repeated cultivation of the same crop in the same field over many years. Owing to limited arable land and rising food demand, CC remains a prevalent agricultural practice in developing countries (Lei et al., 2020; Tan et al., 2021). However, it places significant stress on the soil, leading to the degradation of soil properties (Pascale et al., 2020) and disruption in microbial communities (Beretta-Blanco et al., 2019). These challenges increase the risk of soil-borne diseases, deteriorate microbial structures, and impair crop productivity and quality (Chen et al., 2022a,b; She et al., 2017). Over the past few decades, significant progress has been made in recent decades in understanding the mechanisms underlying CC obstacles (CCOs) in crops such as *Nicotiana tabacum* (Chen et al., 2023), *Citrullus lanatus* (Ding et al., 2021), *Glycine max* (Cui et al., 2024), and *Panax notoginseng* (Wang F. et al., 2022), with a focus on soil health, plant nutrition, microbiology, and agricultural practices. These studies have revealed that the primary soil mechanisms causing CCOs include: (i) secretion of autotoxins from plant root exudates into the soil, leading to allelopathic autotoxicity (Zeeshan Ul Haq et al., 2023); (ii) degradation of soil's physicochemical properties (Song et al., 2018; Wang F. et al., 2022); and (iii) disruption of soil microbial biomass, activity, and community structure (Ashworth et al., 2020; Zhao et al., 2024). These factors lead to plant growth inhibition and soil-borne diseases (Li H. et al., 2019), ultimately affecting crop yield. Understanding the mechanisms underlying CC-induced obstacles is critical to maintaining soil fertility and ensuring sustainable food production.

Soil microorganisms are essential for biogeochemical cycles and energy transfer (Rillig et al., 2019; Xue et al., 2022). Several studies have shown that disturbances in soil microecology and proliferation of pathogenic microorganisms are primary contributors to CCOs (Zhang H. et al., 2022). The CCOs lowers soil pH and suppresses the abundance of beneficial bacteria, including *Sphingomonas*, *Bacillus*, and *Prevotella* (Arafat et al., 2020; Li J. et al., 2023). For instance, continuous tobacco cultivation significantly reduced soil organic matter (SOM), available phosphorus (AP), total nitrogen (TN), and nitrate (NO_3^-), negatively affecting microbial diversity and community

structure (Tan et al., 2021). Furthermore, long-term CCOs diminish soil fertility (Tan et al., 2017), inhibit soil enzyme activity (Li J. et al., 2023), disrupt the microbial balance (Ma et al., 2023), degrade the soil microecological environment (Liao and Xia, 2024), and facilitate the spread of soil-borne pathogens (Chen et al., 2018). These effects increase the prevalence of diseases such as blight and *Verticillium* wilt (Li J. et al., 2023; Tan et al., 2017), which exacerbate plant health challenges and crop productivity. The rhizosphere microbiome plays a crucial role in maintaining plant and soil health by suppressing disease development (de Faria et al., 2021; Trivedi et al., 2021). Complex microbial interactions within the rhizosphere influence the susceptibility of plants to infections and affect their growth (Semenov et al., 2020). Therefore, monitoring the alterations in rhizosphere microbial communities is essential for identifying the root causes of CCOs-induced challenges and developing strategies to enhance plant disease resistance.

In addition to microbial disturbances, CCOs also affect soil metabolite composition and metabolic processes. Changes in soil metabolites, indicative of the physiological and metabolic activities of microbes, offer crucial insights into microbial responses to environmental stress (Cui et al., 2024; Dong et al., 2024; Gong et al., 2023). Metabolomics, a powerful technique for the qualitative and quantitative analysis of low-molecular-weight metabolites, provides a deeper understanding of the biological processes and functional capabilities of microbes (Muthubharathi et al., 2021). This technique has been widely used to monitor soil metabolic responses to stressors, such as salinization (Duan et al., 2023), organic pollutants (Phulpoto et al., 2024), heavy metals (Feng et al., 2024), plastic residues (Dong et al., 2024), and chemical fertilizers (Brown et al., 2021). However, studies focusing on the effects of CC on soil microbial metabolites and metabolic pathways are limited, necessitating further research (Cui et al., 2024).

P. quinquefolius, a medicinal plant valued for its economic and health benefits, is increasingly cultivated across China. Its cultivation follows a continuous 4-year cycle, but CC introduces severe challenges, such as soil-borne diseases, high seedling mortality, stunted plant growth, and reduced yield and quality. However, the microbial mechanisms driving CCOs in *P. quinquefolius* cultivation remain unclear. In this study, we aimed to investigate the effects of different CC durations on

soil physicochemical properties, enzyme activities, microbial biomass and community structures, and metabolite profiles in the rhizosphere of *P. quinquefolius*. The main objectives were to (i) analyze the responses of soil bacterial communities, metabolite composition, and microbial activities across different years of *P. quinquefolius* CCOs, (ii) evaluate potential correlations between microbial community structures and changes in metabolites and metabolic functions, and (iii) elucidate the micro-mechanisms underlying CCOs in *P. quinquefolius*-planted soils. We hypothesized that CCOs significantly altered soil physicochemical properties and microbial community structures, leading to changes in metabolite profiles that contributed to the observed decline in soil health and increased prevalence of soil-borne diseases. Overall, this study applied UHPLC-MS/MS non-targeted metabolomics combined with Illumina high-throughput sequencing technologies to accurately assess the risks of CCOs in *P. quinquefolius* rhizosphere soil and provided the scientific basis for the management of crop cropping practices.

2 Materials and methods

2.1 Experimental design and soil sample collection

The experimental field with long-term CC of *P. quinquefolius* was located in Jixi Village (37°4'N, 122°59'E) in Wendeng County (Shandong, China). This region is characterized by a warm temperate monsoon climate, with an annual average temperature of 11.9 °C and annual precipitation of 767.8 mm. The soil was classified as fluvo aquic, which is a type of soil that is formed under conditions of fluctuating water tables and characterized by a well-developed profile with distinct horizons, good structure, and high fertility (Xin et al., 2019). The *P. quinquefolius* population was maintained at 500,000 ha⁻¹, with 10 cm spacing between plants and 20 cm between rows. Due to serious CCOs, the farmland would be abandoned if the *P. quinquefolius* plants were cultivated for 4 years. To ensure the representativeness and comparability of the samples, four soil sample sites were selected from fields where *P. quinquefolius* had been continuously grown for 1 year (designated as CC1), 2 years (CC2), 3 years (CC3), and 4 years (CC4), respectively. In addition, a control site (CC0) with *Zea mays-Triticum aestivum* rotation cultivation was chosen at the same location to provide a baseline for comparison. The total area of both the *Zea mays-Triticum aestivum* rotation plot and the *P. quinquefolius* CC plot was 120 m × 120 m (14,400 m²). Within each plot, three subplots of 20 m × 20 m were selected for soil sampling, with five 1.0 m × 1.0 m quadrats randomly set up in each subplot for collecting rhizosphere soil samples. The soil samples from each quadrat were mixed to form composite samples, resulting in a total of 15 samples (five subplots × three biological replicates). The distance between each two soil sample sites was more than 500 m, and the soil samplings were collected in September 2023. Importantly, all experimental plots were previously under the same *Zea mays-Triticum aestivum* rotation system with uniform fertilization and management practices, ensuring consistent soil conditions at the start of the study. After passing through a 2-mm sieve, the soil samples were divided into

three groups. The first group was air-dried in laboratory for 2 weeks to analysis the physicochemical properties including soil pH, cation exchange capacity (CEC), SOM, TN, NO₃⁻, ammonium (NH₄⁺), AP, and AK contents AP. The second group of fresh soils was used to count living bacteria, fungi, and Actinomyces; analyze soil enzyme activities; and measure microbial biomass carbon (MBC) and nitrogen (MBN). Immediately after collection, the samples were placed in sealed containers and stored in a -20 °C freezer for no longer than 4 weeks prior to analysis. This method of cryogenic preservation is according to the protocol described by Pavlovska et al. (2021). The third group was preserved at -80 °C for subsequent bacterial community and metabolic function analyses. All analyses were conducted in triplicates.

2.2 Quantification of soil microbial numbers

The soil bacteria, fungi, and Actinomyces in each sample were quantified using the plate counting method (Thornton et al., 1934). Briefly, tenfold serial dilutions were prepared by suspending 10 g of soil in 90 mL of sterile water, mixing thoroughly, and diluting to a final concentration of 10⁻⁶. Aliquots (0.1 mL) of these dilutions were spread onto plates containing potato-dextrose agar for fungi, beef extract peptone agar for bacteria, and Gauze's No. 1 agar for Actinomyces. The plates were incubated at 37 °C for 24 h for fungi and at 25 °C for 48 h for bacteria and Actinomyces (Liao et al., 2018). Subsequently, the number of bacteria, fungi, and Actinomyces was counted.

2.3 Measurement of soil physicochemical properties

Soil pH was determined by measuring the pH of a 1:2.5 soil-to-water mixture using a glass electrode pH meter (PHS-25, Leici, Shanghai, China). Soil CEC was assessed using the silver thiourea method (Pleysier and Juo, 1980). The Walkley-Black method was used to quantify SOM (Walkley and Black, 1934), and an elemental analyzer was used to measure TN, NO₃⁻-N, and NH₄⁺ contents (CHNOS Elemental Analyzer, Vario EL, Elementar, Germany). The Olsen method was used to assess soil AP (Olsen and Sommers, 1982), and flame emission spectrometry was used to evaluate soil available potassium (AK) content (Jackson and Mahmood, 1992).

2.4 Analysis of soil enzyme activities

To assess soil health, we measured the activities of six key soil enzymes: urease (UREA), invertase (INV), alkaline phosphatase (ALP), catalase (CAT), protease (PRO), and polyphenol oxidase (PPO). In this experiment, UREA activity was quantified using a colorimetric assay for ammonium (NH₄⁺) (Kandeler and Gerber, 1988). Briefly, 5.0 g of fresh soil was incubated with 10 mL of 10% urea solution and 1 mL of toluene at 37 °C for 24 h. After incubation, 1 mL of the supernatant was mixed with 4 mL of sodium phenate solution and 3 mL of sodium hypochlorite solution. The absorbance of the resulting blue color was measured

at 578 nm using a spectrophotometer (UV-2600, Shimadzu, Japan). The INV activity was determined using the glucose colorimetric method (Gopal et al., 2007). A 5.0 g soil sample was incubated with 15 mL of 8% sucrose solution and 1 mL of toluene at 37 °C for 24 h. After incubation, 1 mL of the supernatant was mixed with 3 mL of 3,5-dinitrosalicylic acid reagent and heated in a boiling water bath for 5 min. The absorbance was measured at 540 nm. The ALP activity was assessed by monitoring the hydrolysis of p-nitrophenyl phosphate and the release of p-nitrophenol (Tabatabai and Bremner, 1969). A 1.0 g soil sample was incubated with 4 mL of 0.05 M p-nitrophenyl phosphate solution and 1 mL of toluene at 37 °C for 1 h. After incubation, 1 mL of 0.5 M CaCl₂ and 4 mL of 0.5 M NaOH were added, and the mixture was filtered. The absorbance of the filtrate was measured at 400 nm. The CAT activity was measured using the potassium permanganate titration method (Trasar-Cepeda et al., 1999). Specifically, 2.0 g of soil was mixed with 40 mL of distilled water and 5 mL of 0.3% H₂O₂ solution, then incubated at 25 °C for 30 min. The reaction was stopped by adding 5 mL of 1.5 M H₂SO₄. The remaining H₂O₂ was titrated with 0.02 M KMnO₄ solution. The activity of PRO was determined using the leucine aminopeptidase assay (Ladd and Butler, 1972). A 1.0 g soil sample was incubated with 5 mL of 1% casein solution and 1 mL of toluene at 37 °C for 2 h. After incubation, 2 mL of 0.5 M trichloroacetic acid was added to stop the reaction, and the mixture was filtered. The amino acid content in the filtrate was determined using the ninhydrin method, with absorbance measured at 570 nm. The PPO activity was measured using the orthobenzene-triol colorimetric method (Montgomery and Sgarbieri, 1975). For this, 1.0 g of soil was incubated with 5 mL of 0.1 M catechol solution and 1 mL of phosphate buffer (pH 6.0) at 30 °C for 2 h. The reaction was stopped by adding 2 mL of 0.5 M H₂SO₄. The absorbance of the supernatant was measured at 490 nm. Microbial biomass carbon (MBC) and nitrogen (MBN) were quantified using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, 20 g of fresh soil was divided into two subsamples. One subsample was fumigated with ethanol-free chloroform for 24 h at 25 °C in the dark, while the other served as a non-fumigated control. After fumigation, both fumigated and non-fumigated samples were extracted with 80 mL of 0.5 M K₂SO₄ solution by shaking for 30 min and then filtered. The organic carbon and total nitrogen in the extracts were determined using a total organic carbon analyzer (TOC-VCPI, Shimadzu, Japan) and a Kjeldahl nitrogen analyzer (Kjeltec 8400, FOSS, Sweden), respectively. MBC and MBN were calculated using the following equations:

$$MBC = \frac{\text{Fumigated C} - \text{Non-fumigated C}}{K_C}$$

$$MBN = \frac{\text{Fumigated N} - \text{Non-fumigated N}}{K_N}$$

where K_C (0.45) and K_N (0.54) are the conversion factors for carbon and nitrogen, respectively.

2.5 DNA extraction and sequencing

Genomic DNA was extracted from fresh soil (0.5 g) using a FastDNA SPIN Kit (Macherey-Nagel, Germany), according

to the manufacturer's protocol. DNA quality and purity were evaluated using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, United States). The V4–V5 region of the bacterial 16S rRNA gene was amplified using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Bates et al., 2011; Parada et al., 2016). After PCR amplification and purification, paired-end sequencing was performed using the Illumina MiSeq platform. Raw sequencing data were processed using Quantitative Insights Into Microbial Ecology Version 2, employing the DADA2 pipeline to obtain amplicon sequence variants (ASVs). Taxonomic annotation of each ASV was performed using the SILVA database. The raw sequencing read data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under the accession number SUB15654061.

2.6 Non-targeted metabolite analysis

Non-targeted metabolomic profiling was conducted using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Thawed soil samples at 4 °C were mixed with a pre-chilled solvent mixture of methanol, acetonitrile, and water (2:2:1 volume ratio) and added to 100 mg of each sample. This mixture was vortexed, sonicated at low temperature for 30 min, exposed to -20 °C for 10 min, and centrifuged at 14,000 × g for 20 min at 4 °C. The supernatant was vacuum-dried, and the residue was re-dissolved in 100 µL of a 1:1 acetonitrile/water solution and then re-centrifuged at 14,000 × g for 15 min at 4 °C. The supernatant was used as the sample for the UPLC-MS/MS analysis. Quality control (QC) samples prepared by pooling 10 µL of each sample were processed and analyzed with the experimental samples to ensure consistency and reproducibility. The mass spectrometer was operated in both positive and negative electrospray ionization modes. Metabolites with > 50% missing values in any treatment group were excluded. Metabolite identification was achieved by matching the m/z values (within 10 ppm tolerance) and MS/MS spectral data with an in-house database of authenticated standards.

