

Effects of *Ageratina adenophora* Invasion on Soil Nutrients, Enzyme Activities, and Microbial Composition/Function in Karst Areas of Central Guizhou

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Research Article

Keywords: *Ageratina adenophora*, plant invasion, soil microbial community, ecological effect, metagenomics

Posted Date: February 16th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8826938/v1>

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Additional Declarations: No competing interests reported.

Abstract

This study elucidates the regulatory effects of *Ageratina adenophora* invasion on soil nutrient cycling, enzyme activities, and microbial composition/function in karst ecosystems of central Guizhou and reveals the plant–soil–microbe feedback-driven invasion loop. Four invasion gradients were established in Guanling County: CK (uninvaded), L (10–30% cover), M (30–60%), and H (60–90%). Soil nutrients, enzyme activities, metagenomic profiles, and nutrient contents in vegetative organs of *A. adenophora* and native *Artemisia argyi* were analysed. Key results: (1) Soil microbial communities included 7 domains, 198 phyla, 179 classes, 364 orders, 856 families, 3,531 genera, and 22,111 species. No significant differences were detected in soil nutrients, enzyme activities, or microbial composition/structure/diversity across invasion gradients. (2) Invaded soils presented significantly greater soil organic carbon (SOC), total nitrogen (TN), and pH than CK soils. Sucrase and urease activities were significantly elevated. Compared with *A. argyi* leaves, *A. adenophora* leaves contained significantly greater TN and total potassium (TK). (3) Microbial Shannon diversity (H') and Pielou evenness (J) were significantly decreased. *Verrucomicrobia* and *Candidatus Eisenbacteria* were significantly enriched in invaded vs. CK soils. (4) Functional gene (K13038 and K00639) levels were significantly correlated with invasion intensity. Carbohydrate metabolism genes (*GT10* and *CBM4*) were upregulated by 1.5- and 1.7-fold, respectively; the stress resistance gene *merG* was upregulated 2.3-fold. These changes collectively demonstrate that *A. adenophora* invasion increases SOC/TN content and sucrase/urease activity, restructures microbial communities, and upregulates carbohydrate metabolism/stress resistance-related genes, establishing a self-reinforcing "invasive plant–soil–microbe" feedback loop in karst ecosystems.

1. Introduction

The objective of this template is to enable you in an easy way to style your article attractively in a style similar to that of *Computer Physics Communications*. It should be emphasized, however, that the final appearance of your paper in print and in electronic media will very likely *vary to some extent* from the presentation achieved in this Word® document. *Ageratina adenophora*, a globally invasive plant, severely impacts biodiversity, ecosystems, and economies through rapid allelochemical release and efficient nutrient acquisition (Sun *et al.* 2004; Wang *et al.* 2005; Wang and Wang 2006; Zhang *et al.* 2023). To date, it has invaded more than 30 countries worldwide (Changjun *et al.* 2021). Native to Mexico (Wang *et al.* 2005), this species was introduced to Yunnan, China, in the 1940s (Sun *et al.* 2004; Wang and Wang 2006; Zhu *et al.* 2007; Sang *et al.* 2010). Owing to its allelopathic potency and high ecological adaptability, *A. Adenophora* has rapidly colonized Guizhou, Sichuan, Guangxi, and Tibet (Wang *et al.* 2005; Zhang *et al.* 2022). Currently, large-scale infestations occur in Yunnan and Guizhou Provinces, causing significant ecological and economic damage (Sang *et al.* 2010).

The biological traits of *A. adenophora* constitute key drivers of its rapid invasion success. As a perennial herb reaching 1–2 m in height, this species exhibits broad adaptability and light preference, primarily colonizing slopes, roadsides, wastelands, and forest edges (Wang *et al.* 2011). Its high photosynthetic

rate enables rapid biomass accumulation. *A. adenophora* employs both sexual and asexual reproduction, with damaged rhizomes readily sprouting new shoots (Wang *et al.* 2006). A single plant produces tens of thousands of viable seeds dispersed via wind, water, animal, and anthropogenic activities (Wang *et al.* 2006). Critically, allelochemical release underpins invasion success by inhibiting native plants (Li *et al.* 2017). Allelopathy—defined as plant-mediated chemical interactions affecting neighbouring organisms (Arora *et al.* 2024; Kumar *et al.* 2024)—operates through multiple pathways: root exudation, leaf leaching, and litter decomposition (Arora *et al.* 2024). Key allelochemicals include terpenoids (Zhao *et al.* 2009) and phenolic compounds (Xie *et al.* 2010), which suppress the growth and seed germination of native flora, ultimately restructuring the plant community composition and reducing diversity (Rai *et al.* 2023; Wang *et al.* 2025).

The karst region of central Guizhou features unique geological settings and fragile ecosystems characterized by nutrient-poor soils (Wang *et al.* 2019), severe water erosion, and rocky desertification (Chen *et al.* 2018a). Its distinctive geological structure—rugged terrain with thin soil layers—results in poor soil retention capacity, driving both surface soil loss and subsurface leakage (Sun *et al.* 2020). The soils in this region are predominantly calcareous, with low nutrient availability (organic matter, total nitrogen, total phosphorus, and available phosphorus) due to limestone weathering (Li *et al.* 2022a). *A. adenophora* exploits these conditions through exceptional environmental adaptability (Xia *et al.* 2020) and multimechanistic resource competition (Zhao *et al.* 2009; Zheng *et al.* 2012; Shen *et al.* 2020). The unique karst geomorphology and microclimates further facilitate its invasion (Li *et al.* 2022b).

A. adenophora invasion has multifaceted effects on soil nutrients, enzymes, and microbial communities. It modifies soil physicochemical properties, typically increasing nitrogen (N), nitrate ($\text{NO}_3\text{-N}$), ammonium ($\text{NH}_4\text{+N}$), available potassium (AK), and available phosphorus (AP) contents, although reductions in total phosphorus (TP) and potassium (TK) may occur (Deng *et al.* 2015). Concurrently, soil pH shifts are frequently observed (Xia *et al.* 2024). The effects on soil enzyme activities are context dependent and vary by enzyme type and invasion intensity (Darji *et al.* 2024). Crucially, invasion restructures microbial composition, functionality, and diversity (Wan *et al.* 2010; Bajpai and Inderjit 2013; Xiao *et al.* 2014; Chen *et al.* 2015; Balami *et al.* 2017). This includes reduced microbial α diversity, decreased fungal abundance (Xiao *et al.* 2014; Balami *et al.* 2017), and altered community assemblages (Wan-Xue *et al.* 2010).

Southwest China's karst region, characterized by impoverished soils and unique hydrology, constitutes an ecologically fragile zone highly vulnerable to *A. adenophora* invasion (Wang *et al.* 2019). Guanling County in Guizhou exemplifies typical karst mountainous terrain where nutrient limitation prevails. Crucially, how *A. adenophora* maintains invasion dominance by restructuring the soil microbial composition/function to drive a self-reinforcing *allelopathy–nutrient feedback loop* remains unexplored. This study integrates soil physicochemical analyses, enzyme activity assays, and metagenomic sequencing to (1) determine the responses of karst soil nutrients, enzyme activities, and microbial communities to *A. adenophora* invasion; (2) decipher differentiation patterns in microbial community

structure (diversity/composition) and functional genes; and (3) reveal plant–soil–microbe interaction networks, providing theoretical foundations for karst ecological barrier restoration.

2. Materials and Methods

2.1. Site Description

The study area is located in Guanling County (25.908877°N, 105.605618°E), Anshun city, Guizhou Province (Fig. 1). Situated in the upper reaches of the Dabang River, this karst terrain features hilly and mountainous topography at 1,064 m in elevation. The regional climate data indicate that the mean annual temperature is 16.2°C, the average annual frost-free period is 308 days, the mean annual sunshine duration is 1,381.6 hours, and the mean annual precipitation is 1,370 mm. *A. Adenophora* predominantly invades forestlands, roadsides, ditches, and dry farmlands, resulting in contiguous infestations. This area represents one of the most severely invaded zones in Guizhou Province.

2.2. Experimental Design

Genomic DNA was extracted via the TIANamp Soil DNA Kit (DP705, Tiangen Biotech) per the manufacturer's protocol. The concentration of the extracted DNA was assessed with a *Qubit™ 3.0* fluorometer (Invitrogen) with a *dsDNA HS Assay Kit*. The integrity was determined via 1% agarose gel electrophoresis, and library preparation was performed with the VAHTS® Universal Plus DNA Library Prep Kit for Illumina (ND617). Library quality control included 2 factors: the size distribution was determined via a Qsep-400 Bio-Fragment Analyzer, and quantification was performed using a *Qubit 3.0* fluorometer. The final libraries were sequenced on an Illumina NovaSeq 6000 platform with a paired-end 150 bp (PE150) strategy.

2.3. Soil Physicochemical Properties and Plant Nutrient Analyses

Total carbon (TC) and total nitrogen (TN) were determined by elemental analysis (Thermo Fisher Scientific elemental analyser) via high-temperature catalytic combustion in oxygen, converting carbon to CO₂ and nitrogen to N₂. Total phosphorus (TP) was measured via molybdenum-antimony anti-spectrophotometry using a UV-1800PC spectrophotometer (MAPADA, Shanghai). Total potassium (TK) was quantified via flame photometry (FP6410 flame photometer, INESA, Shanghai). The soil organic matter (SOM) content was analysed via the potassium dichromate oxidation–external heating method (DF-101S oil bath), followed by titration. The soil organic carbon (SOC) content was calculated as 58% SOM (based on the dichromate oxidation method). pH was measured potentiometrically (Sartorius PB-1 pH meter) in a 1:2.5 soil:water suspension. Dry matter (DM) was determined by oven drying at 105 ± 5°C to a constant weight. Plant nutrients (TC, TN, TP, and TK) in *A. adenophora* and *A. argyi* were analysed using identical methods as those used for the corresponding soil parameters.