2.7 Data processing and statistical analysis

One-way analysis of variance (ANOVA) was performed using the SPSS 25 software (SPSS Inc., United States), followed by Tukey's HSD *post-hoc* test to identify significant differences ($p < 0.05$) in soil physicochemical properties, enzymatic activities, and microbial biomass among various soil samples. Bacterial alpha diversity indices (ACE, Chao, Shannon, and Simpson) were calculated using the "vegan" package in R, with intergroup diversity differences assessed by the Kruskal–Wallis test. The bacterial community composition at the genus and phylum levels was visualized using Circos software.¹ LEfSe analysis was performed to identify biomarker taxa in different soil samples using

¹ <http://circos.ca/>

the “microbiomeMarker” package. Principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS) based on the Bray–Curtis distance and ANOSIM were used to elucidate differences in bacterial community composition. Redundancy analysis (RDA) was used to evaluate the correlations between the soil microbial community structure and soil microbial biomass, physicochemical properties, and enzymatic activities. The function of the bacterial community was predicted using FAPROTAX and the PICRUST2 software. Bacterial co-occurrence networks based on relative ASV abundance were constructed using the Gephi software (version 10.1).

Statistical analyses of global metabolic variations among comparable groups were performed using Pareto-scaled principal component analysis (PCA) and orthogonal projections to latent structure discriminant analysis (OPLS-DA). Metabolites with significant differences (differential metabolites; DM) among groups were identified based on a variable importance in projection (VIP) score of > 1.0 and a *p*-value of < 0.05. DM was visualized using a heatmap generated using the “pheatmap” R package. The top 10 enriched metabolic pathways were illustrated using bubble plots from the KEGG database. Time-series analysis was performed using the “maSigPro” R package to identify metabolites with significant fluctuations across cultivation periods. Associations between soil bacterial communities and metabolite profiles were assessed using Procrustes analysis (via the “psych” package in R v4.2.2). Additionally, partial least squares-path modeling (PLS-SEM) performed using the “plspm” package was used to evaluate the direct and indirect effects of soil physicochemical properties and enzymatic activities on microbial community composition, metabolite profiles, and biomass.

3 Results

3.1 Effect of *P. quinquefolius* CCOs on soil physicochemical properties

P. quinquefolius CC significantly influenced the rhizospheric soil physicochemical properties (Table 1). Compared to those in the control (CC0), soil pH, CEC, SOM, TN, and NH₄⁺ content in CC1, CC2, CC3, and CC4 treatments exhibited a progressive and significant decrease. Specifically, soil pH decreased by 12.2–28.0%, CEC by 42.6–65.5%, SOM by 8.7–27.3%, TN by 7.6–27.8%, and NH₄⁺ by 16.9–56.6% with increasing years of continuous cropping. This consistent decline across multiple key indicators suggests a severe degradation of soil quality under prolonged monoculture. In contrast, the nitrate (NO₃[−]) content showed a substantial increase, ranging from 36.0 to 75.6% across the CC treatments compared to CC0. This inverse relationship between NH₄⁺ and NO₃[−] suggests altered nitrogen cycling dynamics, potentially due to inhibited nitrification or increased denitrification under CC stress. The contents of AP and AK initially increased, reaching their highest values in the CC2 treatment (52.3 and 152.4 mg kg^{−1}, respectively), but then decreased with further prolonged cropping. These findings collectively highlight a significant and detrimental shift in the soil’s chemical environment, indicating reduced fertility and nutrient imbalance as a direct consequence of continuous *P. quinquefolius* cultivation. Influence of CCOs on soil enzyme activities.

TABLE 1 Changes of soil physicochemical properties in different continuous *Panax quinquefolius* L. cropping treatments.

Treatments	pH value	CEC (cmol g ^{−1})	SOM (g kg ^{−1})	TN (g kg ^{−1})	NO ₃ [−] -N (mg kg ^{−1})	NH ₄ ⁺ -N (mg kg ^{−1})	AP (mg kg ^{−1})	AK (mg kg ^{−1})
CC0	6.29 ± 0.39 a	91.08 ± 0.8 a	16.1 ± 0.8 a	1.44 ± 0.16 a	17.2 ± 1.1 d	26.7 ± 1.8 a	28.1 ± 3.0 d	80.0 ± 1.7 d
CC1	5.52 ± 0.10 b	52.3 ± 0.6 b	14.7 ± 0.2 b	1.33 ± 0.10 ab	23.4 ± 1.7 c	22.2 ± 1.5 b	42.4 ± 2.1 c	91.2 ± 1.7 c
CC2	4.53 ± 0.03 c	48.4 ± 0.03 b	13.6 ± 0.2 bc	1.20 ± 0.08 bc	25.9 ± 1.7 b	21.3 ± 1.1 b	52.3 ± 2.7 a	152.4 ± 4.2 a
CC3	4.75 ± 0.03 c	39.6 ± 2.3 c	12.5 ± 1.0 cd	1.12 ± 0.13 bc	29.3 ± 0.1 a	14.7 ± 1.1 c	46.3 ± 1.5 b	149.6 ± 3.5 a
CC4	4.53 ± 0.07 c	31.4 ± 4.4 d	11.7 ± 0.9 d	1.04 ± 0.11 c	30.2 ± 1.0 a	11.6 ± 0.7 d	41.1 ± 0.04 c	117.6 ± 2.4 b

Values are the mean of three replicates. Different letters in the same column indicate that values are significantly different from each other at *p* ≤ 0.05. CEC, cation exchange capacity; SOM, soil organic matter; TN, total nitrogen; NO₃[−]-N, nitrate-nitrogen; NH₄⁺-N, ammonium-nitrogen; AP, available phosphorus; AK, available potassium.

3.2 Influence of *P. quinquefolius* CCOs on soil enzyme activities

The effects of *P. quinquefolius* CC on the activity of the six key soil enzymes are presented in Table 2. A consistent and progressive decrease in the activities of all measured enzymes-UREA, CAT, PPO, INV, PRO, and ALP-was observed with increasing years of *P. quinquefolius* cultivation. Compared to the CC0 treatment, the activities of UREA, CAT, PPO, INV, PRO, and ALP in CC1, CC2, CC3, and CC4 treatments decreased by 1.8–36.1%, 13.5–60.6%, 13.5–32.7%, 22.6–64.5%, 6.6–94.7%, and 35.3–89.4%, respectively. The most pronounced reductions were observed in PRO and ALP activities, particularly in the CC4 treatment, indicating a severe impairment of protein and phosphorus cycling. This widespread reduction in enzyme activities across all six indicators suggests a significant inhibition of overall soil microbial metabolic activity and a decline in the soil's capacity to facilitate essential biogeochemical processes.

3.3 Effect of *P. quinquefolius* CCOs on soil microbial numbers and biomass

As shown in Supplementary Figure 1, with increasing years of *P. quinquefolius* CC, the number of soil bacteria and fungi in the different sample soils continuously decreased. This decline suggests a negative impact of prolonged monoculture on the overall abundance of these crucial microbial groups. In contrast, the number of Actinomyces species showed a consistent increasing trend, indicating a potential shift in microbial dominance or a greater resilience of Actinomyces to CC stress. Furthermore, MBC and MBN showed no significant differences between the CC0 and

CC1 treatments, suggesting that the initial year of continuous cropping had a limited impact on overall microbial biomass. However, MBC and MBN increased markedly in the CC2, CC3, and CC4 treatments (Supplementary Figure 2). Compared with those in the CC0 treatment, MBC and MBN increased by 19.1 and 35.4% in the CC2 treatment, 26.2 and 63.1% in the CC3 treatment, and 50.8 and 71.8% in the CC4 treatment, respectively. This unexpected increase in MBC and MBN, despite the decrease in bacterial and fungal numbers, could imply a shift toward microbial communities with higher biomass per cell or altered physiological states, possibly in response to accumulating root exudates or changes in nutrient availability.

3.4 Soil microbial community dynamics in response to CCOs

The effect of *P. quinquefolius* CC on soil microbial communities was evaluated by high-throughput 16S rRNA gene sequencing using the Illumina MiSeq platform. Analysis of 15 rhizospheric soil samples generated 862,744 high-quality sequences and identified 48,540 ASVs (Supplementary Table 1). The average number of ASVs in CC0, CC1, CC2, CC3, and CC4 treatments were 4,378; 4,525; 2,545; 2,439; and 2,285; respectively.

The effects of *P. quinquefolius* CC on ACE, Chao, Shannon, and Simpson indices representing the microbial community richness and diversity are shown in Table 3. The Ace and Chao indices showed no significant differences between CC0 and CC1 but were significantly decreased in CC2, CC3, and CC4 treatments. The Shannon index remained unaffected in the CC1 and CC2 treatments but significantly increased in the CC3 and CC4 treatments. Conversely, the Simpson index exhibited a remarkable decrease in the CC4 treatment group. The analyses of PCoA and

TABLE 2 Effects of continuous *Panax quinquefolius* L. cropping on soil enzymatic activities.

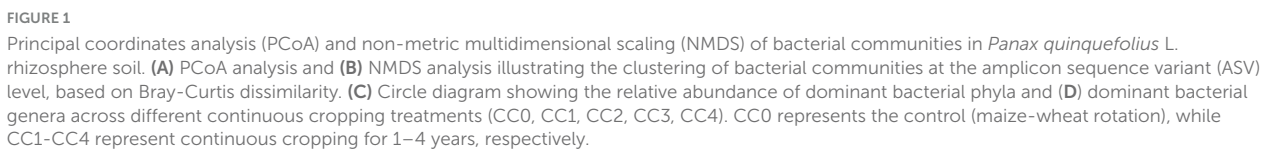
Treatments	UREA ($\mu\text{g d}^{-1}\text{g}^{-1}$)	CAT ($\mu\text{g h}^{-1}\text{g}^{-1}$)	PPO (nmol $\text{h}^{-1}\text{g}^{-1}$)	INV ($\mu\text{g d}^{-1}\text{g}^{-1}$)	PRO ($\mu\text{g h}^{-1}\text{g}^{-1}$)	ALP (nmol $\text{h}^{-1}\text{g}^{-1}$)
CC0	305.6 $\pm$ 6.9 a	60.9 $\pm$ 0.5 a	693.7 $\pm$ 12.9 a	6.2 $\pm$ 0.01 a	7.6 $\pm$ 0.3 a	244.3 $\pm$ 14.2 a
CC1	300.0 $\pm$ 5.2 a	52.7 $\pm$ 1.4 b	600.2 $\pm$ 10.2 b	4.8 $\pm$ 0.1 b	7.1 $\pm$ 0.6 ab	158.0 $\pm$ 3.0 b
CC2	244.8 $\pm$ 3.8 b	40.5 $\pm$ 2.2 c	527.5 $\pm$ 17.9 c	3.9 $\pm$ 0.0 c	6.6 $\pm$ 0.3 b	37.4 $\pm$ 3.1 c
CC3	215.4 $\pm$ 2.5 c	34.1 $\pm$ 1.8 d	529.4 $\pm$ 8.3 c	3.2 $\pm$ 0.1 d	3.7 $\pm$ 0.2 c	32.6 $\pm$ 1.9 c
CC4	195.3 $\pm$ 5.1 d	24.0 $\pm$ 2.0 e	467.2 $\pm$ 9.4 d	2.2 $\pm$ 0.1 e	0.4 $\pm$ 0.1 d	25.9 $\pm$ 1.7 c

Values are the mean of three replicates. Different letters in the same column indicate that values are significantly different from each other at $p \leq 0.05$. UREA, urease activity; CAT, catalase activity; PPO, polyphenol oxidase activity; INV, invertase activity; PRO, protease activity; ALP, alkaline phosphatase activity.

TABLE 3 The richness and diversity of microbial community in in different continuous *Panax quinquefolius* L. cropping soils.

Treatments	Alpha diversity indexes			
	Ace	Chao	Shannon	Simpson
CC0	34.8 $\pm$ 3.3 a	34.7 $\pm$ 3.2 a	2.06 $\pm$ 0.06 b	0.186 $\pm$ 0.017 a
CC1	36.2 $\pm$ 2.1 a	36.0 $\pm$ 2.0 a	2.03 $\pm$ 0.04 b	0.170 $\pm$ 0.003 ab
CC2	28.7 $\pm$ 1.1 b	28.3 $\pm$ 1.2 b	2.04 $\pm$ 0.02 b	0.185 $\pm$ 0.009 a
CC3	26.3 $\pm$ 0.6 b	26.3 $\pm$ 0.6 b	2.14 $\pm$ 0.02 a	0.182 $\pm$ 0.001 ab
CC4	22.9 $\pm$ 0.2 c	22.3 $\pm$ 1.2 c	2.13 $\pm$ 0.03 a	0.163 $\pm$ 0.006 b

Values are the mean of three replicates. Different letters in the same column indicate that values are significantly different from each other at $p \leq 0.05$. Ace, Abundance-based Coverage Estimator; Chao, Chao1 richness estimator; Shannon, Shannon-Wiener diversity index; Simpson, Simpson's diversity index.



Co-occurrence networks of soil bacterial communities were constructed at the ASV level to analyze bacterial co-occurrence patterns across various CC treatments of *P. quinquefolius* (Figure 2). The network revealed five distinct ecological phylotype clusters. Compared with CC0, with increased duration of CC, significant reductions were observed in network metrics: the number of edges decreased from 6,760 to 6,065; the average weighted degree declined from 67.9 to 61.6, and the network density decreased from 0.343 to 0.314. By contrast, the modularity index increased from 0.506 to 0.663. Furthermore, the ratio

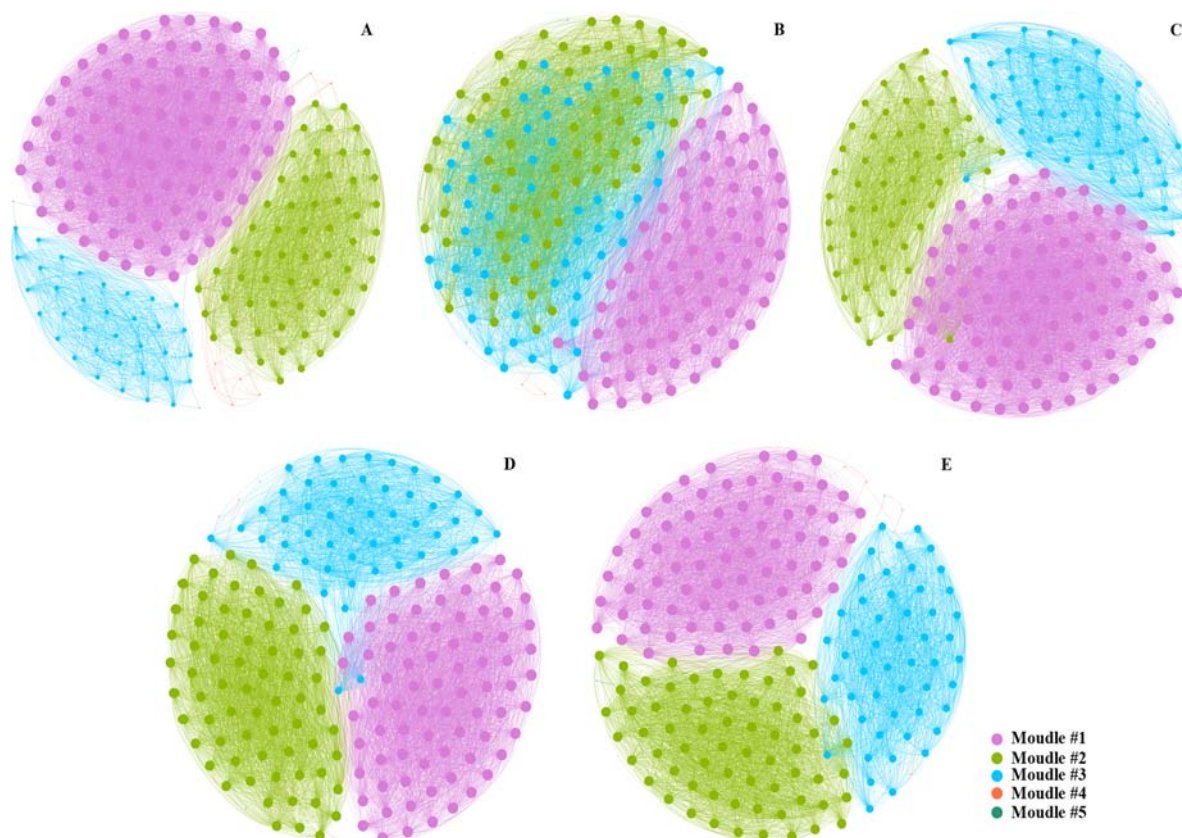


FIGURE 2

Bacterial co-occurrence networks in *Panax quinquefolius* L. rhizosphere soils under continuous cropping. Networks for (A) control (CC0), (B) 1 year (CC1), (C) 2 years (CC2), (D) 3 years (CC3), and (E) 4 years (CC4) of continuous cropping. Each node represents a unique amplicon sequence variant (ASV). The size of each node is scaled by its degree (number of connections). Nodes sharing the same color belong to the same module. Edges between nodes signify strong correlations (Spearman's rank correlation coefficient, $r > 0.08$, $P < 0.05$).

of positive edges decreased in the CC1, CC2, CC3, and CC4 treatments by 0.5, 1.9, 4.3, and 2.6%, respectively, compared to that in CC0 (Supplementary Table 2). These results suggest that *P. quinquefolius* CC significantly disrupts network complexity and destabilizes network stability.

Pairwise comparisons among the five treatments using LEfSe with a threshold of $LDA > 4$ identified 64 biomarkers spanning from the phylum to the genus level (Supplementary Figure 3). The findings indicated that CC treatments significantly reduced the relative abundance of Gemmatimonadota, Actinobacteria, and Proteobacteria phyla compared to that in the control treatment (Figure 3). In the CC1 treatment group, Actinobacteria, Blastocatellia, and Vicinamibacteria were abundant at the class level. The CC2 treatment resulted in the enrichment of Firmicutes and Thermoactinomycetales. Acidobacteriota, Chloroflexi, Acidobacteriae, and Ktedonobacteria were dominant in the CC3 treatment group. In the CC4 treatment, the relative abundance of WPS-2, Bryobacteriales, Ktedonobacteraceae, Bryobacteraceae, and Rhodanobacteraceae was significantly increased.

Evaluation of the relationship between the relative abundances of the top 10 dominant bacterial phyla and various soil environmental variables using RDA revealed that soil environmental variables, including physicochemical characteristics and enzyme activities, accounted for 85.0% of the variation

observed in the bacterial community (Figure 4). On the left side of the RDA1 axis, Acidobacteriota, Chloroflexi, Patescibacteria, and WPS-2 were positively correlated with AP, AK, and NO_3^- -N. Conversely, on the right side of the RDA1 axis, Actinobacteriota, Myxococcota, Bacteroidota, and Gemmatimonadota were positively correlated with pH, CEC, SOM, TN, NO_4^{+} -N, UREA, ALP, CAT, INV, PRO, and PPO. Among all soil environmental factors, AK ($R^2 = 0.89$, $p = 0.001$), pH ($R^2 = 0.64$, $p = 0.004$), and ALP ($R^2 = 0.72$, $p = 0.003$) were identified as the primary factors influencing the structure of the soil bacterial community (Supplementary Table 3).

Mapping the predicted functional profiles to the cluster of orthologous groups (COGs) database using the PICRUSt2 functional prediction tool indicated that the microbial functions across all treatments were primarily enriched in categories such as amino acid transport and metabolism, translation, ribosomal structure and biogenesis, inorganic ion transport and metabolism, and energy production and conversion (Figure 5A). Among these, amino acid transport and metabolism exhibited the highest relative abundance, comprising 10.38% in CC0, 10.54% in CC1, 10.41% in CC2, 10.47% in CC3, and 10.45% in CC4.

Further elucidation of the metabolic capabilities of the microbial communities was achieved by referencing the FAPROTAX database, which identified 30 distinct functional

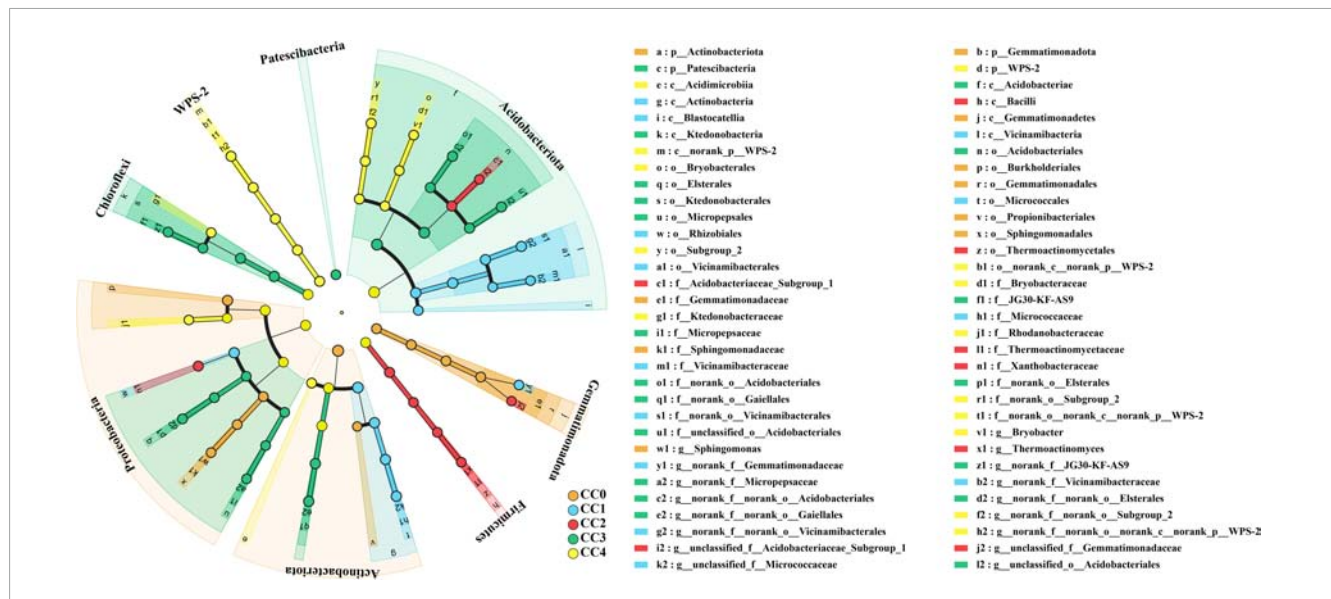


FIGURE 3

Linear discriminant analysis effect size (LEfSe) identifying bacterial biomarkers in *Panax quinquefolius* L. rhizosphere soil across continuous cropping years. The taxa with significantly different relative abundances among the CC0, CC1, CC2, CC3, and CC4 treatments are represented by colored dots ($p < 0.05$). The dots from the center to the outside represents phylum, class, order, family and genus, which have an effect size LDA score > 4 .

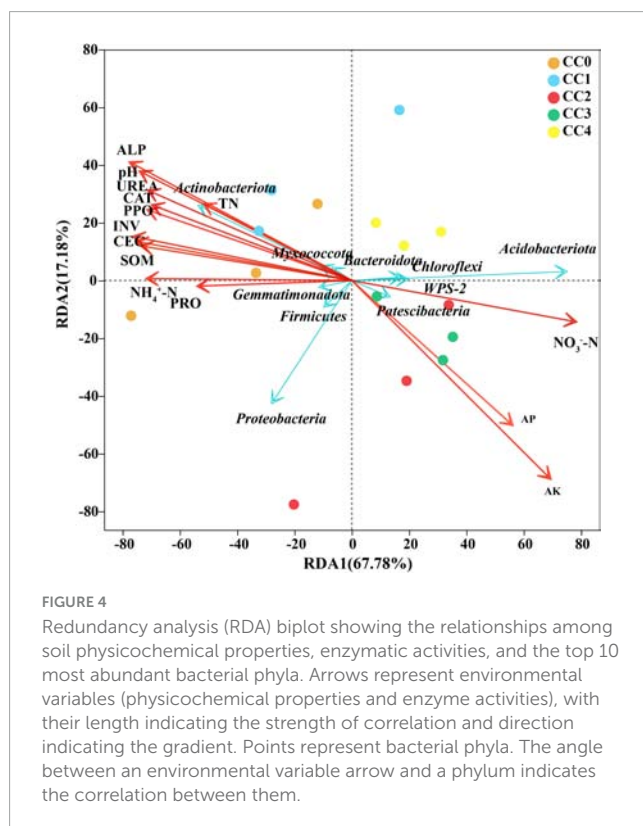


FIGURE 4

Redundancy analysis (RDA) biplot showing the relationships among soil physicochemical properties, enzymatic activities, and the top 10 most abundant bacterial phyla. Arrows represent environmental variables (physicochemical properties and enzyme activities), with their length indicating the strength of correlation and direction indicating the gradient. Points represent bacterial phyla. The angle between an environmental variable arrow and a phylum indicates the correlation between them.

groups within the study period (Figure 5B). Bacteria exhibiting chemoheterotrophy and aerobic chemoheterotrophy constituted a substantial proportion of the soil microbial community with no significant differences among the treatments. Conversely, the nitrogen cycling functions, specifically denitrification, nitrous oxide denitrification, nitrite denitrification, nitrate denitrification,

nitrate respiration, and nitrogen respiration, differed significantly among the CC2, CC3, CC4, CC0, and CC1 treatments.

3.5 Response of microbe non-targeted metabolomic profiling to CCOs

Metabolomic analysis identified 1,040 distinct compounds, including 635 positively and 405 negatively charged metabolites. These compounds were predominantly classified into eicosanoids, fatty acids, amino acids, nucleosides, and phospholipids (Supplementary Figure 4). The results of PCA and OPLS-DA indicated a remarkable differentiation between the control and treatment groups (Supplementary Figure 5), suggesting that *P. quinquefolius* CC duration significantly altered the soil metabolite profile. This alteration, in turn, negatively affected the metabolic pathways and overall functioning of the soil microbial community. Furthermore, the CC2, CC3, and CC4 groups clustered closely together but remained distinct from the CC0 and CC1 groups.

The metabolite profile differences between the treatment and control groups were further analyzed to identify the specific metabolic changes responsible for the observed separation. The PLS-DA score plot showed a clear distinction in both positive and negative metabolites for the CC1 vs. CC0, CC2 vs. CC0, CC3 vs. CC0, and CC4 vs. CC0 groups (Supplementary Figure 6). Using the multivariate OPLS-DA model ($VIP > 1$ and $p < 0.05$), we identified 289 (123 upregulated, 159 downregulated) DMs in CC1 vs. CC0, 364 (140 upregulated, 224 downregulated) in CC2 vs. CC0, 374 (129 upregulated, 245 downregulated) in CC3 vs. CC0, and 386 (143 upregulated, 243 downregulated) in CC4 vs. CC0 (Supplementary Figure 7). These DMs were classified into eight categories: peptides, steroids, carbohydrates, hormones and transmitters, lipids, nucleic acids, organic acids, and vitamins and

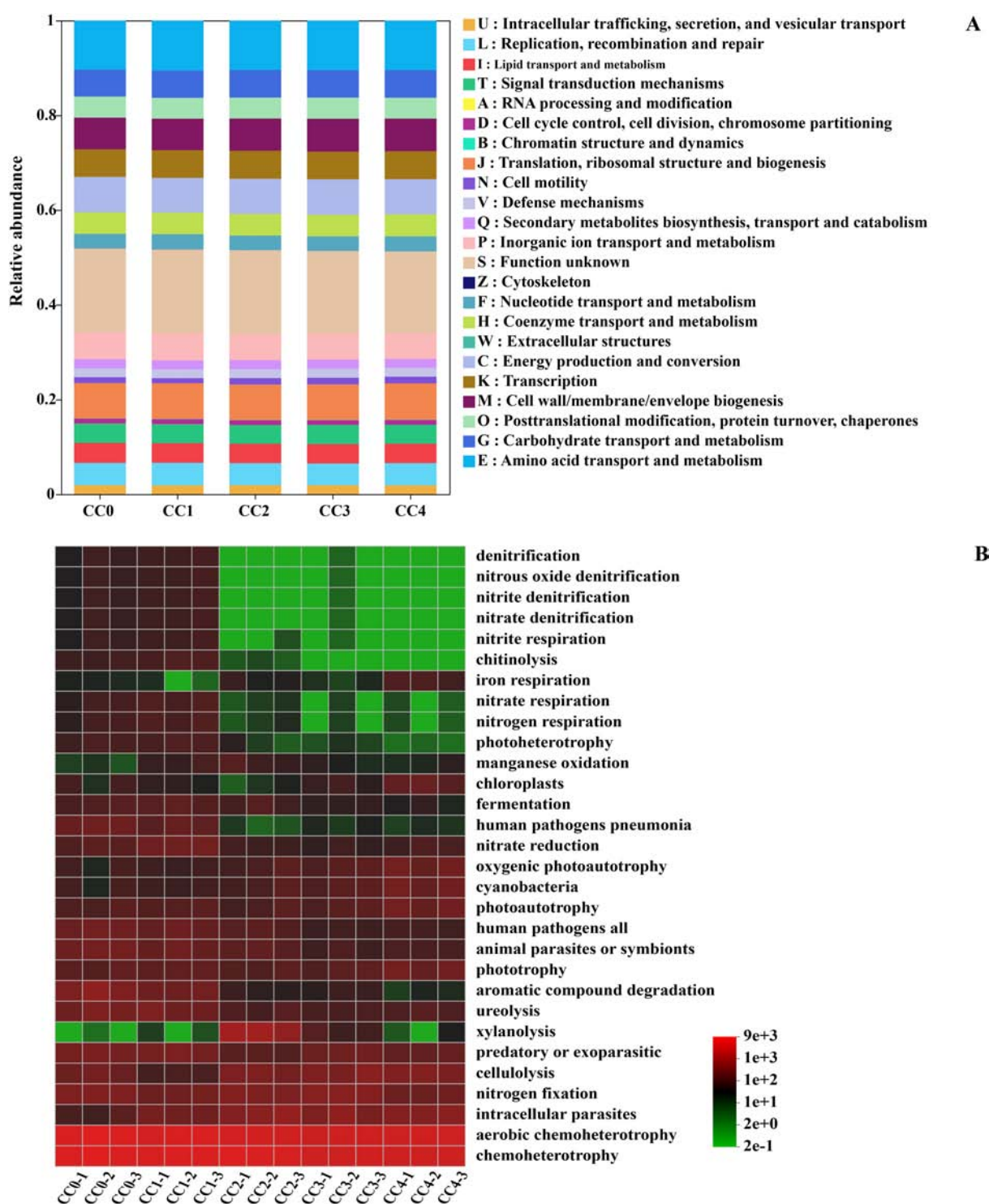


FIGURE 5

Predicted metabolic functions of the bacterial community in *Panax quinquefolius* L. rhizosphere soil under continuous cropping. (A) Functional prediction based on PICRUSt2 analysis, showing enriched metabolic pathways. (B) Functional prediction based on FAPROTAX analysis, illustrating specific biogeochemical functions. Both analyses compare the bacterial communities across different continuous cropping treatments.

cofactors (Supplementary Figure 8). A Venn diagram comparing metabolic sets (Supplementary Figure 9) identified 80 unique DMs in CC1 vs. CC0, 33 in CC2 vs. CC0, 27 in CC3 vs. CC0, 34 in CC4 vs. CC0, and 137 common DMs across all the groups. The top 50 DMs in each group are illustrated in cluster heatmaps (Figure 6).