2.4. Enzyme Activity Assays

All enzymatic analyses were performed using a SpectraMax single-mode reader for absorbance measurements. The specific methods used included urease (UE), which is quantified by indophenol blue colorimetry on the basis of ammonia production from urea hydrolysis and reacts with phenol–sodium hypochlorite to form indophenol blue. Phosphatase (PHO) was measured via disodium phenyl phosphate colorimetry, which detects phenol release through chromogenic reactions. Catalase (CAT) was assessed by the UV absorption method at 240 nm, H₂O₂ consumption was directly quantified after enzymatic decomposition. Sucrase (SUC) and amylase (AMS) were determined using 3,5-dinitrosalicylic acid (DNS) colorimetry at 540 nm, where reducing sugars from substrate hydrolysis react with DNS to form reddish-brown compounds.

2.5. Metagenomic Sequencing Procedures

Genomic DNA was extracted via the TIANamp Soil DNA Kit (DP705, Tiangen Biotech) per the manufacturer's protocol. The concentration of the extracted DNA was assessed with a *Qubit™ 3.0* fluorometer (Invitrogen) with a *dsDNA HS Assay Kit*. The integrity was determined via 1% agarose gel electrophoresis, and library preparation was performed with the VAHTS® Universal Plus DNA Library Prep Kit for Illumina (ND617). Library quality control included 2 factors: the size distribution was determined via a Qsep-400 Bio-Fragment Analyzer, and quantification was performed using a *Qubit 3.0* fluorometer. The final libraries were sequenced on an Illumina NovaSeq 6000 platform with a paired-end 150 bp (PE150) strategy.

2.6. Sequence Processing and Assembly

The raw sequencing reads underwent rigorous quality control to generate clean reads for downstream analysis. Quality trimming was performed with fastp (v0.23.4) (Chen *et al.* 2018b) to remove low-quality bases. For host decontamination, clean reads were aligned to host genomes using Bowtie2 (Langmead and Salzberg 2012), with subsequent removal of host-derived sequences. For *de novo* assembly, processed reads were assembled with MEGAHIT (Li *et al.* 2015), and contigs ≥ 300 bp were retained. For assembly evaluation, quality was assessed via the QAST (Gurevich *et al.* 2013). For gene catalogue construction, contigs were clustered into nonredundant gene catalogues via MMseq2 (v12-113e3; Mirdita *et al.* 2019) at 90% sequence identity and 80% coverage thresholds. The predicted genes were functionally annotated against Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), evolutionary genealogy of genes: Nonsupervised Orthologous Groups (eggNOG), Carbohydrate-Active enZymes (CAZy), and the Comprehensive Antibiotic Resistance Database (CARD).

2.7. Data Analysis

Statistical analyses were performed using Microsoft Excel 2019. Student's t tests were used to assess differences in alpha diversity indices, soil nutrients, enzyme activities, and species abundance between groups. Nonmetric multidimensional scaling (NMDS) based on Bray–Curtis distance matrices was used to visualize community composition variations. All graphical outputs were generated with R software (v4.3.1) via the ggplot2 and vegan packages.

3. Results

3.1. Soil Nutrient Alterations

A. adenophora invasion significantly altered the soil nutrient content and pH (Fig. 2). Compared with the uninvaded control (CK), the invaded soils presented greater pH ($P < 0.05$), elevated soil organic carbon and total nitrogen ($P < 0.05$), and nonsignificant increases in soil organic matter, total carbon, total potassium, total phosphorus, and dry matter. These results indicate that invasion establishes an allelopathy–nutrient positive feedback loop by selectively enhancing key nutrients (SOC and TN) coupled with slight alkalinization, potentially favouring invasive growth.

3.2. Impact of *A. adenophora* Invasion on Soil Enzyme Activities

A. adenophora invasion significantly altered soil enzyme activities (Fig. 3), with generally higher values in invaded soils than in the uninvaded control. The soil urease and phosphatase activities tended to increase with invasion intensity. Specifically, sucrase and urease activities were significantly elevated in invaded soils compared with those in CK soils ($P < 0.05$). These changes suggest that invasion accelerates nutrient turnover through the selective activation of carbon/nitrogen-metabolizing enzymes (SUC and UE) to sustain competitive dominance, whereas the delayed response of phosphorus-cycling enzymes (nonsignificant PHO change) indicates a selective nutrient acquisition strategy.

3.3. Differences in Nutrient Contents between *A. adenophora* and *A. argyi*

Significant differences in nutrient content were observed between the invasive *A. adenophora* and the native plant *A. argyi* (Fig. 4). Compared with *A. argyi*, *A. adenophora* generally presented higher total nitrogen and total potassium contents ($p < 0.05$), whereas *A. argyi* presented significantly greater total carbon contents in stem tissues. Both species displayed organ-specific nutrient allocation patterns, but *A. adenophora* presented more pronounced nutrient enrichment in leaf tissues.

3.4. Species Composition and Gene Counts

A total of 12 soil samples were collected, including soil microorganisms spanning 7 domains, 198 phyla, 179 classes, 364 orders, 856 families, 3,531 genera, and 22,111 species. Analysis of gene count differences between *A. adenophora*-invaded and non-invaded conditions revealed minimal redundant gene numbers and the lowest proportion of unique redundant genes under invasion (Fig. 5a and b).

Compositional analysis revealed domain-level dominance by bacteria (83.8%), with minor components including Archaea, Fungi, Viruses, Metazoa, Eukaryota, and Viridiplantae ($< 0.1\%$), alongside unclassified and unassigned taxa (Fig. 5c). At the phylum level, the community was predominantly composed of *Proteobacteria* (31.2%), *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Verrucomicrobia*, *Planctomycetes*, and *Nitrospirae* (1.3%) (Fig. 5d). The ten most abundant phyla were

exclusively bacteria, with *Proteobacteria*, *Acidobacteria* and *Gemmatimonadetes* collectively constituting 56.7% of the community. Functionally, *Proteobacteria*—the dominant phylum—mediates carbon/nitrogen cycling, symbiosis, and pathogenesis; *Acidobacteria* represents oligotrophic specialists that decompose recalcitrant organics (cellulose) in acidic soils; and *Gemmatimonadetes* dominates arid soils with putative involvement in phosphate transport and metabolism. This microbial profile aligns with the oligotrophic, drought-prone karst environment of central Guizhou, indicating that *A. adenophora* invasion restructures soil microbiomes by selecting extremophile-adapted taxa.

(a) Venn diagram. Ellipse colours denote distinct groups, with overlaps indicating shared non-redundant gene counts and non-overlapping areas representing group-specific non-redundant genes. (b) Non-redundant gene counts (box plot). The group categories are displayed on the x-axis, and the gene counts are displayed on the y-axis. Box plot elements: box tops/bottoms = upper/lower quartiles (interquartile range, IQR); central line = median; whiskers = minimum/maximum values within 1.5×IQR; outlying points beyond whiskers represent outliers. The numeric labels on the intergroup connectors indicate t test p values ($p > 0.05$ not displayed). (c) Domain-level taxonomic composition. The samples are arranged on the x-axis, with relative abundance percentages on the y-axis. The colour-coded bars represent taxa (one hue per taxon), and the bar length reflects the relative abundance proportions. For optimal visualization, only the top 10 most abundant taxa are shown; the remaining taxa are merged as "Others." "Unassigned" denotes taxonomically unannotated species. (d) Phylum-level composition showing the top 10 abundant taxa.

3.5. Divergence in Species Composition

Analysis of the microbial communities in *A. adenophora*-invaded and non-invaded soils revealed significant compositional divergence ($P < 0.05$), with hierarchical clustering segregating the 12 soil samples into two distinct groups. Under heavy invasion (H), *Candidatus* Doudnabacteria and *Candidatus* Kerfeldbacteria presented the strongest positive correlations, whereas *Candidatus* Lindowbacteria and *Candidatus* Cloacimonetes presented minimal associations in control (CK) soils. Invasion conditions reduced the abundances of *Actinobacteria* and *Candidatus* Dormibacteraeota but increased those of most other taxa (Fig. 6a). Among the 15 most abundant phyla, *Verrucomicrobia*, *Actinobacteria*, *Candidatus* Eisenbacteria, and *Bacteroidetes* dominated with marked intergroup differences. *Actinobacteria* peaked in CK, whereas *Verrucomicrobia* and *Candidatus* Eisenbacteria were significantly enriched in invaded soils (Fig. 6b). These results collectively confirm profound invasion-induced microbial restructuring, identifying *Candidatus* Doudnabacteria and *Candidatus* Kerfeldbacteria as the taxa most responsive to *A. adenophora* invasion.

(a) Phylum-level divergent taxa heatmap. Displayed taxa that passed differential abundance testing ($p < 0.05$). The left dendrogram clusters divergent taxa, the top dendrogram clusters samples, and the central heatmap visualizes abundance gradients. (b) Phylum-level divergent abundance bar plot. Left section: mean abundance bars (x-axis = mean proportion, y-axis = taxon names); centre: p value asterisks (* $p < 0.05$, ** $p \leq 0.001$); right: actual p values. The top 15 taxa were selected by a descending p value and sorted by descending abundance.

3.6. Species Diversity Composition

To assess the impact of *A. adenophora* invasion on microbial diversity, alpha and beta diversity analyses were conducted. Alpha diversity was evaluated using the Chao1 richness index, Shannon diversity index, Simpson dominance index, and Pielou evenness index, whereas beta diversity was evaluated via principal component analysis (PCA) and nonmetric multidimensional scaling (NMDS) to resolve intersample compositional differences.

Significant intergroup differences emerged in the alpha diversity indices ($p < 0.05$). Compared with the control soils, the invaded soils presented significantly lower Shannon diversity and Pielou evenness indices than the control soils (CK, $p < 0.05$), indicating microbial diversity loss and uneven species distribution following invasion. Conversely, the Simpson dominance and Chao1 richness indices did not significantly differ across groups ($p > 0.05$) (Fig. 7a-d). These results collectively confirmed the greater microbial biodiversity and relative species richness in the CK soils than in the CK soils, indicating that *Adenophora* invasion profoundly restructured the microbial communities.