The confirmed DMs were mapped to the KEGG database to identify their corresponding metabolic pathways. Twenty metabolic pathways with p -values of < 0.05 in each group are shown in Figure 7. The calculated differential abundance score (DA score) was significantly downregulated in most pathways

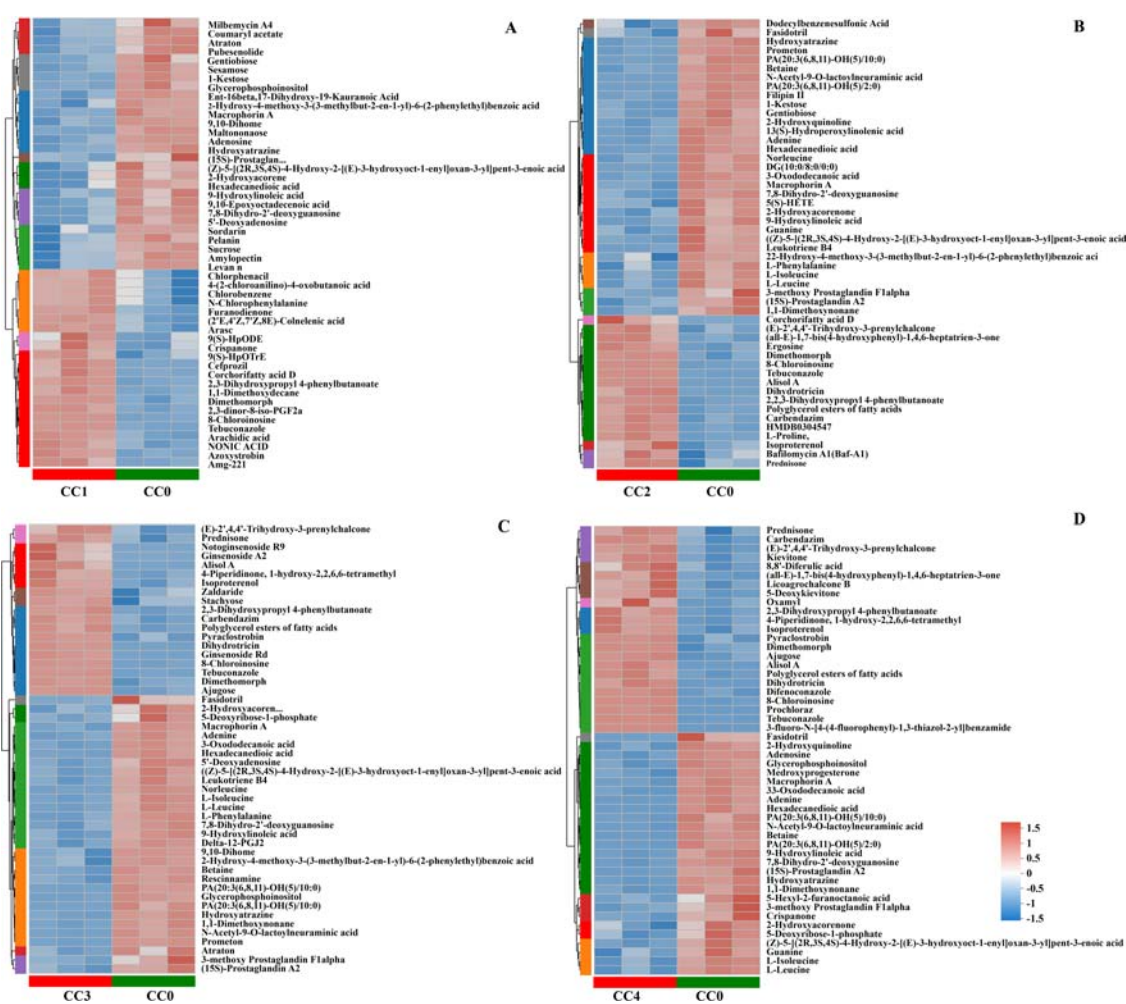


FIGURE 6

Cluster heatmaps of differentially abundant metabolites (DMs) in *Panax quinquefolius* L. rhizosphere soil. Heatmaps illustrate the relative abundance of DMs when comparing (A) 1 year continuous cropping (CC1) vs. control (CC0), (B) 2 years continuous cropping (CC2) vs. control (CC0), (C) 3 years continuous cropping (CC3) vs. control (CC0), and (D) 4 years continuous cropping (CC4) vs. control (CC0).

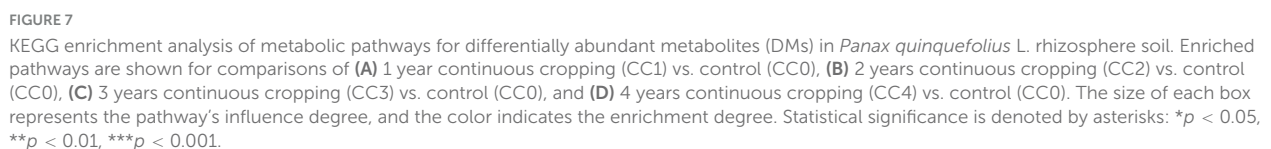
across the four groups (DA score < 0). In the CC1 vs. CC0 groups, DMs were enriched in phenylpropanoid biosynthesis, pyrimidine metabolism, atrazine degradation, and arachidonic acid metabolism (Figure 7A). For CC2 vs. CC0 groups, pyrimidine, arachidonic acid, alpha-linolenic acid, and nucleotide metabolisms were enriched (Figure 7B). The CC3 vs. CC0 group showed enrichment in pyrimidine metabolism; phenylpropanoid biosynthesis; various plant secondary metabolite biosynthesis; stilbenoid, diarylheptanoid, and gingerol biosynthesis; and phenylalanine metabolism (Figure 7C). In the CC4 vs. CC0 group, the primary metabolic pathways were nucleotide, stilbenoid, diarylheptanoid and gingerol, phenylpropanoid, and pyrimidine metabolism (Figure 7D).

A time series analysis using maSigPro was conducted to identify metabolite changes across the five periods of *P. quinquefolius* CC. The heatmap (Figure 8) revealed significant stereoselectivity of metabolites among the different cropping years. Ten significant gene clusters were also identified. The metabolites in Clusters 2, 3, 5, 8, and 10 increased with prolonged cropping, whereas those in Clusters 1, 4, 6, 7, and 9 decreased. According

to the HMDB classification, upregulated metabolites included organoheterocyclic compounds, benzenoids, and organic acids and derivatives in Cluster 2; phenylpropanoids, polyketides, lipids, and lipid-like molecules in Cluster 3; benzenoids, nucleosides, nucleotides, analogs, and organic acids and derivatives in Cluster 4; lipids, organic acids, and organoheterocyclic compounds in Cluster 8; and phenylpropanoids and polyketides in Cluster 10. Downregulated metabolites were primarily lipids, organic acids, and organoheterocyclic compounds in Cluster 1; lipids and organic acids in Cluster 4; organic acids and organoheterocyclic compounds in Cluster 6; and lipids and organoheterocyclic compounds in Clusters 7 and 9.

3.6 Association between the microbes and metabolites in the rhizosphere soil of *P. quinquefolius*

Procrustes analysis was conducted to identify the potential relationships between soil bacteria and metabolites



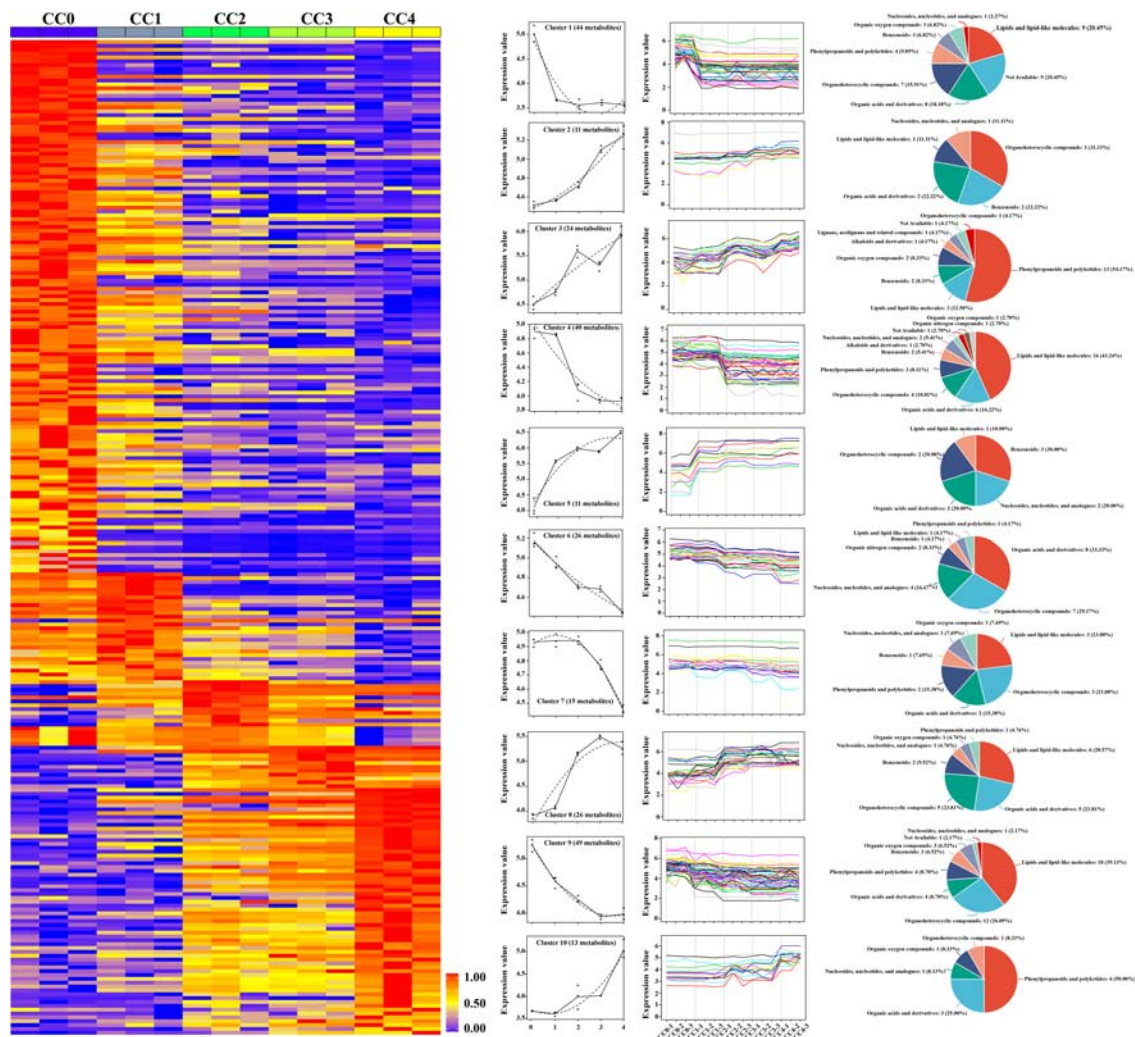


FIGURE 8

Time-series expression patterns of differential metabolites in *Panax quinquefolius* L. rhizosphere soil across continuous cropping years. This heatmap, generated by maSigPro chronological analysis, illustrates the dynamic changes in metabolite abundance over 0, 1, 2, 3, and 4 years of continuous cropping. Metabolites are grouped into ten distinct clusters based on their expression patterns. The accompanying pie charts show the classification of identified metabolites within each cluster according to the Human Metabolome Database (HMDB).

(Supplementary Figure 10). The analysis revealed no significant relationship between the CC1 and CC0 groups (Supplementary Figure 10A, $M2 = 0.713$, $p > 0.05$); however, significant correlations were found between CC2 and CC0 (Supplementary Figure 10B, $M2 = 0.393$, $p < 0.05$), CC3 vs. CC0 (Supplementary Figure 10C, $M2 = 0.248$, $p < 0.05$), and CC4 vs. CC0 groups (Supplementary Figure 10D, $M2 = 0.291$, $p < 0.05$). Pearson's correlation analysis further examined the relationships within the paired microbiome-metabolome datasets (Figure 9). In the CC1 vs. CC0 group, Bacteroidota was negatively correlated with pubesinoid, sordarin, amylopectin, levan n, and sucrose. Firmicutes showed a significant positive correlation with dimethomorph, amg-221, and 8-chlorinosine but a negative correlation with ent-16beta,17-dihydroxy-19-kauranoic acid, gentiobiose, 9,10-dihome, sesamose, 1-ketose, adenosine, hydroxyatrazine, pubesinoid, amylopectin, and levan n. In the CC2 vs. CC0 group, Actinobacteriota, Bacteroidota, Myxococcota, and Patescibacteria were highly correlated with most metabolites (except corchorifatty acid

D). In the CC3 vs. CC0 group, Myxococcota was significantly negatively correlated with 9,10-dihome, betaine, hydroxyatrazine, 1,1-dimethoxynonane, 3-methoxy prostaglandin F1 α , L-isoleucine, 7,8-dihydro-2'-deoxyguanosine, macrophorin A, 3-oxododecanoic acid, and hexadecanedioic acid but positively correlated with other metabolites. Acidobacteriota and Patescibacteria showed opposite correlations compared to Myxococcota. In the CC4 vs. CC0 group, all the selected metabolites were highly correlated with Gemmatimonadota, Myxococcota, Patescibacteria, and WPS-2.

3.7 Correlations between soil metabolites, microbes, and environmental factors

Mantel's test demonstrated the relationships between the bacterial community, diversity, metabolite profiles, soil physiochemical properties, enzyme activities, and microbial

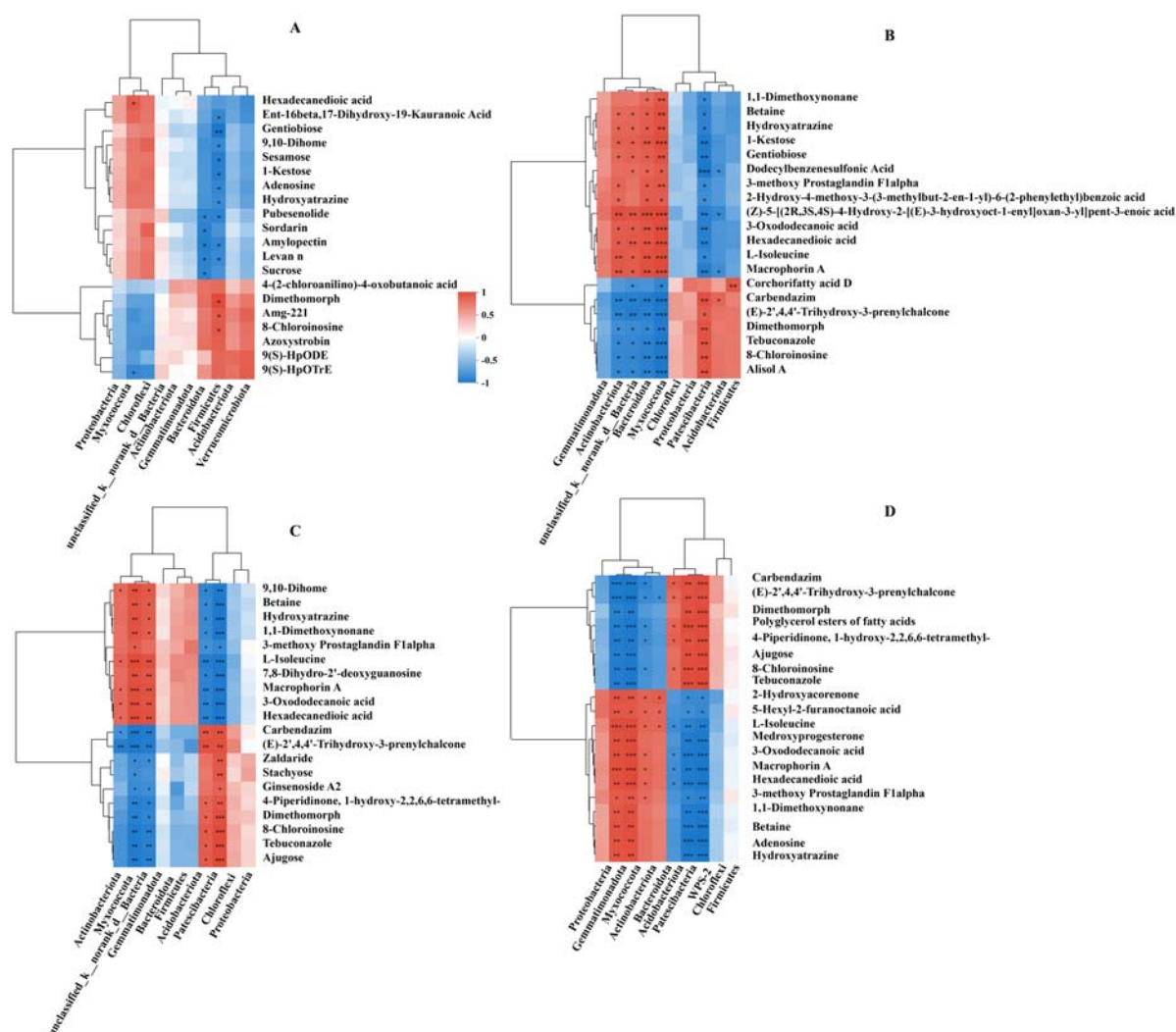


FIGURE 9

Pearson correlation analysis between dominant soil bacterial phyla and altered soil metabolites in *Panax quinquefolius* L. rhizosphere soil.

Correlation matrices are presented for comparisons of (A) 1 year continuous cropping (CC1) vs. control (CC0), (B) 2 years continuous cropping (CC2) vs. control (CC0), (C) 3 years continuous cropping (CC3) vs. control (CC0), and (D) 4 years continuous cropping (CC4) vs. control (CC0). Color intensity indicates the strength and direction of correlation (e.g., red for positive, blue for negative). Statistical significance is denoted by asterisks:

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

biomass (Supplementary Figure 11). Soil environmental factors were significantly correlated with the bacterial community composition, diversity, and metabolite profiles, with UREA, ALP, and MBN notably influencing the bacterial community. Metabolites were strongly associated with UREA, ALP, pH, and AK but showed a weaker correlation with PRO and TN. Bacterial diversity was significantly related to all environmental factors except for AP. The analysis of PLS-SEM revealed the effect of *P. quinquefolius* CC on the rhizosphere microbial community (Figure 10). The goodness-of-fit index of the model was 0.82, suggesting a well-constructed influence pathway. *P. quinquefolius* CC negatively affected soil physicochemical properties (path coefficient = -0.80, $p < 0.001$) and soil enzymatic activities (path coefficient = 0.46, $p < 0.001$). Changes in soil physicochemical properties did not directly affect microbial community composition, metabolites, or microbial biomass, whereas soil enzymatic activity positively influenced microbial

community composition and negatively affected metabolites and microbial biomass. Additionally, microbial community composition positively affected microbial biomass and metabolites, whereas changes in metabolites did not directly influence microbial biomass (path coefficient = 0.11, $p > 0.05$).

4 Discussion

4.1 Detrimental effects of CCOs on soil physicochemical properties and enzyme activities

Continuous cultivation of the same crop significantly alters soil physicochemical properties, including pH and nutrient levels, thereby affecting subsequent crop health and growth

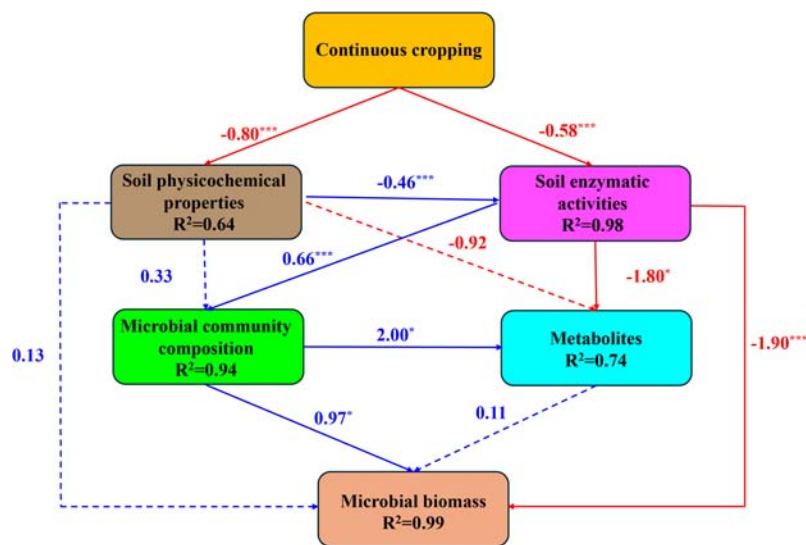


FIGURE 10

Partial least squares path modeling (PLS-SEM) illustrating the effects of continuous *Panax quinquefolius* L. cropping on soil physicochemical properties, enzymatic activities, bacterial communities, metabolite profiles, and microbial biomass. Red lines indicate a positive correlation (promoting effect), while blue lines indicate a negative correlation (inhibition effect). The numbers adjacent to the lines represent the standardized path coefficients. Statistical significance is denoted by asterisks: * $p < 0.05$, *** $p < 0.001$.

(Zhao et al., 2024). Studies have revealed a notable decrease in pH in soils continuously cultivated with *Camellia sinensis* (Arafat et al., 2020), *Nicotiana tabacum* (She et al., 2017), and *Cucumis melo* (Li J. et al., 2023). Tan et al. (2021) demonstrated that *Nicotiana tabacum* CC for 10 years depleted soil nutrients such as SOM, AP, TN, and NO_3^- -N. Similarly, *Zea mays* CCOs reduced trace elements such as manganese and iron in *Zea mays* systems (Jiao et al., 2019). Consistent with these studies, our study also revealed significant decreases in soil pH, SOM, CEC, TN, and NH_4^+ -N contents with prolonged *P. quinquefolius* cultivation. Soil acidification in CC systems results from excessive chemical N fertilizers, accumulation of root exudates, such as propionic, palmitic, and palmitoleic acids, and bio-decomposition of plant residues (Wang F. et al., 2022). Furthermore, CC depletes soil nutrients through plant uptake and inhibits C and N mineralization (Gentry et al., 2013), impairing nutrient transformation and cycling. This reduces the nutrient chelation capacity of the soil, consequently increasing nutrient loss via leaching and runoff (Hussain et al., 2019). However, AP and AK levels were higher in CCOs treatments than those in the control. In agreement with this, Gu et al. (2020) showed an increase in total phosphorus, total potassium, AP, and AK with continuous Chinese chive cropping years, likely due to the high application of chemical P and K fertilizers, reduced soil CEC, and inhibited plant cation absorption capacity.

Soil enzymes are vital for nutrient cycling and energy transformation and serve as bioindicators for biochemical processes (Gong et al., 2019; Wyszowska et al., 2019). CCOs have been shown to reduce soil enzyme activity, which indicates a decline in soil quality (Gong et al., 2019; Qin et al., 2017). This study observed a decrease in the levels of UREA, CAT, PPO, INV, PRO, and ALP with increasing years of *P. quinquefolius* CCOs, suggesting significant inhibition of soil microbial metabolic activity by CCOs. Soil CAT, crucial for microbial metabolism and detoxification of reactive oxygen species, showed reduced

activity in continuously cropped soil, indicating restrained aerobic organism activity and inhibited scavenging of toxic substances (Cai et al., 2019). Soil UREA and PRO are key enzymes in the soil N cycle that catalyze urea decomposition and protein conversion into oligopeptides and amino acids (Greenfield et al., 2021). Soil ALP influences the catabolic transformation and bioavailability of organic P (Vives-Peris et al., 2020), whereas INV hydrolyzes sucrose to glucose and fructose, providing essential energy for microbial metabolism (Cheng et al., 2021). Soil PPO is involved in recalcitrant SOC decomposition (Sinsabaugh et al., 2005), and both enzymes participate in the soil C cycle (Abad-Valle et al., 2016). The reduction in UREA, PRO, ALP, INV, and PPO activity indicates that CCOs restrained the catabolic transformation and biological efficiency of N, P, and C metabolism in soil microecosystems, leading to reduced soil fertility for plant growth. Furthermore, CC-induced challenges suppress the expression of functional genes associated with key enzyme activities (Zhao et al., 2021), reduce the number of enzyme-producing microorganisms (Li J. et al., 2023), and alter microbial diversity and abundance (Yang et al., 2018), thereby resulting in low enzyme activity in long-term CC soil systems. We observed a strong positive correlation between soil enzyme activity and pH during *P. quinquefolius* CC. These results indicate that the decrease in soil pH due to CCOs may further contribute to the reduction in soil enzyme activity in continuously cropped soils (Liu et al., 2021).

4.2 Disruption of soil microbial community structure caused by CCOs

Soil microorganisms are essential for biogeochemical cycling, organic matter turnover, nutrient availability, and the promotion of plant growth (Mohanram and Kumar, 2019). The abundance, diversity, metabolic activity, and community structure

of rhizosphere microorganisms indicate the soil health (Dong et al., 2016). Studies have shown that long-term CC negatively affects soil microbial populations, diversity, and richness compared with short-term CC practices (Li J. et al., 2023). Under CC, rhizospheric microbial populations showed a decreased diversity of beneficial bacteria and fungi and an increased diversity of pathogenic bacteria (Zhang H. et al., 2022). Gao et al. (2021) found a significant decrease in the total bacterial population in sweet potato fields with longer monoculture duration. Our study showed that *P. quinquefolius* CC significantly reduced bacterial and fungal numbers while increasing those of Actinomycetes, potentially affecting ecosystem function stability. Previous studies have highlighted the importance of soil microbial diversity in determining soil quality (Lehmann et al., 2020). An elevated Shannon index indicates increased microbial diversity, whereas a high Simpson index indicates reduced diversity (Zheng et al., 2018). Higher Chao1 and Ace indices indicate increased bacterial richness (Guo et al., 2021). In this study, the Ace, Chao, and Simpson indices significantly decreased, whereas the Shannon index showed an upward trend with increasing CC duration. These results indicated that *P. quinquefolius* CC reduced bacterial richness but increased bacterial diversity in the rhizosphere soil. Taxonomic classification of 16S rRNA gene sequences identified Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, and Firmicutes as the dominant bacterial phyla in these soils. Proteobacteria, the most abundant phylum in soil (Militon et al., 2010), is essential for the global cycling of C, N, and S (Bezza and Chirwa, 2017). This phylum also degrades organic pollutants, suppresses plant diseases, and promotes plant growth (Feng et al., 2015). As Proteobacteria consists of nutrient-rich, fast-proliferating bacteria in soils high in organic C and N, their reduced presence in CC soils, due to decreased SOM and TN, hampers nutrient and energy cycling, weakening plant resilience to soil-borne diseases. Actinobacteria, which are associated with disease suppression, are more abundant in disease-suppressive soils than in disease-conducive soils (Mendes et al., 2011). The reduced abundance of Actinobacteria confirmed the presence of pathogenic microorganisms in CC samples. Acidobacteriota, an anaerobic phototrophic group, thrives in nutrient-poor environments (Ward et al., 2009) and is highly sensitive to nitrogen availability (Fierer et al., 2011). Their growth is inhibited in nitrogen-rich legume soils (Che et al., 2022). The inverse relationship between TN and NH_4^+ -N contents and Acidobacteriota abundance corroborates this conclusion and may explain the increased Acidobacteriota abundance under nutrient-deficient conditions in *P. quinquefolius* CC. Chloroflexi, a phylum of oligotrophic bacteria with high stress tolerance, is prevalent in harsh nutrient-poor arid soils (Huang et al., 2019). An increase in Chloroflexi abundance typically signifies reduced soil quality, suggesting a decline in soil nutrients in *P. quinquefolius* CC. This finding aligns with that in previous studies on tomatoes (Jiang et al., 2024), *P. notoginseng* (Wang F. et al., 2022), and cut chrysanthemum (Li J. et al., 2023) under long-term monoculture, where CCOs increased the relative abundance of Chloroflexi. Similar to Proteobacteria, Firmicutes significantly suppresses soil-borne diseases, such as wilt (Lee et al., 2020). In this study, the relative abundance of Firmicutes increased during the first 2 years of *P. quinquefolius* CC and then declined in the third and fourth years, highlighting their role in pathogen antagonism during the initial cropping period. LEfSe

analysis identified significant bacterial taxa linked to soil bacterial community changes over different years in *P. quinquefolius* CC, with distinct taxa in each group. The key microbial biomarkers shifted from Gemmatimonadota, Actinobacteriota, and Proteobacteria, thriving in favorable soil conditions, to Acidobacteriota, Chloroflexi, and WPS-2, which endure extreme environments, indicating soil quality deterioration over successive cropping years. Our study demonstrated that *P. quinquefolius* CC decreased the relative abundance of beneficial bacterial taxa such as Gaiellales, Vicinamibacteria, Sphingobacteriales, and Sphingomonas while increasing the abundance of pathogenic bacteria such as Acidobacteriales and Xanthomonadaceae.

Soil microorganism composition and dynamics are influenced by changes in soil environmental factors (Tan et al., 2021). Studies have demonstrated that bacterial populations thrive in high pH environments, whereas bacterial diversity decreases significantly under acidic conditions (Kim et al., 2020). Degruene et al. (2015) indicated that a soil pH variation of < 0.5 units can affect bacterial growth. Ma et al. (2022) also reported that continuous *Lilium brownii* cropping reduced rhizosphere microbial diversity and soil pH value. Furthermore, SOM and nutrients such as AP, TN, and NH_4^+ -N significantly affect bacterial community variation in continuous *Nicotiana tabacum* cropping soils (Tan et al., 2021). In this study, RDA results showed that soil bacterial community structure was mainly influenced by pH, AK, and ALP activity, suggesting that soil acidification, K levels, and organic P transformation are crucial factors limiting microbial diversity and composition in *P. quinquefolius* CC soils. Additionally, pH was positively correlated with the abundance of Gemmatimonadetes and Actinobacteria and negatively correlated with that of Acidobacteriota, consistent with the findings of previous studies (Conradie and Jacobs, 2021; Ma et al., 2023).