The PCA results demonstrated a cumulative explanatory power of 75.25% (PC1 = 61.74%, PC2 = 13.51%), effectively capturing intersample compositional divergence. Visualization revealed clear segregation between the *A. adenophora*-invaded and control (CK) groups in ordination space, indicating distinct community structures. Conversely, the proximity among the different invasion-intensity groups suggested high compositional similarity (Fig. 7e). NMDS analysis corroborated these findings, with a stress value of 0.0001 (reliability threshold: stress < 0.2 ; lower values indicate higher precision), confirming exceptional ordination reliability and strong concordance with the PCA results (Fig. 7f). Overall, these results indicate that *A. adenophora* invasion significantly impacts microbial biodiversity and relative species richness, while minimal separation among invasion gradients reflects high community similarity.

(a) Shannon index variation. X-axis: experimental groups; Y-axis: alpha diversity index. (b) Simpson index variation. X-axis: groups; Y-axis: alpha diversity index. (c) Chao1 index variation. X-axis: groups; Y-axis: alpha diversity index. (d) Pielou evenness index variation. X-axis: groups; Y-axis: alpha diversity index. (e) Principal component analysis (PCA) of species abundance. Points represent sample-specific compositions; colour coding denotes groups. X-axis: PC1 with explanatory power (%); Y-axis: PC2 with explanatory power (%). (f) Nonmetric multidimensional scaling (NMDS) ordination. Points correspond to samples; colours indicate groups. Stress values < 0.1 indicate acceptable ordination, whereas values < 0.05 demonstrate excellent representativeness.

3.7. Functional Gene Annotation

eggNOG functional classification revealed that among the top 20 categories, [S]: Function unknown predominated, whereas [R]: General function prediction represented only the most abundant annotated function, and [N]: Cell motility was the least frequent (Fig. 8a). The CAZy distribution was as follows: GH (32%), GT (38.9%), PL (1.7%), CE (5.1%), AA (1.8%), and CBM (20.5%), with GT being the most prevalent

and PL the least abundant (Fig. 8b). KEGG pathway analysis at level 2 identified four major categories: metabolism, genetic information processing, environmental information processing, and cellular processing. The global and overview maps revealed the primary metabolic pathways (highest relative abundance), followed by carbohydrate metabolism (sugar synthesis/degradation and monosaccharide/polysaccharide metabolism) and amino acid metabolism (third highest) (Fig. 8c). Gene profiling revealed that profiles indicating the following 20 types of antibiotic resistance were the most enriched: multidrug, tetracycline, macrolide, peptide, glycopeptide, aminoglycoside, aminoglycoside, aminocoumarin, fluoroquinolone, mupirocin, nitroimidazole, pleuromutilin, rifamycin, lincosamide, fosfomycin, carbapenem, phenicol, disinfecting agents and antiseptics, sulfonamide and elfamycin resistance. Notably, multidrug, tetracycline and macrolide resistance-related genes exhibited the highest degree of enrichment (Fig. 8d).

(a) eggNOG functional classification. X-axis: eggNOG categories; Y-axis: relative abundance of functional genes. (b) CAZy distribution. The colour-coded sectors represent carbohydrate-active enzyme classes, with sector areas proportional to relative abundance. (c) KEGG pathway annotation at level 2. X-axis: relative abundance of functional genes; Y-axis: level-2 functional categories. (d) Antibiotic resistance gene abundance. X-axis: antibiotic resistance types; Y-axis: relative abundance of resistance genes.

3.8. Functional Gene Diversity

Microbial functional gene diversity critically influences key biogeochemical processes and metabolic functions. In addition to the global and overview maps, carbohydrate metabolism, amino acid metabolism, and energy metabolism presented the highest gene abundances (Fig. 9a). CAZy annotation revealed GT2 (glycosyltransferases, cellulose synthase), GT4 (glycosyltransferases), and CBM50 (carbohydrate-binding modules targeting pectin compounds, such as pectate and galacturonate) as predominantly enriched categories (Fig. 9b), which drive microbial decomposition of plant cell walls, host–microbe interactions, and sugar metabolism regulation. The CARD annotation encompassed 10 antibiotic resistance classes: nitroimidazole, mupirocin, fluoroquinolone, aminocoumarin, aminoglycoside, glycopeptide, peptide, macrolide, tetracycline, and multidrug (Fig. 9c). Functional gene PCA demonstrated a cumulative explanatory power of 71.23% (PC1 = 52.45%, PC2 = 18.78%), indicating robust ordination efficacy (Fig. 9d).

(a) Functional composition scatter plot. X-axis: KEGG level-2 metabolic pathways; Y-axis: relative abundance of functional gene categories. (b) CAZy enzyme abundance bubble plot. X-axis: sample names; Y-axis: enzyme classes. The bubble size corresponds to the magnitude of the relative abundance. (c) Circos diagram of the CARD antibiotic resistome. Outer ring: right semicircle = samples/groups, left semicircle = resistance gene types (radial scale = abundance proportion). Inner ribbons connect resistance genes to samples/groups, revealing gene functional composition per sample and sample distribution per gene; ribbon width indicates proportional distribution. (d) Functional gene PCA ordination. Points represent samples; colour coding denotes groups. X-axis: PC1 with explanatory power (%); Y-axis: PC2 with explanatory power (%).

Heatmap analysis revealed significant differences between *A. adenophora*-invaded and control (CK) soils (Fig. 10a), with 26 functional genes segregating into two distinct clusters. Sixteen genes presented significant invasion-associated enrichment, whereas ten presented CK-specific associations. Pathways significantly linked to invasion included photosynthesis; linoleic acid metabolism; one carbon pool by folate; streptomycin biosynthesis; polyketide sugar unit biosynthesis; metabolic pathways; the sulphur relay system; lysine biosynthesis; folate biosynthesis; the phosphotransferase system (PTS); aminoacyl-tRNA biosynthesis; acarbose and validamycin biosynthesis; nicotinate and nicotinamide metabolism; riboflavin metabolism; terpenoid backbone biosynthesis; and lipopolysaccharide biosynthesis.

Heatmap analysis at the KEGG Orthology (KO) level revealed significant compositional divergence between *A. adenophora*-invaded and control (CK) soils ($P < 0.05$, Fig. 10b), with metabolic enzymes segregating into two distinct clusters. The invasion-associated KO terms included K00773, K03655, K11749, K01409, K02652, K02038, K07263, K01710, K02346, K07300, K03321, K04042, K14415, K03639, K01928, K02669, K06941, K02666, K01662, K03750, K06180, K06969, K00639, K03587, K03820, K03545, K00099, K08483, K02517, K01933, K01754, K03631, K00943, K02037, K03833, K01893, K02005, K02653, K00703, K02662, K13038, K04066, K00973, K01885, K02039, K01924, K00812, K01952, K07462, K01961, K03572, K00602, K07391, K00821, K01887, K00806, K00104, and K03564. CK-associated terms included K00135, K01869, K01902, K00252, K01939, K02314, K01434, K01692, K03799, K01687, K00265, K03496, K00525, K03046, K02358, K03544, K06994, K00915, K01621, K00164, K02112, K10112, K01251, K00162, K01426, K05343, K02274, K01915, K01652, K01214, K01897, K07045, K15371, K04043, K00666, K03694, K14162, K01113, K03553, K02600, K02519, and K03466. Notably, K13038 and K00639 encode flavonoid synthases and polyketide synthases that produce allelochemicals that inhibit seed germination in competing species, whereas K02038, K01933, and K01893 encode nutrient transporters and nitrogenases that monopolize nitrogen/phosphorus resources.

Heatmap analysis of carbohydrate-active enzyme families revealed significant compositional divergence between *A. adenophora*-invaded and control (CK) soils ($P < 0.05$, Fig. 10c), with enzymatic profiles segregating into two distinct clusters. The invasion-associated CAZy families included GT105, GT89, PL39, GH92, CBM42, GT9, GH85, CBM16, CBM67, CBM4, CBM6, PL0, GH50, AA5, GH120, GH81, GH144, GH64, CBM64, GH160, CBM0, CBM26, CBM57, PL9, CBM21, GH142, PL2, GT3, GH128, CBM60, GH141, GH2, and GH30. The CK-associated families included GH3, GT28, GH100, GT1, PL7, GH77, GH1, GT58, AA3, AA12, GH76, GH4, GH101, and GH15. The number of invasion-enriched families was 1.2× (GT105), 1.7× (CBM42), 1.7× (PL9), and 1.5× (GH144) greater than that in the CK. *A. Adenophora* invasion leverages GT105 and CBM42 for complex exopolysaccharide synthesis, PL9 and GH144 for plant polysaccharide degradation, AA5 for phenolic detoxification, and CBM60 for stress resistance, whereas CK communities primarily utilize GH3/GH15 for carbon acquisition.

Heatmap analysis at KEGG level 3 revealed significant compositional divergence between *A. adenophora*-invaded and control (CK) soils ($P < 0.05$, Fig. 10d), with differentially abundant genes clustering into two distinct groups. The invasion-associated genes included *cutA*, ALU1-P, *eefA*, *zwS/hydG*, *pmpM*, *galE*, *actA*, *copR*, *crdA*, *smdA*, *vmeF*, *mepA*, *norM/pmpM*, *merG*, *recG*, *nczA*, *smdB*,

G2alt, and *troD*. The CK-associated genes included *ctpD*, *actR*, *pgpA/ltpgpA*, *emrB*, *adeE*, *bepC*, *yfmP*, *fetB/ybbM*, *adeG*, *srpC*, and *silR*. The number of invasion-enriched genes was 2.3-fold (*merG*), 1.4-fold (*norM*), 1.1-fold (*recG*), 1.3-fold (*smdB*), 1.1-fold (*zraS*), and 1.1-fold (*copR*) greater than that in the CK soils. Mechanistically, invasion-associated *merG* and *norM* confer heavy metal resistance, *recG* and *smdB* facilitate oxidative damage repair, while *zraS* and *copR* encode two-component regulatory systems, collectively enhancing adaptive advantages during *A. adenophora* invasion.

KEGG level-3 bar plot analysis (Fig. 10e) revealed significant intergroup differences ($P < 0.05$), with aminoacyl-tRNA biosynthesis exhibiting the highest abundance among the top 15 pathways, whereas biosynthesis of ansamycins was the lowest. The control (CK) values were significantly lower than those of the invaded groups. Concurrently, genus-level functional gene profiling (Fig. 10d) revealed the following order of abundance: *copR* > *gale* > *vmeF* > *smdB* > *actA* > *adeG* > *actR* > *norM/pmpM* > ALU1-P > *crdA* > *emrB* > *trod* > *pgpA/ltpgpA* > *yfmP* > *fetB/ybbM*. The invasion-enriched genes *copR*, *gale*, *vmeF*, *smdB*, and *actA* presented 1.1-fold, 1.23-fold, 1.19-fold, 1.21-fold, and 1.45-fold greater abundances than CK, respectively.

(a) Differential functional gene abundance heatmap. (b) KEGG Orthology (KO) level differential abundance heatmap. (c) CAZy family-level differential abundance heatmap. (d) Differential functional gene abundance heatmap. (e) KEGG level-3 differential gene abundance bar plot. (f) Genus-level differential gene abundance bar plot. Displayed genes that passed differential abundance testing ($p < 0.05$). The left dendrogram clusters the differentially abundant genes; the top dendrogram clusters the samples; the central heatmap visualizes the abundance gradients.

Correlation network analysis (Fig. 11a) identified *metabolic pathways*, *biosynthesis of secondary metabolites*, and *microbial metabolism in diverse environments* as the most abundant pathways, exhibiting strong positive/negative correlations with the top 20 functional genes across multiple metabolic processes. The RDA ordination (Fig. 11b) revealed a cumulative explanatory power of 57.65% (Axis 1: 34.98%; Axis 2: 22.67%), indicating moderate functional gene interpretability. Significant positive correlations included *Biosynthesis of antibiotics*, *purine metabolism*, *biosynthesis of secondary metabolites*, and *metabolic pathways* with sucrase (SUC) and phosphatase (PHO); *biosynthesis of amino acids* and *two-component system* with total potassium (TK), total phosphorus (TP), and catalase (CAT); and *carbon metabolism* and *microbial metabolism in diverse environments* with urease (UE), total carbon (TC), soil organic matter (SOM), soil organic carbon (SOC), total nitrogen (TN), and ammonium nitrogen (AMS) contents. A functional gene–environment heatmap analysis (Fig. 11c) segregated the soil parameters into two clusters: **Cluster 1** (positively correlated with TK, TP, SUC, PHO, and CAT): fructose and mannose metabolism; one carbon pool by folate; streptomycin biosynthesis; biosynthesis of secondary metabolites; base excision repair; protein export; glycerophospholipid metabolism; biosynthesis of amino acids; homologous recombination; mismatch repair; porphyrin and chlorophyll metabolism; ribosome; bacterial secretion system; two-component system; vitamin B6 metabolism; chlorocyclohexane and chlorobenzene degradation; nicotinate and nicotinamide metabolism; glycerolipid metabolism; polyketide sugar unit biosynthesis; terpenoid backbone biosynthesis;

lipopolysaccharide biosynthesis; riboflavin metabolism; metabolic pathways; thiamine metabolism; lysine biosynthesis; folate biosynthesis; sulphur relay system; biotin metabolism; phenylalanine, tyrosine and tryptophan biosynthesis; ubiquinone and other terpenoid-quinone biosynthesis; fatty acid biosynthesis; fatty acid metabolism; biosynthesis of unsaturated fatty acids; carbon fixation in prokaryotes; aminoacyl-tRNA biosynthesis; inositol phosphate metabolism; peptidoglycan biosynthesis; amino sugar and nucleotide sugar metabolism; and D-glutamine and D-glutamate metabolism. **Cluster 2** (positively correlated with SOC, TC, SOM, UE, AMS, and TN): residual functional categories.

(a) Correlation network. Circles represent functional genes, with size denoting abundance; connecting lines indicate intergene correlations, where line thickness corresponds to correlation strength, and colour (red = positive, green = negative) signifies directionality. (b) RDA ordination of functional genes: (1) blue arrows denote environmental factors; arrow length indicates influence strength on community variation; (2) arrow angle relative to axes reflects factor–axis correlation (smaller angles = higher correlation); (3) black points represent functions; proximity to an arrow indicates stronger factor–function interaction; (4) points aligned with arrow direction indicate positive factor–function covariation, whereas opposite alignment indicates negative covariation; (5) axis scales derived from regression values of samples against environmental factors; (6) RDA1/RDA2 axes represent primary ordination vectors, with labelled percentages indicating cumulative explanatory power for community structure. (c) Function–environment heatmap. X-axis: environmental factors; Y-axis: functional genes.

3.9. Soil Nutrients and Enzymes as Determinants of Microbial Diversity

Soil nutrients and enzymes critically influence microbial diversity. The RDA ordination revealed a cumulative explanatory power of 69.96% (RDA1 = 47.9%, RDA2 = 22.06%), demonstrating robust interpretability. Significant positive correlations emerged between *Viridiplantae*, *Archaea*, *Fungi*, *Eukaryota* and catalase (CAT), total phosphorus (TP), phosphatase (PHO), and total potassium (TK), while *Bacteria* were positively correlated with TK, soil organic carbon (SOC), total carbon (TC), soil organic matter (SOM), urease (UE), and sucrase (SUC) (Fig. 12a). Species–environment heatmap analysis segregated the taxa into two clusters: Cluster 1 (positively correlated with PHO, TP, TK, CAT, and SUC): *candidate division NC10*, *Candidatus Rokubacteria*, *Crenarchaeota*, *Nitrospinae*, *Candidatus Aenigmarchaeota*, *einococcus Thermus*, *Abditibacteriota*, *Thaumarchaeota*, *Chloroflexi*, *Candidatus Roizmanbacteria*, *Acidobacteria*, *Candidatus Tectomicrobia*, *Actinobacteria*, *andidatus Dormibacteraeota*, *andidatus Giovannonibacteria*, *andidatus Microgenomates*, *andidate division WWE3*, *yanobacteria*, *tribacterota*, *andidatus Blackallbacteria*, *alneolaeota*, *andidatus Magasanikbacteria*, *roviricota*, *andidatus Saccharibacteria*, and *andidatus_Kaiserbacteria*. Cluster 2 was positively correlated with SOC, TC, SOM, UE, ammonium nitrogen (AMS), and TN: residual taxa (Fig. 12b).

(a) RDA ordination diagram (displaying species). (1) Blue arrows represent environmental factors; arrow length indicates the strength of the factor's influence on community variation, with longer arrows denoting greater influence. (2) The angle between an arrow and an ordination axis reflects the factor–

axis correlation, where smaller angles indicate stronger correlations. (3) Black dots denote species; closer proximity between a species point and an arrow signifies a stronger species–environmental factor interaction. (4) Species points aligned with an arrow indicate a positive correlation between the factor and species variation, whereas those in the opposite direction indicate a negative correlation. (5) Scale ticks on the horizontal and vertical axes represent values generated for samples during regression analysis with environmental factors. (6) The RDA1 and RDA2 axes represent the two variables with the highest explanatory power; their axis labels indicate the proportion of environmental variation explained by each axis, and a larger combined sum corresponds to greater explanatory capacity for overall community structure and species distribution patterns. (b) Species–environmental factor correlation heatmap: Environmental factors are displayed on the horizontal axis (showing the top 20 species with the smallest p values), with species on the vertical axis.

4. Discussion

4.1. Synergistic Drive by Soil Nutrients and Allelopathy

A. adenophora synergistically drives its invasion by altering soil nutrients and releasing allelochemicals. Compared with native species, invasive plants often exhibit increased biomass, increased nitrogen availability, altered nitrogen fixation rates, and the production of litter with higher decomposition rates in invaded habitats (Ehrenfeld 2003). During the initial invasion phase, *A. adenophora* likely promotes its own growth while inhibiting native plants by increasing the soil nutrient content, particularly the nitrogen content (Deng *et al.* 2015; Jiao and Huang 2024). This study demonstrated that *A. adenophora* invasion led to significant accumulation of soil SOC and TN ($p < 0.05$) and weak alkalization of the soil pH (Fig. 2). This may stem from the regulation of soil mineralization processes by allelochemicals, which aligns with the significant correlations observed with the functional genes K00639 and K13038. In areas invaded by exotic species, litter decomposition rates and nutrient availability typically increase (Standish *et al.* 2004; Martin *et al.* 2010), which could also contribute to elevated soil nutrient levels. The allelochemicals released by *A. adenophora* can directly suppress the growth of native plants, reducing their ability to compete for resources (Inderjit *et al.* 2011; Jiao and Huang 2024; Wu *et al.* 2025). Furthermore, the TN and TK contents in *A. adenophora* leaves were greater than those in *A. argyi* leaves (Fig. 4), suggesting that its leaf litter may also be an important supplementary source of soil nitrogen.