Identifying symbiotic patterns in microbial communities is vital for understanding the potential relationships between microorganisms in terrestrial ecosystems (Banerjee et al., 2019). Co-occurrence network analysis is widely used to examine microbial community stability under external disturbances such as soil contamination (Song et al., 2024), chemical fertilizer application (Zhang H. et al., 2022), and CCOs (Zhang H. et al., 2022). In this study, co-occurrence network analysis revealed that CC treatments significantly reduced edges, average weighted degree, and network density while increasing the modularity index. These findings indicate that compared to non-CC, CCOs reduce network complexity and stability, resulting in a fragmented bacterial community (Li J. et al., 2023). Because complex soil microbial communities generally exhibit greater resistance and resilience to environmental changes than less diverse ones (Allison and Martiny, 2008), continuous *P. quinquefolius* cultivation makes the soil bacterial community more sensitive and difficult to restore when facing soil environmental changes. Additionally, with prolonged CC, the proportion of positive correlations among bacterial interactions in the rhizosphere soil network decreased from 54.5 to 50.2%, indicating increased competitive interactions among bacterial assemblages within the CC system.

Prediction of the effects of *P. quinquefolius* CC on microbial biogeochemical functions revealed that amino acid transport and metabolism exhibited the highest relative abundances across all treatments and also demonstrated an increasing trend with increased CC duration. Similar findings have been observed in

continuous *Sesamum indicum* cropping (Lyu et al., 2023) and *P. notoginseng* (Wang F. et al., 2022), where CCOs led to an increase in amino acid transport and metabolism. Amino acids are crucial metabolites driving microbial metabolism and biosynthesis (Lin et al., 2017). Under adverse conditions, microbial communities can sustain high cell growth by reprogramming essential amino acid metabolism (Yang et al., 2021). The upregulation of amino acid transport and metabolism highlights the role of amino acids in the regulation of microbial metabolic activity, offering insights into enhancing microbial resistance to CCOs in consecutive *P. notoginseng* monocultures. The analyze of FAPROTAX effectively predicts microbial community functions in N, S, P, and C biotransformations under stress (Sansupa et al., 2021). In our study, the FAPROTAX functional prediction indicated that functional genes related to N cycling, including denitrification, nitrate, and nitrogen respiration, were significantly affected by the CC treatment. Soil N cycling disturbances were mainly due to changes in bacterial taxa, such as the order Burkholderiales, family Nitrosomonadaceae, and class Acidimicrobiia, which are associated with N cycling and beneficial plant growth (Garrido-Oter et al., 2018). The decline in the relative abundance of soil bacteria due to *P. quinquefolius* CC suppressed nitrogen fixation and conversion, which is insufficient for plant growth. The phylum Nitrospirae and class Nitrospiria are crucial for the ammonia- and nitrite-oxidizing processes. The reduction of Nitrospirae and Nitrospiria indicates dissimilatory NO_3^- reduction to NH_4^+ and inhibited denitrification (Jakus et al., 2021). These findings may explain the increased NO_3^- -N content and decreased NH_4^+ -N levels in the *P. quinquefolius* CC treatments.

4.3 Metabolomic changes induced by CCOs

Soil metabolites originate from plant root exudates, microbial metabolites, and organic matter decomposition (Cheng et al., 2018), and changes in their composition and content reflect microbial responses to environmental stress (Wu et al., 2022). In the present study, metabolomics analysis identified 289 DMs in the CC1 vs. CC0 group, 364 DMs in the CC2 vs. CC0 group, 374 DMs in the CC3 vs. CC0 group, and 386 DMs in the CC4 vs. CC0 group. The identified metabolites were categorized as carbohydrates, nucleic acids, steroids, hormones and transmitters, peptides, vitamins, and cofactors, indicating responses to CCOs. Mapping of these DMs into the KEGG database revealed significant enrichment in phenylpropanoid biosynthesis (map00940) and pyrimidine metabolism (map00240) pathways across groups, consistent with previous studies (Shen et al., 2024). The phenylpropanoid pathway is crucial for the biosynthesis of compounds that protect cells from oxidative damage under abiotic stress, such as phenols, flavonoids, lignin, coumarin, hydroxycinnamic acid, cinnamic acid, ferulic acid, coumaroylshikimic acid, and caffeoylshikimic acid (Neelam et al., 2020; Sharma et al., 2019). The pyrimidine metabolism pathway is vital for microbial energy metabolism, such as the electron transport chain and TCA cycle (Xue et al., 2023), which not only participates in cell energy supply, DNA synthesis, and cell division and proliferation (Hu et al., 2022) but also plays a crucial role in

improving cellular adaptation mechanisms in response to external stresses (Li J. et al., 2023). The downregulation of phenylpropanoid biosynthesis and pyrimidine metabolism in *P. quinquefolius* CC treatments suggests that the stress resistance of microbes and plants to CCOs was weakened due to the inhibited biosynthesis of phenolic compounds and shortened energy supply. Furthermore, the significant downregulation of various pathways, including starch and sucrose metabolism, tyrosine metabolism, isoflavonoid biosynthesis, nucleotide metabolism, and biosynthesis of various plant secondary metabolites, indicated that *P. quinquefolius* CC extensively affected primary and secondary metabolism in plant defense against pathogens (Lyu et al., 2023; Shen et al., 2024).

Time-series analysis revealed the temporal dynamics of soil metabolites in different CC treatments, demonstrating distinct variations among the five CC treatments. Of the 259 identified metabolites, 174 were downregulated and 85 were upregulated with increased CC duration. The downregulated metabolites were primarily lipids and lipid-like molecules, whereas the upregulated metabolites comprised organic acids and derivatives, phenylpropanoids, and polyketides. Lipids and lipid-like molecules, which are vital for cellular biofilms, membrane transport (Bramkamp and Lopez, 2015), and cellular signaling (Sunshine and Iruela-Arispe, 2017), are crucial for microbial resistance to environmental stress by maintaining membrane fluidity and homeostasis (Stankeviciute et al., 2019). The reduction in these metabolites suggests that CCOs impair the stress resistance of soil microorganisms. Accumulation of organic acids fosters pathogenic fungi and inhibits plant growth-promoting rhizobacteria (Wu et al., 2017), supported by the negative correlation between organic acids (e.g., betaine, 3-oxododecanoic acid, and L-isoleucine) and the abundance of Patescibacteria, WPS-2, and Acidobacteriota phyla. Furthermore, organic acid accumulation may lower soil pH by releasing H^+ ions (Zhao et al., 2024). Phenylpropanoids and polyketides are key plant metabolites that detoxify harmful substances and enhance abiotic stress tolerance (Frémont et al., 2022). The increased levels of phenylpropanoids and polyketides (e.g., glicophenone, xanthomicrol, and demethoxycurcumin) suggest a photoprotective mechanism against CC-induced challenges in *P. quinquefolius*.

4.4 Microbe-metabolite interactions and the contribution to CCOs

As key drivers of soil metabolic activity, soil microorganisms influence metabolite stereoselectivity (Song et al., 2020). Exploring the relationship between differential metabolites and rhizosphere microbes is therefore crucial. Co-occurrence patterns between bacterial communities and metabolic profiles were examined in soils with varying CC durations. Procrustes analysis revealed an M2 value of 0.713 ($p = 0.291$) for CC1 vs. CC0 groups, indicating no significant correlation between bacterial communities and metabolic profiles during the first year of *P. quinquefolius* growth. As continuous cultivation increased, M2 decreased to 0.393 ($p = 0.045$) in the CC2 vs. CC0 group, 0.248 ($p = 0.021$) in the CC3 vs. CC0 group, and 0.291 ($p = 0.038$) in the CC4 vs. CC0 group, confirming a significant correlation between metabolism and bacterial communities after 2–4 years of cultivation. This

correlation was strengthened with prolonged *P. quinquefolius* cropping. This finding aligns with those of previous studies on continuous soybean cropping (Cui et al., 2024), long-term *Camellia oleifera* cultivation (Lu et al., 2022), and heatmap correlation analysis. The positive association between certain metabolites may be attributed to their presence in microbial secretions, whereas the negative correlation between microbial taxa and metabolites can be explained by the specific metabolic consumption of these metabolites by microbes (Li H. et al., 2019).

The composition and dynamics of soil microorganisms and metabolites are significantly influenced by changes in soil environmental factors; however, their potential relationships remain unclear. The analysis of PLS-SEM was used to model the direct and indirect relationships between CCOs, soil physicochemical properties, enzymatic activities, microbial community composition, metabolites, and microbial biomass. The structural equation model showed that *P. quinquefolius* CC significantly and directly affected the soil physicochemical properties and enzymatic activities. These enzymatic activities significantly and positively influenced the microbial community and negatively affected soil metabolite profiles. Conversely, soil physicochemical properties showed no obvious relationship with the metabolite profiles of microbial community composition. These findings suggest that soil enzymatic activities, in conjunction with soil physicochemical properties, play a crucial role in regulating soil microorganisms, metabolite composition, and dynamics. Similarly, Zhao et al. (2017) concluded that the degradation of soil enzyme activity due to continuous cropping is a primary cause of *P. notoginseng* replanting failure. Microbial biomass was positively influenced by the microbial community and negatively influenced by soil enzymatic activities, suggesting that *P. quinquefolius* CC significantly altered the soil bacterial community composition, primarily because of enzymatic activity constraints, leading to disturbances in soil microbial biomass accumulation.

4.5 Limitations and considerations

It is important to note that the comparison between the *P. quinquefolius* monoculture soils (CC1–CC4) and the maize-wheat rotation soil (CC0) introduces a potential confounding variable: crop species. The observed differences in soil properties, microbial communities, and metabolite profiles between CC0 and the CC treatments may not be solely attributable to the CC practice. Instead, these differences could be partly due to the fundamental differences between the rhizosphere environments created by *Zea mays*/*Triticum aestivum* and *P. quinquefolius*. *Zea mays* and *Triticum aestivum*, as cereal crops, have different root exudates, nutrient demands, and associated microbial communities compared to *P. quinquefolius*, a medicinal plant. Therefore, the observed changes in soil characteristics and microbial dynamics could be influenced by both the CC practice and the specific crop species involved. To more accurately isolate the effects of CCOs on soil health and microbial ecology, future studies should consider using a more comparable control, such as a rotation system involving *P. quinquefolius* with other crops (e.g., legumes or other medicinal plants known to alleviate CCOs) (Cui et al., 2024; Zeeshan Ul Haq et al., 2023), or a fallow control within

the same field type. Additionally, while our study provides a comprehensive snapshot of the rhizosphere at specific time points, the dynamic nature of soil microbial communities and metabolite profiles suggests that more frequent, longitudinal sampling could offer deeper insights into the temporal progression of CCOs (Dong et al., 2016). Future research could also benefit from integrating metagenomic or metatranscriptomic approaches to provide a more detailed functional understanding of the microbial community's response to continuous cropping stress (Pang et al., 2021; Wang et al., 2025).

5 Conclusion

This study elucidates the microecological mechanisms underlying CCOs in *P. quinquefolius*, demonstrating a consequential decline in soil health marked by deteriorated physicochemical properties, dysbiosis of the microbial community, and pronounced alterations in metabolite profiles. Specifically, CCOs resulted in reduced soil pH, CEC, SOM, TN, and enzymatic activities, while concurrently promoting the proliferation of pathogenic taxa and disrupting beneficial microbial interactions. The inhibitory effects of CCOs were particularly evident in key microbial functions, such as impairing amino acid metabolism and nitrogen cycling processes, thus providing new mechanistic insights into strategies for enhancing microbial resilience. Metabolomic profiling further revealed significant disruptions in phenylpropanoid biosynthesis and pyrimidine metabolism, reflecting shifted microbial metabolic activities under continuous cropping stress. By integrating non-targeted metabolomics with high-throughput sequencing, this work offers a comprehensive perspective on soil ecosystem dysfunction and deliver novel insights for developing sustainable management practices aimed at mitigating CCOs and preserving soil health. Future research should prioritize long-term strategies to improve soil fertility and suppress soil-borne diseases in *P. quinquefolius* cropping systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JS: Writing – original draft. QH: Software, Writing – original draft, Investigation. KC: Data curation, Methodology, Writing – original draft. XS: Conceptualization, Writing – review & editing. QZ: Conceptualization, Funding acquisition, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research and/or publication of this article. This work was supported

by the Open Project of the State Key Laboratory of Crop Biology (2022KF06), and the Open Fund Project of the Ministry of Education Key Laboratory for Conservation and Utilization of Forest Resources in Southwest Mountains (KLESWFU-202001).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to

ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2025.1698779/full#supplementary-material>