4.2. Selective Activation of Enzyme Activities

The invasion process of *A. adenophora* is closely linked to soil nutrients, microbial communities, and enzyme activities (Li *et al.* 2017). Its invasion leads to significant changes in soil nutrients and enzyme activities (Sun *et al.* 2013; Deng *et al.* 2015). *A. adenophora* invasion alters the structure and diversity of soil microbial communities, which in turn affects the activities of enzymes involved in nutrient transformation (Wan-Xue *et al.* 2010; Balamir *et al.* 2017). Microorganisms acquire nutrients by secreting specific extracellular enzymes to degrade soil organic matter (Sinsabaugh *et al.* 2009). This study revealed that under *A. adenophora* invasion, SUC (sucrase) and UE (urease) activities were significantly

greater than those in the control group (CK) ($p < 0.05$) (Fig. 3), indicating that invasion preferentially stimulates the carbon and nitrogen metabolic enzyme systems, accelerating the decomposition of carbohydrates and urea to support rapid growth. In contrast, PHO (phosphatase) activity did not change significantly, suggesting that phosphorus acquisition is not the core of its competitive advantage. This finding, combined with the finding that extracellular polysaccharide synthesis is dominated by certain genes, such as GT105 and CBM42 (Fig. 10c), collectively indicates efficient monopolization of carbon resources.

4.3. Functional Remodelling of Microbial Communities

A. adenophora invasion remodels microbial communities through multiple pathways, thereby affecting soil nutrient cycling and ecosystem functions (Wan-Xue *et al.* 2010; Zhao *et al.* 2019; Li *et al.* 2022a; Wu *et al.* 2025). During plant invasion, bacterial diversity is a primary response variable, with invasive plants often leading to an increase in bacterial diversity (Torres *et al.* 2021). *A. adenophora* invasion shifts the dominant soil microbial composition from *Aeromicrobium* and *Marmoricola* to *Reyranella* and *Bradyrhizobium* (Li *et al.* 2022c). Post-invasion, *A. adenophora* selectively enriches less abundant genera, such as *Clostridium* and *Enterobacter*. At the phylum level, the community shifts from *Bacteroidetes*-dominated to *Proteobacteria*-dominated (Chen *et al.* 2019). This study demonstrated that under *A. adenophora* invasion, the soil microbial Shannon index was lower ($p < 0.05$) (Fig. 7a), and the species composition significantly differed ($p < 0.05$) (Fig. 7e), indicating that invasion resulted in the selection of a reduced number of dominant bacterial groups, such as *Verrucomicrobia*, *Proteobacteria*, and *Acidobacteria*.

4.4. Interactive Feedback in the *A. adenophora*–Soil–Microbe System

A. adenophora establishes an invasive plant–soil feedback mechanism by altering soil properties and microbial communities. Its post-invasion secretions and allelochemicals can inhibit the growth of other plants, thereby reducing competition (Li *et al.* 2017; Kumar *et al.* 2024). Simultaneously, by modifying soil nutrient availability and enzyme activities, *A. adenophora* affects the nutrient uptake of other plants, resulting in a competitive advantage (Deng *et al.* 2015). Furthermore, invasion by *A. adenophora* increases the number of microbes beneficial to its invasion (Chen *et al.* 2019), increasing its invasive capacity and forming a positive invasive plant–soil–microbe feedback loop (Fang *et al.* 2019). As litter serves as a source of nutrient input, invasion by exotic species increases litter decomposition rates and nutrient availability (Standish *et al.* 2004; Martin *et al.* 2010). This further establishes a positive feedback loop of "high-quality litter input → stimulation of enzyme activity → enrichment of functional microbes → nutrient recycling," ultimately leading to intensified invasion.

5. Conclusion

This study reveals the response mechanism of the "invasive plant–soil physicochemical properties–microorganism" system during *A. adenophora* invasion into the karst ecosystem of central Guizhou. At the biochemical level, *A. adenophora* invasion significantly increased the SOC and TN contents and pH

($p < 0.05$), creating a weakly alkaline, nitrogen-enriched environment. This reflects a "carbon–nitrogen prioritization" strategy under karst regional conditions while simultaneously forming a weakly alkaline soil environment favourable for *A. adenophora* survival. Under invasion conditions, sucrase (SUC) and urease (UE) activities were significantly greater than those in the control group (CK) and were positively correlated with the abundances of *Proteobacteria* and *Gemmatimonadetes*. At the microbial level, *A. adenophora* invasion reduced the microbial Shannon index ($p < 0.05$) but enriched bacteria, such as *Verrucomicrobia* and *Candidatus Eisenbacteria*. It upregulated genes involved in nutrient acquisition (GT105/CBM42), allelochemical synthesis (K00639/K13038), and stress resistance (merG/recG), enhancing niche competitive advantages. *A. adenophora* invasion establishes a self-reinforcing feedback loop of "*A. adenophora* invasion → soil nutrient enrichment and enzyme stimulation → microbial functional adaptation," providing a favourable foundation for its invasion success.

Declarations

Declaration of Competing Interest

We affirm that no competing financial interests or personal relationships exist which could be reasonably construed as influencing the research presented in this work.

Funding

This study was supported by the Youth Science and Technology Development Project of Guizhou Academy of Sciences (Grant No. 52000024P00280H10021H), Guizhou Provincial Key Laboratory of Agricultural Biosafety (Grant No. QKHZSYS[2026]024) and the earmarked fund for Guizhou Modern Agriculture Research System (Grant No. GZSTCYJSTX[2025]–01).

Author Contribution

L is responsible for providing the ideas and funding sources, W is mainly in charge of the methods, and W is responsible for the overall conception of the text and the experiments.

Acknowledgments

We extend our sincere gratitude to Dr. Weijie Li for his support and Dr. Jiaguo Wang for his assistance. We acknowledge AJE (www.aje.cn) for professional language editing services.

Data availability

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Figures

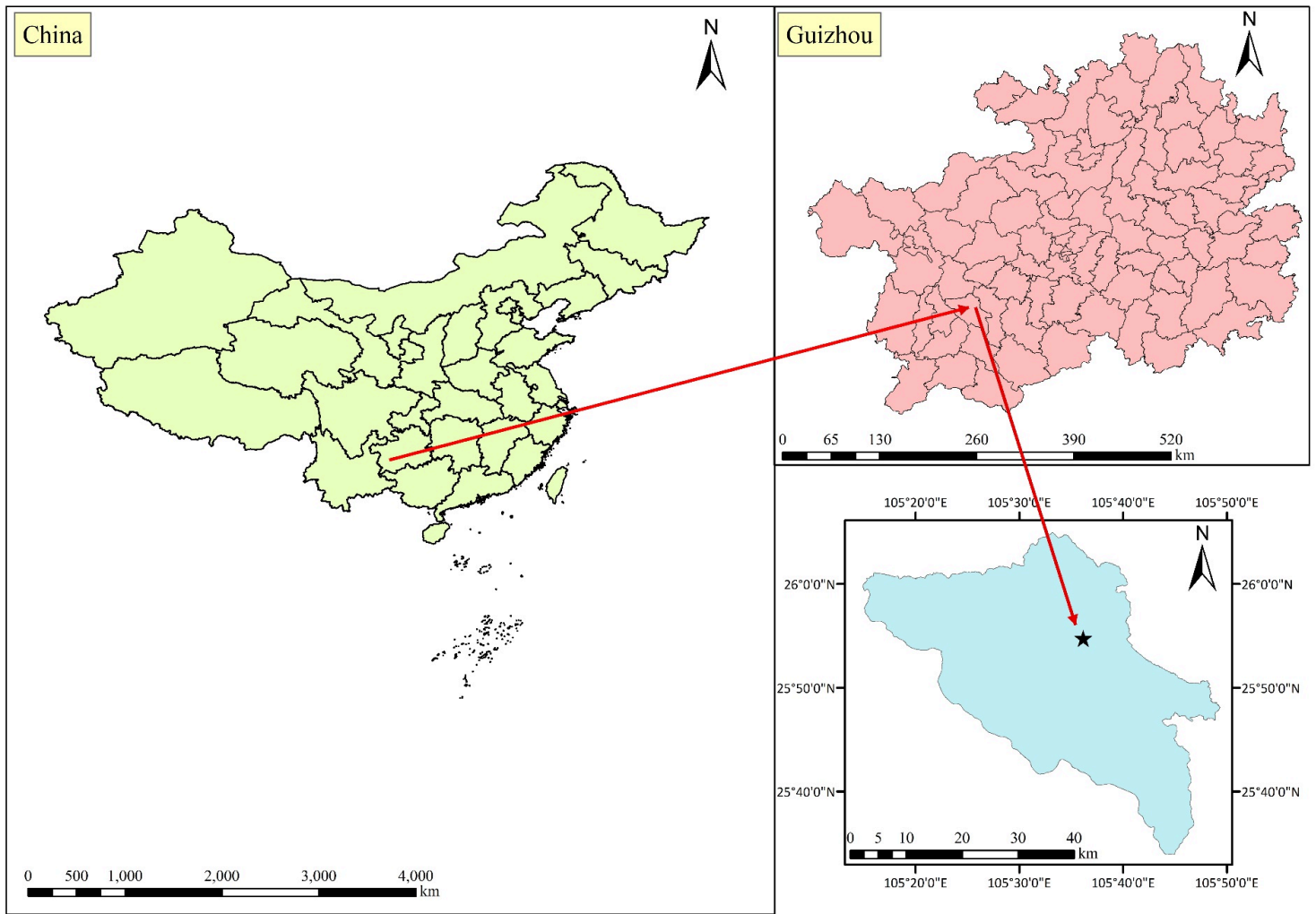


Figure 1

Location map of the study area.

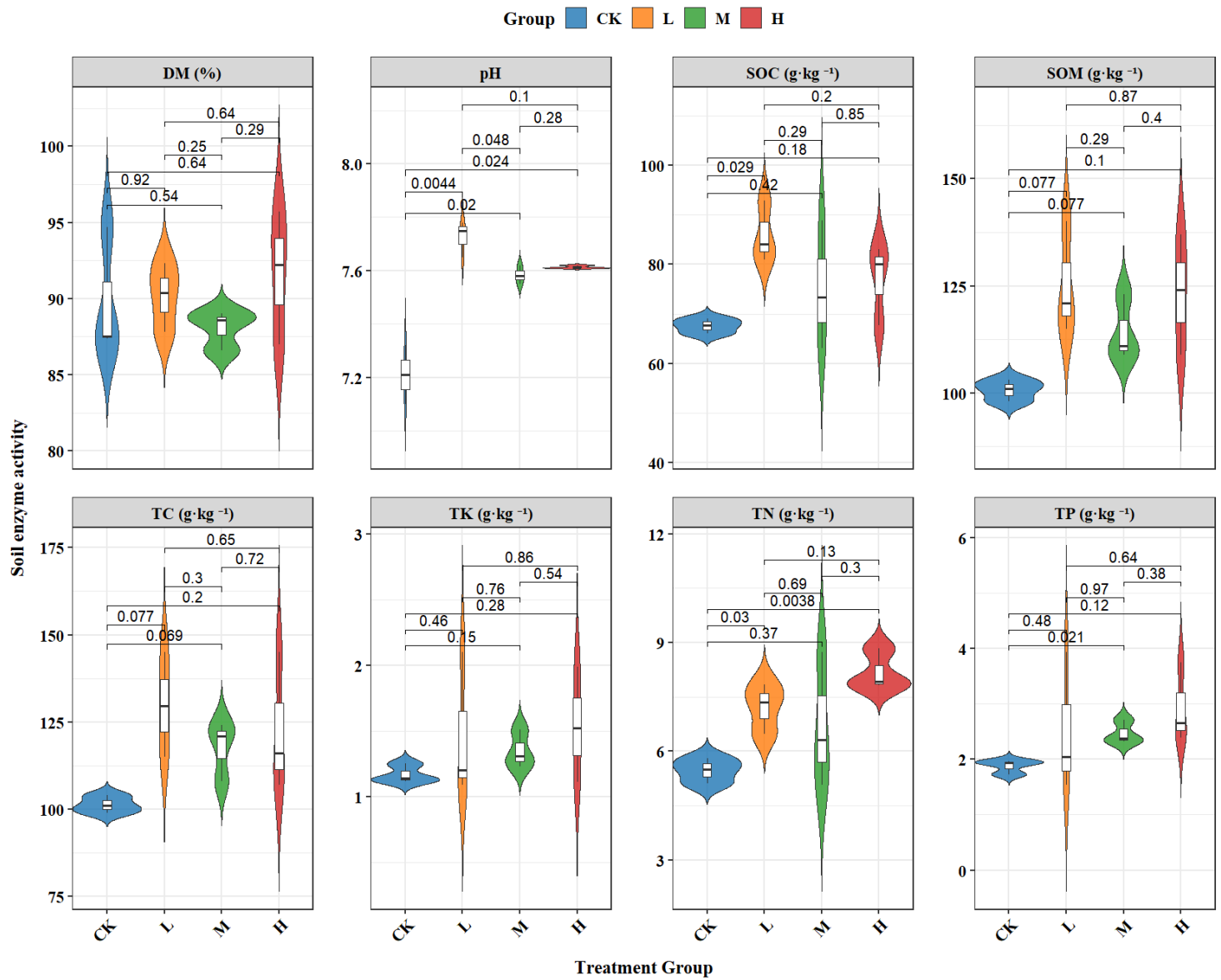


Figure 2

Effects of *A. adenophora* invasion on the soil nutrient content.

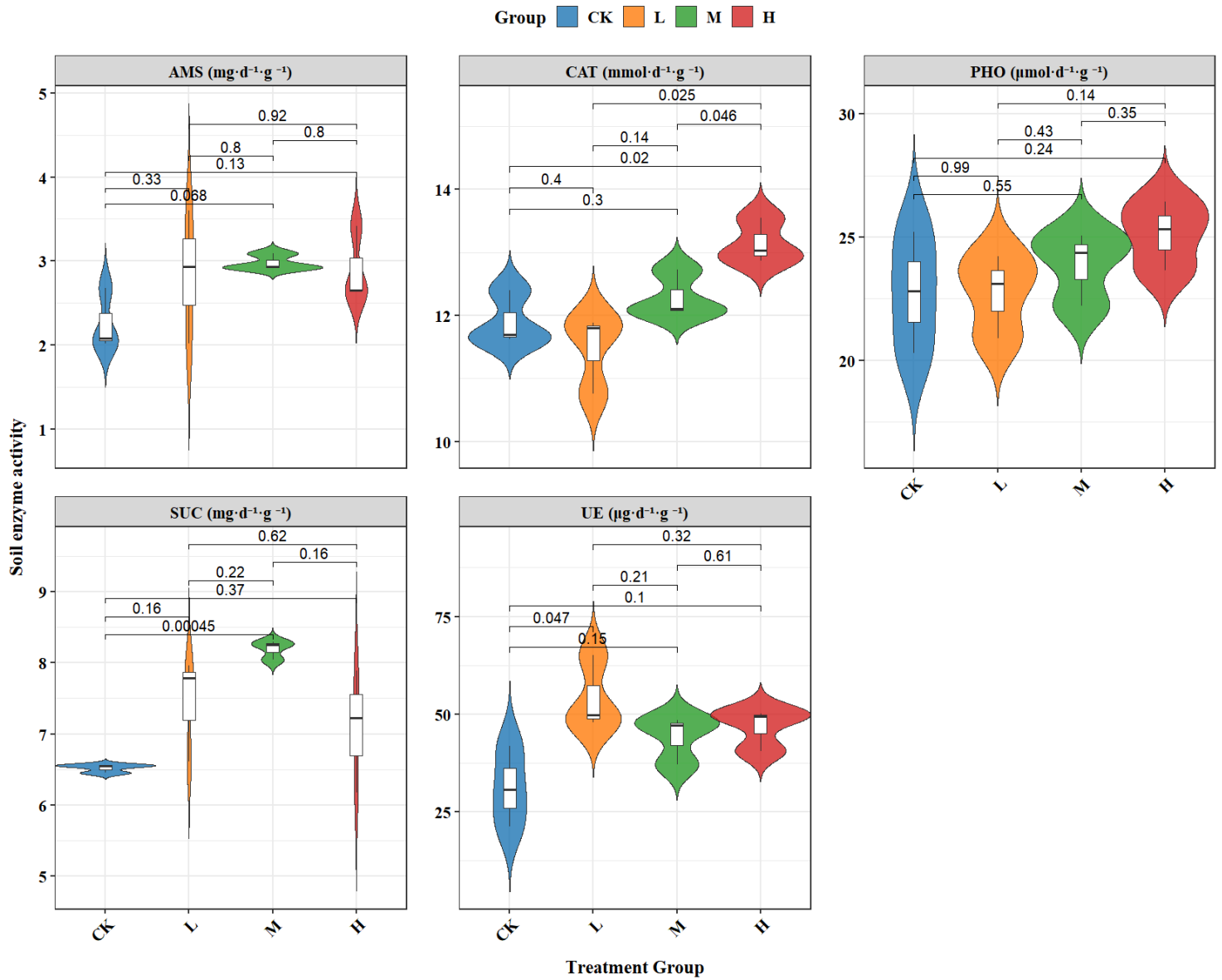


Figure 3

Effects of *A. adenophora* invasion on soil enzyme activities.

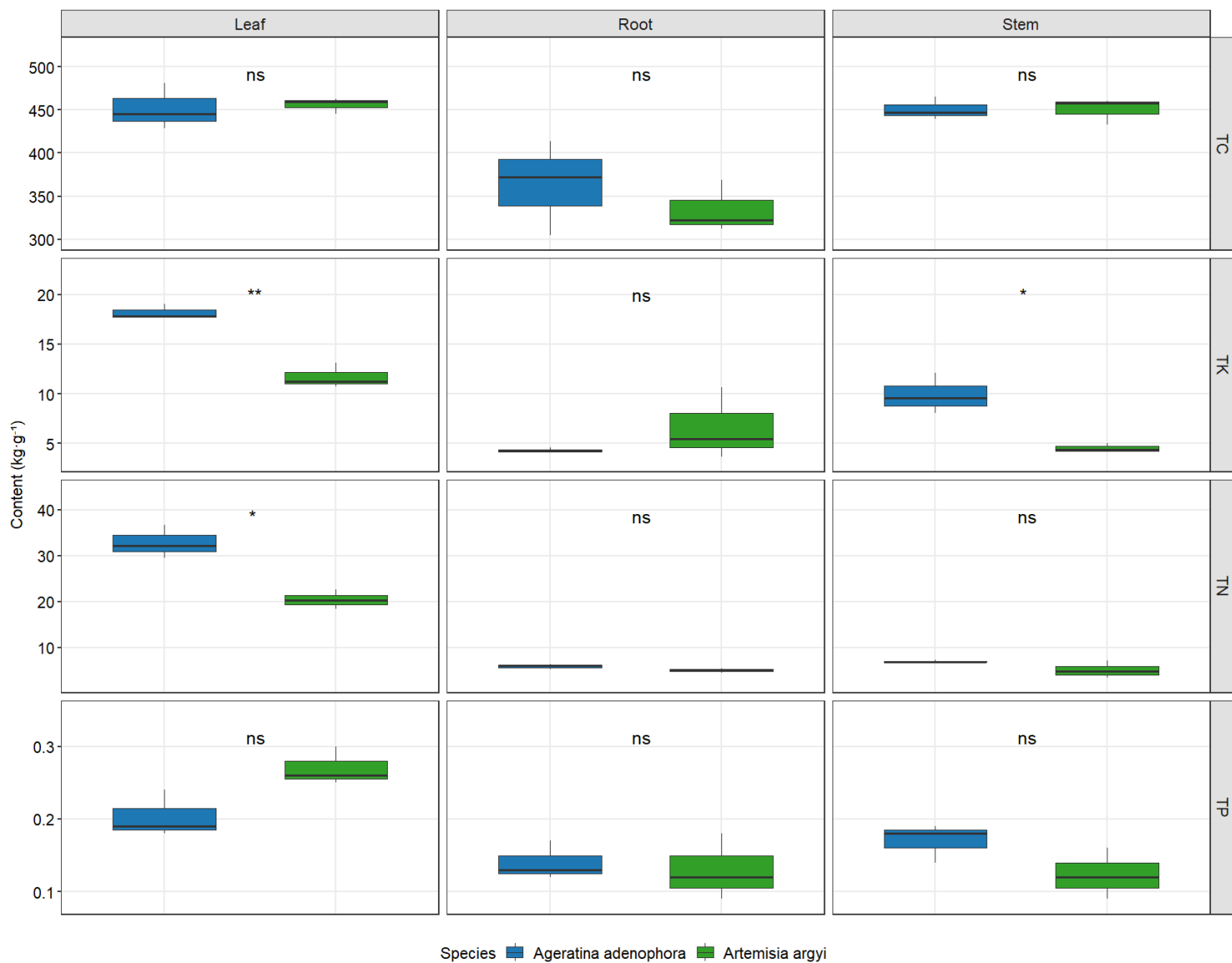


Figure 4

Nutrient contents of *A. adenophora* and *A. argyi*.