References

- Abad-Valle, P., Álvarez-Ayuso, E., Murciego, A., and Pellitero, E. (2016). Assessment of the use of sepiolite amendment to restore heavy metal polluted mine soil. *Geoderma* 280, 57–66. doi: 10.1016/j.geoderma.2016.06.015
- Allison, S., and Martiny, J. (2008). Colloquium paper: Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U S A* 105, 11512–11519. doi: 10.1073/pnas.0801925105
- Arafat, Y., Ud Din, I., Tayyab, M., Jiang, Y., Chen, T., Cai, Z., et al. (2020). Soil sickness in aged tea plantation is associated with a shift in microbial communities as a result of plant polyphenol accumulation in the tea gardens. *Front. Plant Sci.* 11:601. doi: 10.3389/fpls.2020.00601
- Ashworth, A. J., Owens, P. R., and Allen, F. L. (2020). Long-term cropping systems management influences soil strength and nutrient cycling. *Geoderma* 361:114062. doi: 10.1016/j.geoderma.2019.114062
- Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A., Gatteringer, A., et al. (2019). Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *ISME J.* 13, 1722–1736. doi: 10.1038/s41396-019-0383-2
- Bates, S. T., Berg-Lyons, D., Caporaso, J. G., Walters, W. A., Knight, R., and Fierer, N. (2011). Examining the global distribution of fungal communities using high-throughput sequencing. *Environ. Microbiol.* 13, 2839–2845. doi: 10.1038/ismej.2010.171
- Beretta-Blanco, A., Pérez, O., and Carrasco-Letelier, L. (2019). Soil quality decrease over 13 years of agricultural production. *Nutr. Cycl. Agroecosyst.* 114, 45–55. doi: 10.1007/s10705-019-09990-3
- Bezza, F. A., and Chirwa, E. M. N. (2017). The role of lipopeptide biosurfactant on microbial remediation of aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil. *Chem. Eng. J.* 309, 563–576. doi: 10.1016/j.cej.2016.10.055
- Bramkamp, M., and Lopez, D. (2015). Exploring the existence of lipid rafts in bacteria. *Microbiol. Mol. Biol. Rev.* 79, 81–100. doi: 10.1128/MMBR.00036-14
- Brown, R. W., Chadwick, D. R., Zang, H., and Jones, D. L. (2021). Use of metabolomics to quantify changes in soil microbial function in response to fertiliser nitrogen supply and extreme drought. *Soil Biol. Biochem.* 160:108351. doi: 10.1016/j.soilbio.2021.108351
- Cai, M., Hu, C., Wang, X., Zhao, Y., Jia, W., Sun, X., et al. (2019). Selenium induces changes of rhizosphere bacterial characteristics and enzyme activities affecting chromium/selenium uptake by pak choi (*Brassica campestris* L. ssp. *Chinensis* Makino) in chromium contaminated soil. *Environ. Pollut.* 249, 716–727. doi: 10.1016/j.envpol.2019.03.079
- Che, T., Xu, Y., Li, Y., Wei, Z., Zang, X., Zhang, X., et al. (2022). Mixed planting reduces the shaping ability of legume cover crop on soil microbial community structure. *Appl. Soil Ecol.* 178:104581. doi: 10.1016/j.apsoil.2022.104581
- Chen, S., Qi, G., Luo, T., Zhang, H., Jiang, Q., Wang, R., et al. (2018). Continuous-cropping tobacco caused variance of chemical properties and structure of bacterial network in soils. *Land. Degrad. Dev.* 29, 4106–4120. doi: 10.1002/ldr.3167
- Chen, Y., Du, J., Li, Y., Tang, H., Yin, Z., Yang, L., et al. (2022a). Evolutions and managements of soil microbial community structure drove by continuous cropping. *Front. Microbiol.* 13:839494. doi: 10.3389/fmicb.2022.839494
- Chen, Y., Wei, T., Sha, G., Zhu, Q., Liu, Z., Ren, K., et al. (2022b). Soil enzyme activities of typical plant communities after vegetation restoration on the Loess Plateau. *China. Appl. Soil Ecol.* 170:104292. doi: 10.1016/j.apsoil.2021.104292
- Chen, Y., Yang, L., Zhang, L., Li, J., Zheng, Y., Yang, W., et al. (2023). Autotoxins in continuous tobacco cropping soils and their management. *Front. Plant Sci.* 14:1106033. doi: 10.3389/fpls.2023.1106033
- Cheng, C., Gao, M., Zhang, Y., Long, M., Wu, Y., and Li, X. (2021). Effects of disturbance to moss biocrusts on soil nutrients, enzyme activities, and microbial communities in degraded karst landscapes in southwest China. *Soil Biol. Biochem.* 152:108065. doi: 10.1016/j.soilbio.2020.108065
- Cheng, N., Peng, Y., Kong, Y., Li, J., and Sun, C. (2018). Combined effects of biochar addition and nitrogen fertilizer reduction on the rhizosphere metabolomics of maize (*Zea mays* L.) seedlings. *Plant. Soil* 433, 19–35. doi: 10.1007/s11104-018-3811-6
- Conradie, T., and Jacobs, K. (2021). Distribution patterns of Acidobacteriota in different fynbos soils. *PLoS One* 16:e0248913. doi: 10.1371/journal.pone.0248913
- Cui, X., Yuan, J., Yang, X., Wei, C., Bi, Y., Sun, Q., et al. (2024). Biochar application alters soil metabolites and nitrogen cycle-related microorganisms in a soybean continuous cropping system. *Sci. Total Environ.* 917:170522. doi: 10.1016/j.scitotenv.2024.170522
- de Faria, M. R., Costa, L. S. A. S., Chiaramonte, J. B., Bettiol, W., and Mendes, R. (2021). The rhizosphere microbiome: Functions, dynamics, and role in plant protection. *Trop. Plant Pathol.* 46, 13–25. doi: 10.1007/s40858-020-00390-5
- Degrune, F., Dufrêne, M., Colinet, G., Massart, S., Taminiau, B., Bodson, B., et al. (2015). A novel sub-phylum method discriminates better the impact of crop management on soil microbial community. *Agron. Sustain. Dev.* 35, 1157–1166. doi: 10.1007/s13593-015-0291-4
- Ding, S., Zhou, D., Wei, H., Wu, S., and Xie, B. (2021). Alleviating soil degradation caused by watermelon continuous cropping obstacle: Application of urban waste compost. *Chemosphere* 262:128387. doi: 10.1016/j.chemosphere.2020.128387
- Dong, D., Guo, Z., Wu, F., Yang, X., and Li, J. (2024). Plastic residues alter soil microbial community compositions and metabolite profiles under realistic conditions. *Sci. Total Environ.* 906:167352. doi: 10.1016/j.scitotenv.2023.167352
- Dong, L., Xu, J., Feng, G., Li, X., and Chen, S. (2016). Soil bacterial and fungal community dynamics in relation to *Panax notoginseng* death rate in a continuous cropping system. *Sci. Rep.* 6:31802. doi: 10.1038/srep31802

- Duan, M., Yan, R., Wang, Q., Zhou, B., Zhu, H., Liu, G., et al. (2023). Integrated microbiological and metabolomics analyses to understand the mechanism that allows modified biochar to affect the alkalinity of saline soil and winter wheat growth. *Sci. Total Environ.* 866:161330. doi: 10.1016/j.scitotenv.2022.161330
- Feng, C., Huang, L., Yu, H., Yi, X., and Wei, C. (2015). Simultaneous phenol removal, nitrification and denitrification using microbial fuel cell technology. *Water Res.* 76, 160–170. doi: 10.1016/j.watres.2015.03.001
- Feng, H., Wang, Y., Li, N., Qian, Z., Chen, T., Chen, X., et al. (2024). Effects of biochar pyrolysis temperature on uranium immobilization in soil remediation: Revealed by 16S rDNA and metabolomic analyses. *J. Hazard Mater.* 466:133502. doi: 10.1016/j.jhazmat.2024.133502
- Fierer, N., Lauber, C., Ramirez, K., Zaneveld, J., Bradford, M., and Knight, R. (2011). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J.* 6, 1007–1017. doi: 10.1038/ismej.2011.159
- Frémont, A., Sas, E., Sarrazin, M., Gonzalez, E., Brisson, J., Pitre, F., et al. (2022). Phytochelatin and coumarin enrichment in root exudates of arsenic-treated white lupin. *Plant. Cell. Environ.* 45, 936–954. doi: 10.1111/pce.14163
- Gao, Z., Hu, Y., Han, M., Xu, J., Wang, X., Liu, L., et al. (2021). Effects of continuous cropping of sweet potatoes on the bacterial community structure in rhizospheric soil. *BMC Microbiol.* 21:102. doi: 10.1186/s12866-021-02120-6
- Garrido-Oter, R., Nakano, R., Dombrowski, N., Ma, K., McHardy, A., et al. (2018). Modular traits of the rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. *Cell Host Microbe* 24, 155–167.e5. doi: 10.1016/j.chom.2018.06.006
- Gentry, L. F., Ruffo, M. L., and Below, F. E. (2013). Identifying factors controlling the continuous corn yield penalty. *Agron. J.* 105, 295–303. doi: 10.2134/agronj2012.0246
- Gong, X., Feng, Y., Dang, K., Jiang, Y., Qi, H., and Feng, B. (2023). Linkages of microbial community structure and root exudates: Evidence from microbial nitrogen limitation in soils of crop families. *Sci. Total Environ.* 881:163536. doi: 10.1016/j.scitotenv.2023.163536
- Gong, X., Liu, C., Li, J., Luo, Y., Yang, Q., Zhang, W., et al. (2019). Responses of rhizosphere soil properties, enzyme activities and microbial diversity to intercropping patterns on the Loess Plateau of China. *Soil Tillage Res.* 195:104355. doi: 10.1016/j.still.2019.104355
- Gopal, M., Gupta, A., Arunachalam, V., and Magu, S. (2007). Impact of azadirachtin, an insecticidal allelochemical from neem on soil microflora, enzyme and respiratory activities. *Bioresour. Technol.* 98, 3154–3158. doi: 10.1016/j.biortech.2006.10.010
- Greenfield, L. M., Puissant, J., and Jones, D. L. (2021). Synthesis of methods used to assess soil protease activity. *Soil Biol. Biochem.* 158:108277. doi: 10.1016/j.soilbio.2021.108277
- Gu, Y., Wang, Y., Wang, P., Wang, C., Ma, J., Yang, X., et al. (2020). Study on the diversity of fungal and bacterial communities in continuous cropping fields of Chinese chives (*Allium tuberosum*). *Biomed. Res. Int.* 2020:3589758. doi: 10.1155/2020/3589758
- Guo, Q., Xiao, M., Ma, Y., Niu, H., and Zhang, G. (2021). Polyester microfiber and natural organic matter impact microbial communities, carbon-degraded enzymes, and carbon accumulation in a clayey soil. *J. Hazard Mater.* 405:124701. doi: 10.1016/j.jhazmat.2020.124701
- Hu, Z., Yan, W., Yang, C., Huang, X., Hu, X., Li, Y., et al. (2022). Integrative analysis of transcriptome and metabolome provides insights into the underlying mechanism of cold stress response and recovery in two tobacco cultivars. *Environ. Exp. Bot.* 200:104920. doi: 10.1016/j.envexpbot.2022.104920
- Huang, Y., Zhao, Y., Wang, J., Zhang, M., Jia, W., and Qin, X. (2019). LDPE microplastic films alter microbial community composition and enzymatic activities in soil. *Environ. Pollut.* 254:112983. doi: 10.1016/j.envpol.2019.112983
- Hussain, M. Z., Bhardwaj, A. K., Basso, B., Robertson, G. P., and Hamilton, S. K. (2019). Nitrate leaching from continuous corn, perennial grasses, and poplar in the US midwest. *J. Environ. Qual.* 48, 1849–1855. doi: 10.2134/jeq2019.04.0156
- Jackson, K., and Mahmood, T. (1992). Atomic absorption, atomic emission, and flame emission spectrometry. *Anal. Chem.* 66, 252R–279R. doi: 10.1021/ac00084a012
- Jakus, N., Blackwell, N., Straub, D., Kappler, A., and Kleindienst, S. (2021). Presence of Fe(II) and nitrate shapes aquifer-originating communities leading to an autotrophic enrichment dominated by an Fe(II)-oxidizing Gallionellaceae sp. *FEMS Microbiol. Ecol.* 97:fiab145. doi: 10.1093/femsec/fiab145
- Jiang, Y., Wang, J., Liu, L., Chen, L., Yin, X., Tan, W., et al. (2024). Synergistic changes in bacterial community composition, function, and soil characteristics of tomato rhizosphere soil under long-term monoculture conditions. *Rhizosphere* 31:100950. doi: 10.1016/j.rhisph.2024.100950
- Jiao, X., Zhang, X., Lu, X., Qin, R., Bi, Y., and Gao, W. (2019). Effects of maize rotation on the physicochemical properties and microbial communities of American ginseng cultivated soil. *Sci. Rep.* 9:8615. doi: 10.1038/s41598-019-44530-7
- Kandeler, E., and Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol. Fertil. Soils* 6, 68–72. doi: 10.1007/BF00257924
- Kim, S., Waldman, W., Kim, T., and Rillig, M. (2020). Effects of different microplastics on nematodes in the soil environment: Tracking the extractable additives using an ecotoxicological approach. *Environ. Sci. Technol.* 54, 13868–13878. doi: 10.1021/acs.est.0c04641
- Ladd, J. N., and Butler, J. H. A. (1972). Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol. Biochem.* 4, 19–30. doi: 10.1016/0038-0717(72)90038-7
- Lee, S., Kong, H., Song, G., and Ryu, C. (2020). Disruption of Firmicutes and Actinobacteria abundance in tomato rhizosphere causes the incidence of bacterial wilt disease. *ISME J.* 15, 330–347. doi: 10.1038/s41396-020-00785-x
- Lehmann, J., Bossio, D., Kögel-Knabner, I., and Rillig, M. (2020). The concept and future prospects of soil health. *Nat. Rev. Earth Environ.* 1, 544–553. doi: 10.1038/s43017-020-0080-8
- Lei, H., Liu, A., Hou, Q., Zhao, Q., Guo, J., and Wang, Z. (2020). Diversity patterns of soil microbial communities in the *Sophora flavescens* rhizosphere in response to continuous monocropping. *BMC Microbiol.* 20:272. doi: 10.1186/s12866-020-01956-8
- Li, H., Yuan, G., Zhu, C., Zhao, T., Zhang, R., Wang, X., et al. (2019). Soil fumigation with ammonium bicarbonate or metasodium under high temperature alleviates continuous cropping-induced Fusarium wilt in watermelon. *Sci. Hortic.* 246, 979–986. doi: 10.1016/j.scienta.2018.11.090
- Li, J., Cheng, X., Chu, G., Hu, B., and Tao, R. (2023). Continuous cropping of cut chrysanthemum reduces rhizospheric soil bacterial community diversity and co-occurrence network complexity. *Appl. Soil Ecol.* 185:104801. doi: 10.1016/j.apsoil.2022.104801
- Li, M., Wang, J., Zhou, Q., and Yaseen, M. (2023). Effects of continuous melon cropping on rhizospheric fungal communities. *Rhizosphere* 27:100726. doi: 10.1016/j.rhisph.2023.100726
- Li, X., Qu, C., Bian, Y., Gu, C., Jiang, X., and Song, Y. (2019). New insights into the responses of soil microorganisms to polycyclic aromatic hydrocarbon stress by combining enzyme activity and sequencing analysis with metabolomics. *Environ. Pollut.* 255:113312. doi: 10.1016/j.envpol.2019.113312
- Li, Y., Tian, Q., Wang, Z., Li, J., Liu, S., Chang, R., et al. (2023). Integrated analysis of transcriptomics and metabolomics of peach under cold stress. *Front. Plant Sci.* 14:1153902. doi: 10.3389/fpls.2023.1153902
- Liao, J., and Xia, P. (2024). Continuous cropping obstacles of medicinal plants: Focus on the plant-soil-microbe interaction system in the rhizosphere. *Sci. Hortic.* 328:112927. doi: 10.1016/j.scienta.2024.112927
- Liao, P., Liu, P., Wang, Y., Huang, C., Lan, L., Yang, Y., et al. (2018). Stereoscopic cultivation of *Panax notoginseng*: A new approach to overcome the continuous cropping obstacle. *Ind. Crops Prod.* 126, 38–47.
- Lin, R., Liu, W., Piao, M., and Zhu, H. (2017). A review of the relationship between the gut microbiota and amino acid metabolism. *Amino Acids* 49, 2083–2090. doi: 10.1007/s00726-017-2493-3
- Liu, S., Wang, Z., Niu, J., Dang, K., Zhang, S., Wang, S., et al. (2021). Changes in physicochemical properties, enzymatic activities, and the microbial community of soil significantly influence the continuous cropping of *Panax quinquefolius* L. (American ginseng). *Plant. Soil* 463, 427–446. doi: 10.1007/s11104-021-04911-2
- Lu, S., He, Y., Chen, Y., Chen, L., Wang, Z., Yuan, J., et al. (2022). Co-analysis of rhizosphere metabolomics and bacterial community structures to unfold soil ecosystem health in *Camellia oleifera* land under long-term cultivation. *Appl. Soil Ecol.* 171:104336. doi: 10.1016/j.apsoil.2021.104336
- Lyu, F., Zhang, W., Wang, R., Lyu, R., Lin, H., Zhang, Z., et al. (2023). Rhizosphere bacterial community composition was significantly affected by continuous cropping but not by sesame genotype. *Rhizosphere* 27:100750. doi: 10.1016/j.rhisph.2023.100750
- Ma, W., Liao, X., Wang, C., and Zhang, Y. (2022). Effects of four cropping patterns of *Lilium brownii* on rhizosphere microbiome structure and replant disease. *Plants* 11:824. doi: 10.3390/plants11060824
- Ma, X., Zhang, Y., Wei, F., Zhao, L., Zhou, J., Qi, G., et al. (2023). Applications of *Chaetomium globosum* CEF-082 improve soil health and mitigate the continuous cropping obstacles for *Gossypium hirsutum*. *Ind. Crops Prod.* 197:116586. doi: 10.1016/j.indcrop.2023.116586
- Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J., et al. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332, 1097–1100. doi: 10.1126/science.1203980
- Milton, C., Boucher, D., Vachelard, C., Perchet, G., Barra, V., Troquet, J., et al. (2010). Bacterial community changes during bioremediation of aliphatic hydrocarbon-contaminated soil. *FEMS Microbiol. Ecol.* 74, 669–681. doi: 10.1111/j.1574-6941.2010.00982.x
- Mohanram, S., and Kumar, P. (2019). Rhizosphere microbiome: Revisiting the synergy of plant-microbe interactions. *Ann. Microbiol.* 69, 307–320. doi: 10.1007/s13213-019-01448-9
- Montgomery, M. W., and Sgarbieri, V. C. (1975). Isoenzymes of banana polyphenol oxidase. *Phytochemistry* 14, 1245–1249. doi: 10.1016/S0031-9422(00)98602-3
- Muthubharathi, B., Gowripriya, T., and Balamurugan, K. (2021). Metabolomics: Small molecules that matter more. *Mol. Omics* 17, 210–229. doi: 10.1039/d0mo00176g