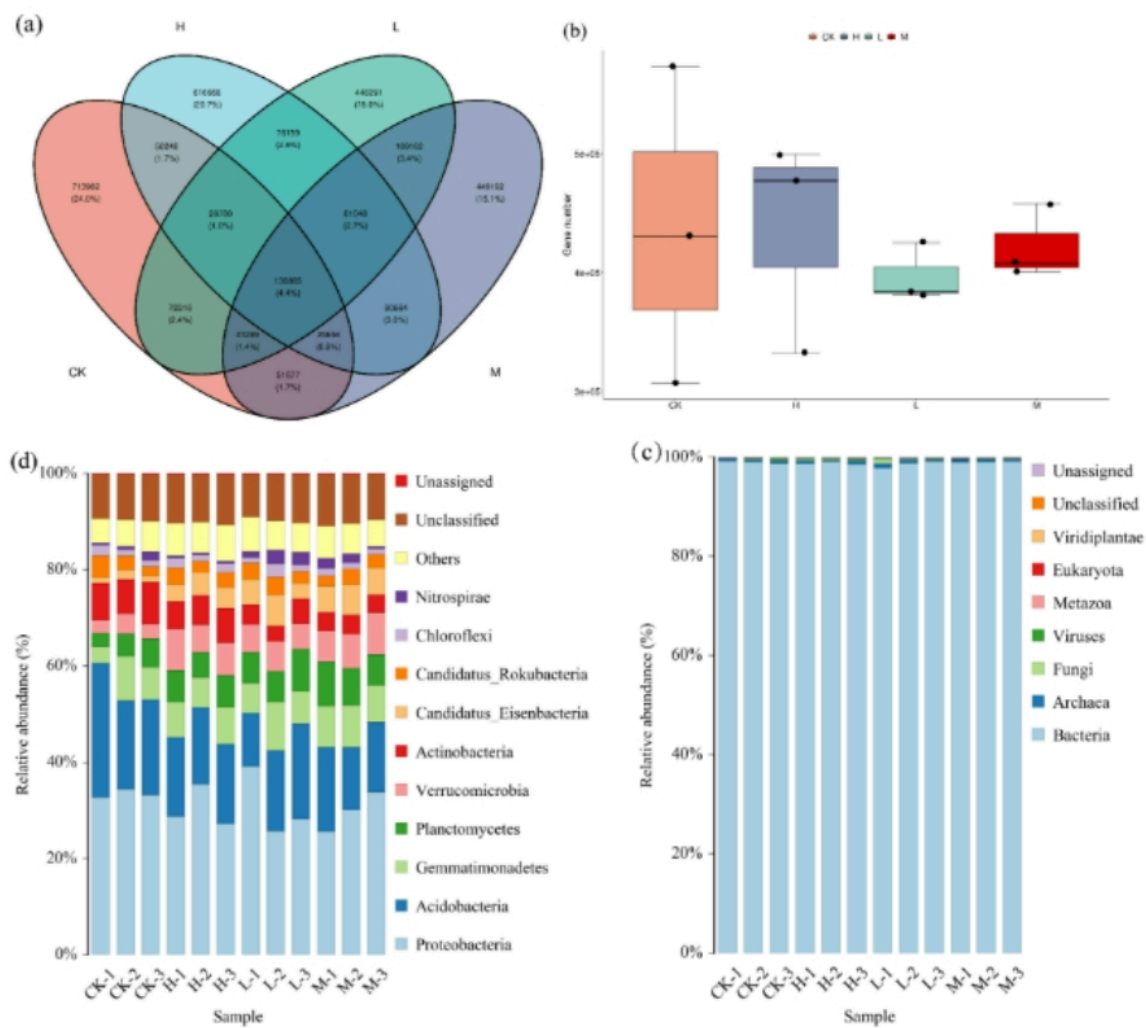


Figure 5

Non-redundant genes and species composition.

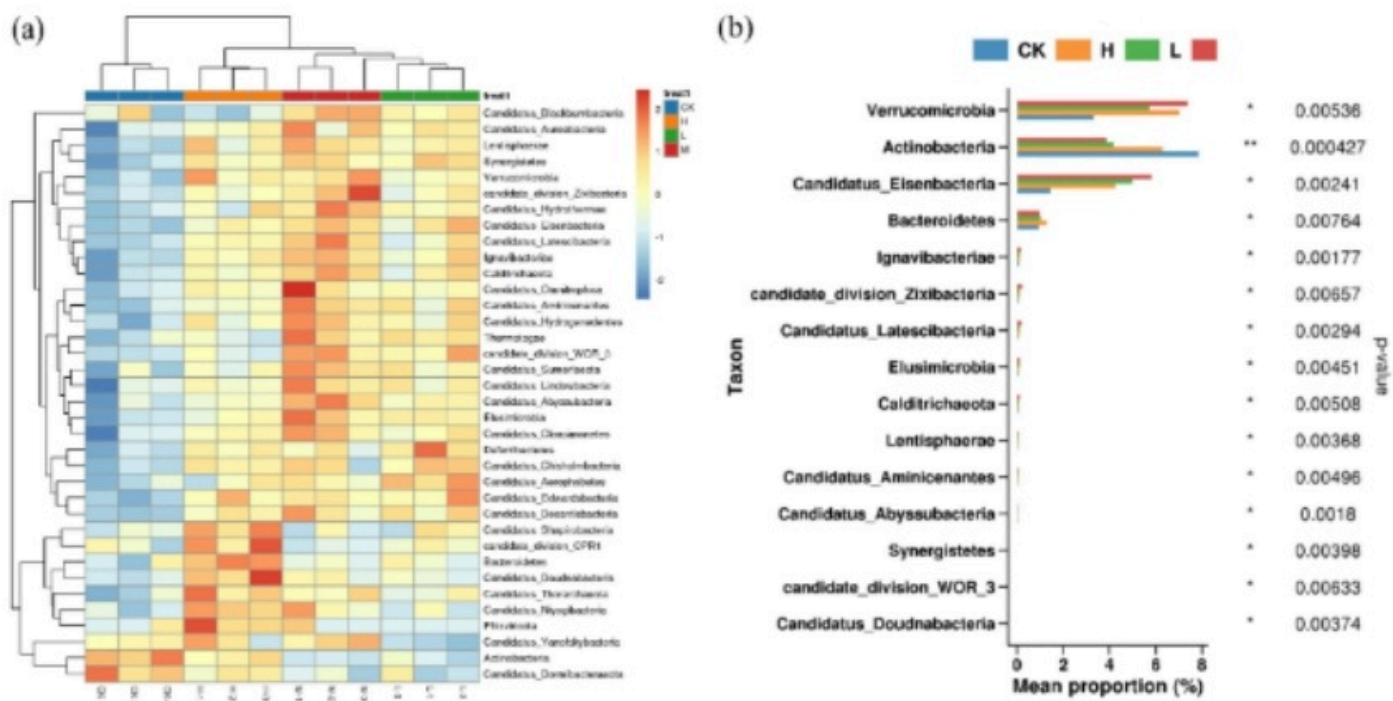


Figure 6

Species composition divergence.