- Neelam, Khatkar, A., and Sharma, K. K. (2020). Phenylpropanoids and its derivatives: Biological activities and its role in food, pharmaceutical and cosmetic industries. *Crit. Rev. Food Sci. Nutr.* 60, 2655–2675. doi: 10.1080/10408398.2019.1653822
- Olsen, S. R., and Sommers, L. E. (1982). "Phosphorus," in *Methods of soil analysis*, 2nd Edn, Part 2, eds A. L. Page and R. H. Miller (Madison: ASA and SSSA).
- Pang, Z., Dong, F., Liu, Q., Lin, W., Hu, C., and Yuan, Z. (2021). Soil metagenomics reveals effects of continuous sugarcane cropping on the structure and functional pathway of rhizospheric microbial community. *Front. Microbiol.* 12:627569. doi: 10.3389/fmicb.2021.627569
- Parada, A., Needham, D., and Fuhrman, J. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18, 1403–1414. doi: 10.1111/1462-2920.13023
- Pascale, A., Proietti, S., Pantelides, I., and Stringlis, I. (2020). Modulation of the root microbiome by plant molecules: The basis for targeted disease suppression and plant growth promotion. *Front. Plant Sci.* 10:1741. doi: 10.3389/fpls.2019.01741
- Pavlovskaya, M., Prekrasna, I., Parnikoza, I., and Dykyi, E. (2021). Soil sample preservation strategy affects the microbial community structure. *Microbes Environ.* 36:ME20134. doi: 10.1264/jsme2.ME20134
- Phulpoto, I., Qi, Z., Qazi, M., and Yu, Z. (2024). Biosurfactants-based mixed polycyclic aromatic hydrocarbon degradation: From microbial community structure toward non-targeted metabolomic profile determination. *Environ. Int.* 184:108448. doi: 10.1016/j.envint.2024.108448
- Pleysier, J. L., and Juo, A. S. R. (1980). A single-extraction method using silver-thiourea for measuring exchangeable cations and effective CEC in soils with variable charges. *Soil Sci.* 129, 205–211. doi: 10.1097/00010694-198004000-00002
- Qin, S., Yeboah, S., Cao, L., Zhang, J., Shi, S., and Liu, Y. (2017). Breaking continuous potato cropping with legumes improves soil microbial communities, enzyme activities and tuber yield. *PLoS One* 12:e0175934. doi: 10.1371/journal.pone.0175934
- Rillig, M., de Souza Machado, A., Lehmann, A., and Klümper, U. (2019). Evolutionary implications of microplastics for soil biota. *Environ. Chem.* 16, 3–7. doi: 10.1071/EN18118
- Sansupa, C., Wahdan, S. F. M., Hossen, S., Disayathanoowat, T., Wubet, T., and Purahong, W. (2021). Can we use functional annotation of prokaryotic taxa (FAPROTAX) to assign the ecological functions of soil bacteria? *Appl. Sci.* 11:688. doi: 10.3390/app11020688
- Semenov, M. V., Krasnov, G. S., Semenov, V. M., and van Bruggen, A. H. C. (2020). Long-term fertilization rather than plant species shapes rhizosphere and bulk soil prokaryotic communities in agroecosystems. *Appl. Soil Ecol.* 154:103641. doi: 10.1016/j.apsoil.2020.103641
- Sharma, A., Shahzad, B., Kumar, V., Kohli, S., Sidhu, G., Bali, A., et al. (2019). Phytohormones regulate accumulation of osmolytes under abiotic stress. *Biomolecules* 9:285. doi: 10.3390/biom9070285
- She, S., Niu, J., Zhang, C., Xiao, Y., Chen, W., Dai, L., et al. (2017). Significant relationship between soil bacterial community structure and incidence of bacterial wilt disease under continuous cropping system. *Arch. Microbiol.* 199, 267–275. doi: 10.1007/s00203-016-1301-x
- Shen, Y., Zhang, H., Zhan, Y., Zhang, T., Li, Q., Wang, E., et al. (2024). Transcriptomics and metabolomics of the molecular mechanisms of ginseng's response to the continuous cropping obstacle. *Physiol. Mol. Plant Pathol.* 133:102329. doi: 10.1016/j.pmpp.2024.102329
- Sinsabaugh, R. L., Gallo, M. E., Lauber, C., Waldrop, M. P., and Zak, D. R. (2005). Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. *Biogeochem* 75, 201–215. doi: 10.1007/s10533-004-7112-1
- Song, X., Li, C., Qiu, Z., Wang, C., and Zeng, Q. (2024). Ecotoxicological effects of polyethylene microplastics and lead (Pb) on the biomass, activity, and community diversity of soil microbes. *Environ. Res.* 252:119012. doi: 10.1016/j.envres.2024.119012
- Song, X., Pan, Y., Li, L., Wu, X., and Wang, Y. (2018). Composition and diversity of rhizosphere fungal community in *Coptis chinensis* Franch. continuous cropping fields. *PLoS One* 13:e0193811. doi: 10.1371/journal.pone.0193811
- Song, Y., Li, X., Yao, S., Yang, X., and Jiang, X. (2020). Correlations between soil metabolomics and bacterial community structures in the pepper rhizosphere under plastic greenhouse cultivation. *Sci. Total Environ.* 728:138439. doi: 10.1016/j.scitotenv.2020.138439
- Stankeviciute, G., Guan, Z., Goldfine, H., and Klein, E. (2019). Caulobacter crescentus adapts to phosphate starvation by synthesizing anionic glycolipids and a novel glycosphingolipid. *mBio* 10:e00107-19. doi: 10.1128/mBio.00107-19
- Sunshine, H., and Iruela-Arispe, M. (2017). Membrane lipids and cell signaling. *Curr. Opin. Lipidol.* 28, 408–413. doi: 10.1097/MOL.0000000000000443
- Tabatabai, M. A., and Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307. doi: 10.1016/0038-0717(69)90012-1
- Tan, G., Liu, Y., Peng, S., Yin, H., Meng, D., Tao, J., et al. (2021). Soil potentials to resist continuous cropping obstacle: Three field cases. *Environ. Res.* 200:111319. doi: 10.1016/j.envres.2021.111319
- Tan, Y., Cui, Y., Li, H., Kuang, A., Li, X., Wei, Y., et al. (2017). Rhizospheric soil and root endogenous fungal diversity and composition in response to continuous *Panax notoginseng* cropping practices. *Microbiol. Res.* 194, 10–19. doi: 10.1016/j.micres.2016.09.009
- Thornton, H. G., Gray, P. H. H., and Russell, E. J. (1934). The numbers of bacterial cells in field soils, as estimated by the ratio method. *Proc. R. Soc. B Biol. Sci.* 115, 522–543. doi: 10.1098/rspb.1934.0057
- Trasar-Cepeda, C., Camiña, F., Leirós, M. C., and Gil-Sotres, F. (1999). An improved method to measure catalase activity in soils. *Soil Biol. Biochem.* 31, 483–485. doi: 10.1016/S0038-0717(98)00153-9
- Trivedi, P., Leach, J., Tringe, S., Sa, T., and Singh, B. (2021). Author Correction: Plant-microbiome interactions: From community assembly to plant health. *Nat. Rev. Microbiol.* 19:72. doi: 10.1038/s41579-020-00490-8
- Vance, E. D., Brookes, P. C., and Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707. doi: 10.1016/0038-0717(87)90052-6
- Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., and Pérez-Clemente, R. (2020). Root exudates: From plant to rhizosphere and beyond. *Plant Cell Rep.* 39, 3–17. doi: 10.1007/s00299-019-02447-5
- Walkey, A., and Black, I. A. (1934). An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29–38. doi: 10.1097/00010694-193401000-00003
- Wang, F., Zhang, X., Wei, M., Wang, Y., Liang, Z., and Xia, P. (2022). Appropriate crop rotation alleviates continuous cropping barriers by changing rhizosphere microorganisms in *Panax notoginseng*. *Rhizosphere* 23:100568. doi: 10.1016/j.rhisp.2022.100568
- Wang, R., Zhang, Z., Lv, F., Lin, H., Wei, L., and Xiao, Y. (2022). Optimizing the bacterial community structure and function in rhizosphere soil of sesame continuous cropping by the appropriate nitrate ammonium ratio. *Rhizosphere* 23:100550. doi: 10.1016/j.rhisp.2022.100550
- Wang, X., Alami, M., Gong, S., Cheng, Q., Chen, C., Li, X., et al. (2025). Utilizing Microbial inoculants to alleviate continuous cropping obstacles: Insights into the *Metabolites* and transcriptomic responses of *Pinellia ternata*. *Metabolites* 15:189. doi: 10.3390/metabo15030189
- Ward, N., Challacombe, J., Janssen, P., Henrissat, B., Coutinho, P., Wu, M., et al. (2009). Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Appl. Environ. Microbiol.* 75, 2046–2056. doi: 10.1128/AEM.02294-08
- Wu, C., Ma, Y., Wang, D., Shan, Y., Song, X., Hu, H., et al. (2022). Integrated microbiology and metabolomics analysis reveal plastic mulch residue affects soil microorganisms and their metabolic functions. *J. Hazard Mater.* 423:127258. doi: 10.1016/j.jhazmat.2021.127258
- Wu, H., Wu, L., Zhu, Q., Wang, J., Qin, X., Xu, J., et al. (2017). The role of organic acids on microbial deterioration in the *Radix pseudostellariae* rhizosphere under continuous monoculture regimes. *Sci. Rep.* 7:3497. doi: 10.1038/s41598-017-03793-8
- Wyszkowska, J., Borowik, A., Olszewski, J., and Kucharski, J. (2019). Soil bacterial community and soil enzyme activity depending on the cultivation of *Triticum aestivum*, *Brassica napus*, and *Pisum sativum* ssp. *arvense*. *Diversity* 11:246. doi: 10.3390/d11120246
- Xin, X., Zhang, X., Chu, W., Mao, J., Yang, W., Zhu, A., et al. (2019). Characterization of fluvo-aquic soil phosphorus affected by long-term fertilization using solution 31P NMR spectroscopy. *Sci. Total Environ.* 692, 89–97. doi: 10.1016/j.scitotenv.2019.07.221
- Xue, P., Liu, X., Shi, X., Yuan, H., Wang, J., Zhang, J., et al. (2023). Stereoselective accumulation and biotransformation of chiral fungicide epoxiconazole and oxidative stress, detoxification, and endogenous metabolic disturbance in earthworm (*Eisenia foetida*). *Sci. Total Environ.* 858:159932. doi: 10.1016/j.scitotenv.2022.159932
- Xue, P., Liu, X., Zhao, L., Zhang, J., and He, Z. (2022). Integrating high-throughput sequencing and metabolomics to investigate the stereoselective responses of soil microorganisms to chiral fungicide cis-epoxiconazole. *Chemosphere* 300:134198. doi: 10.1016/j.chemosphere.2022.134198
- Yang, M., Yang, D., and Yu, X. (2018). Soil microbial communities and enzyme activities in sea-buckthorn (*Hippophae rhamnoides*) plantation at different ages. *PLoS One* 13:e0190959. doi: 10.1371/journal.pone.0190959
- Yang, W., Li, C., Wang, S., Zhou, B., Mao, Y., Rensing, C., et al. (2021). Influence of biochar and biochar-based fertilizer on yield, quality of tea and microbial community in an acid tea orchard soil. *Appl. Soil Ecol.* 166:104005. doi: 10.1016/j.apsoil.2021.104005
- Zeeshan Ul Haq, M., Yu, J., Yao, G., Yang, H., Iqbal, H. A., Tahir, H., et al. (2023). A systematic review on the continuous cropping obstacles and control strategies in medicinal plants. *Int. J. Mol. Sci.* 24:12470. doi: 10.3390/ijms241512470

- Zhang, H., Zheng, X., Wang, X., Xiang, W., Xiao, M., Wei, L., et al. (2022). Effect of fertilization regimes on continuous cropping growth constraints in watermelon is associated with abundance of key ecological clusters in the rhizosphere. *Agric. Ecosyst. Environ.* 339:108135. doi: 10.1016/j.agee.2022.108135
- Zhang, L., He, Y., Lin, D., Yao, Y., Song, N., and Wang, F. (2022). Co-application of biochar and nitrogen fertilizer promotes rice performance, decreases cadmium availability, and shapes rhizosphere bacterial community in paddy soil. *Environ. Pollut.* 308:119624. doi: 10.1016/j.envpol.2022.119624
- Zhao, J., Li, Y., Wang, B., Huang, X., Yang, L., Lan, T., et al. (2017). Comparative soil microbial communities and activities in adjacent Sanqi ginseng monoculture and maize-Sanqi ginseng systems. *Appl. Soil Ecol.* 120, 89–96. doi: 10.1016/j.apsoil.2017.08.002
- Zhao, X., Elcin, E., He, L., Vithanage, M., Zhang, X., Wang, J., et al. (2024). Using biochar for the treatment of continuous cropping obstacle of herbal remedies: A review. *Appl. Soil Ecol.* 193:105127. doi: 10.1016/j.apsoil.2023.105127
- Zhao, Y., Qin, X. M., Tian, X. P., Yang, T., Deng, R., and Huang, J. (2021). Effects of continuous cropping of *Pinellia ternata* (Thunb.) Breit. on soil physicochemical properties, enzyme activities, microbial communities and functional genes. *Chem. Biol. Technol. Agric.* 8:43. doi: 10.1186/s40538-021-00243-6
- Zheng, W., Zhao, Z., Gong, Q., Zhai, B., and Li, Z. (2018). Responses of fungal–bacterial community and network to organic inputs vary among different spatial habitats in soil. *Soil Biol. Biochem.* 125, 54–63. doi: 10.1016/j.soilbio.2018.06.029