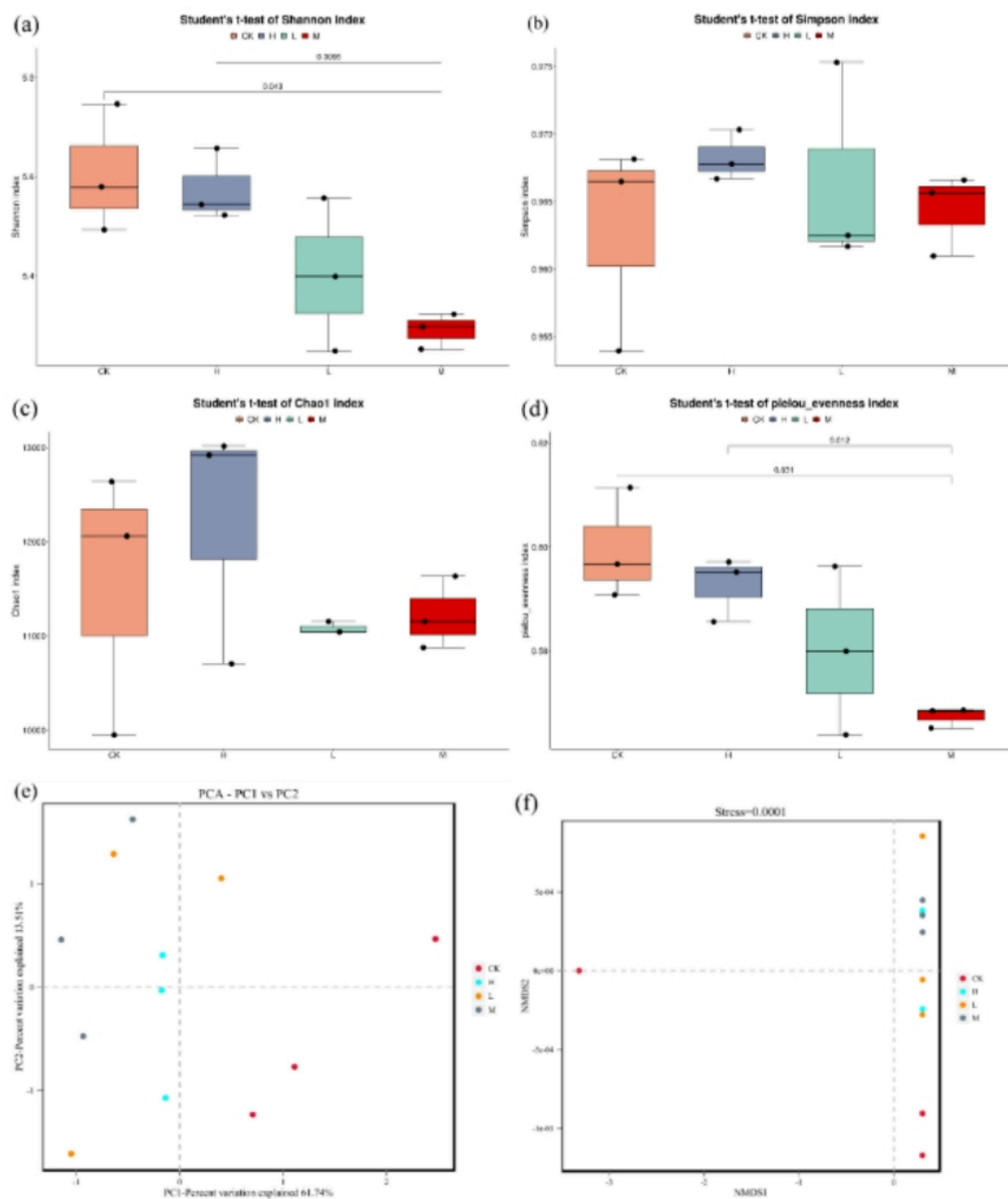


Figure 7

Variations in soil microbial alpha and beta diversity.

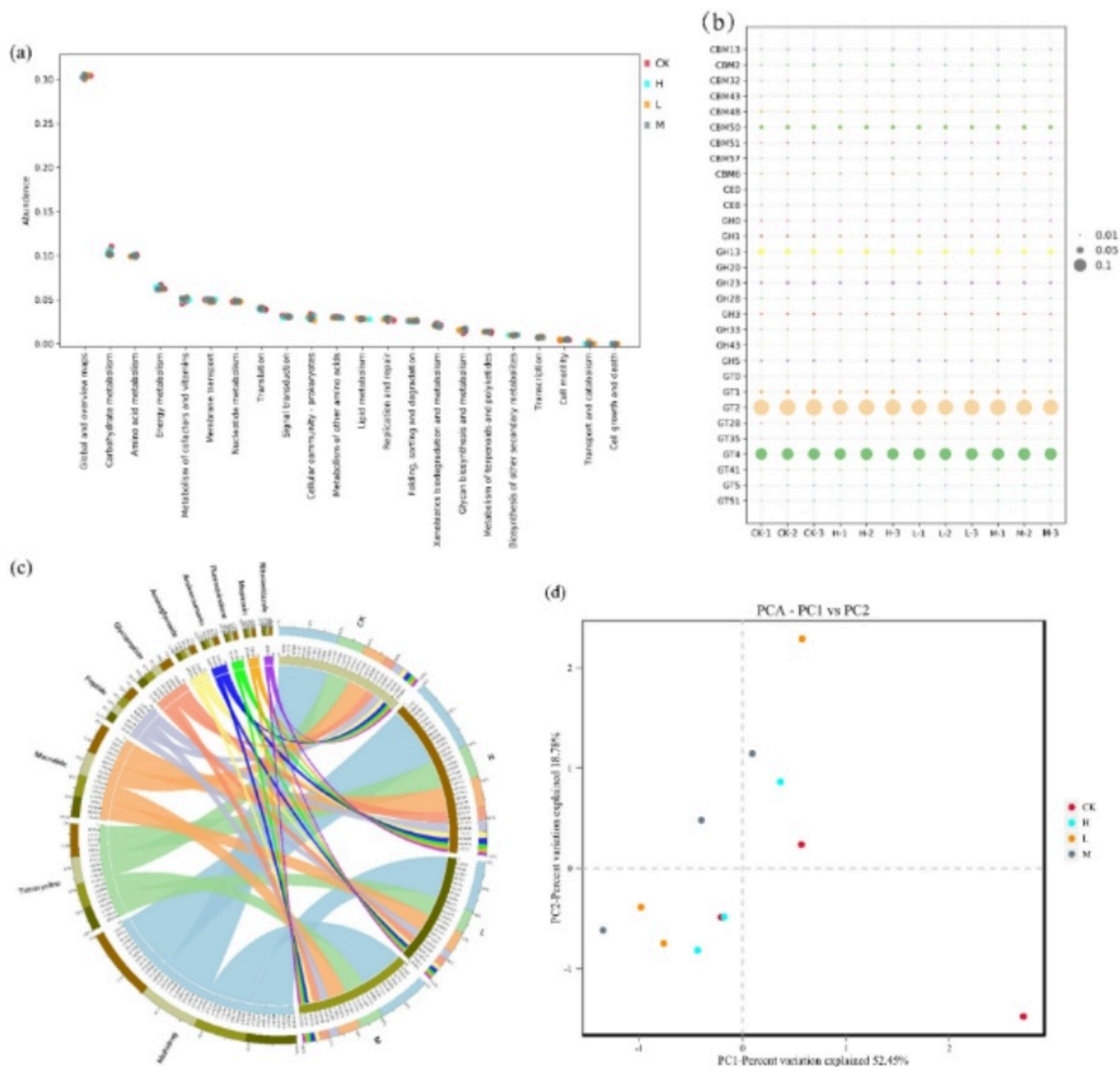


Figure 9

Functional gene composition and diversity analysis.

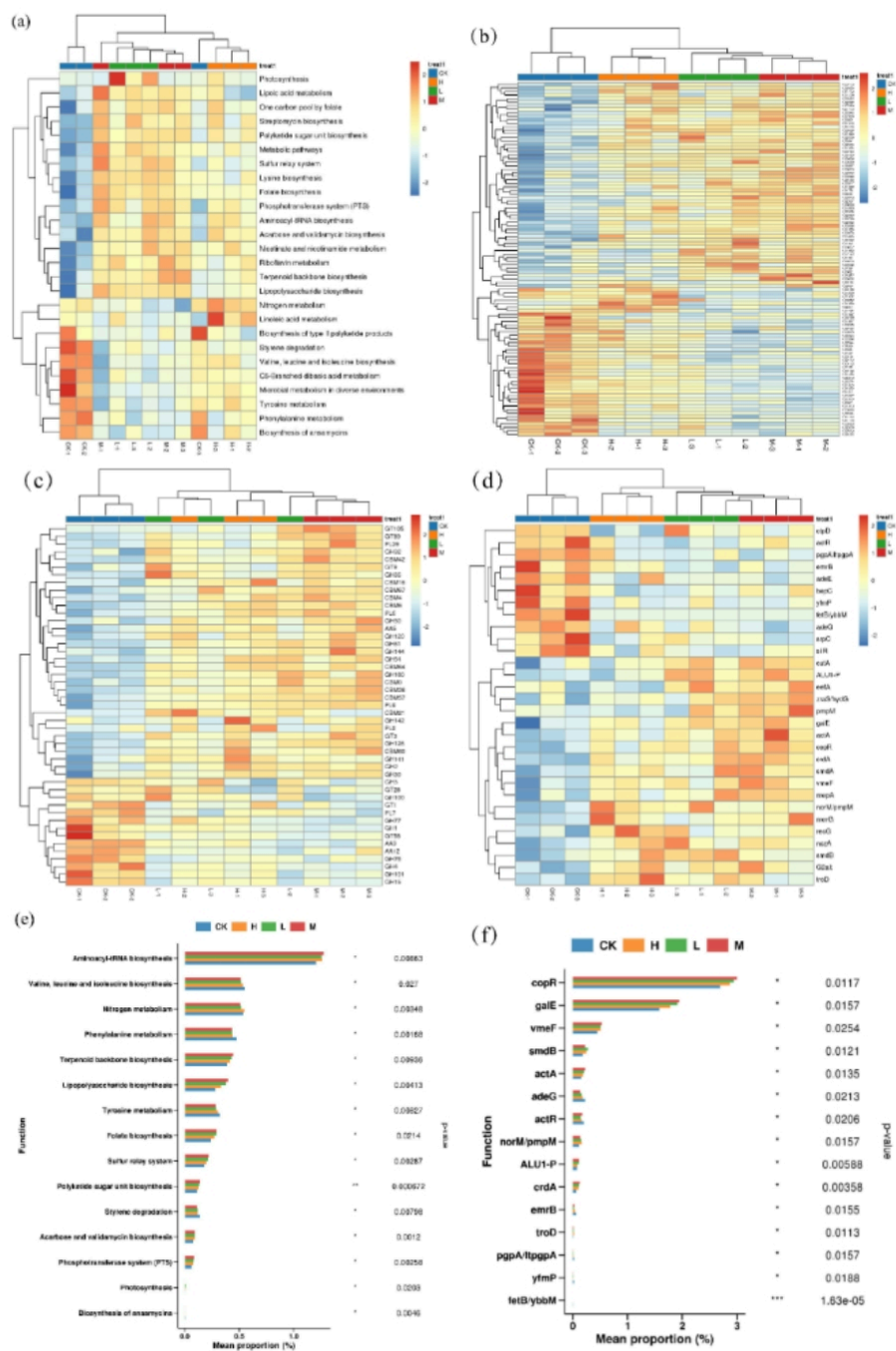


Figure 10

Differential functional genes.

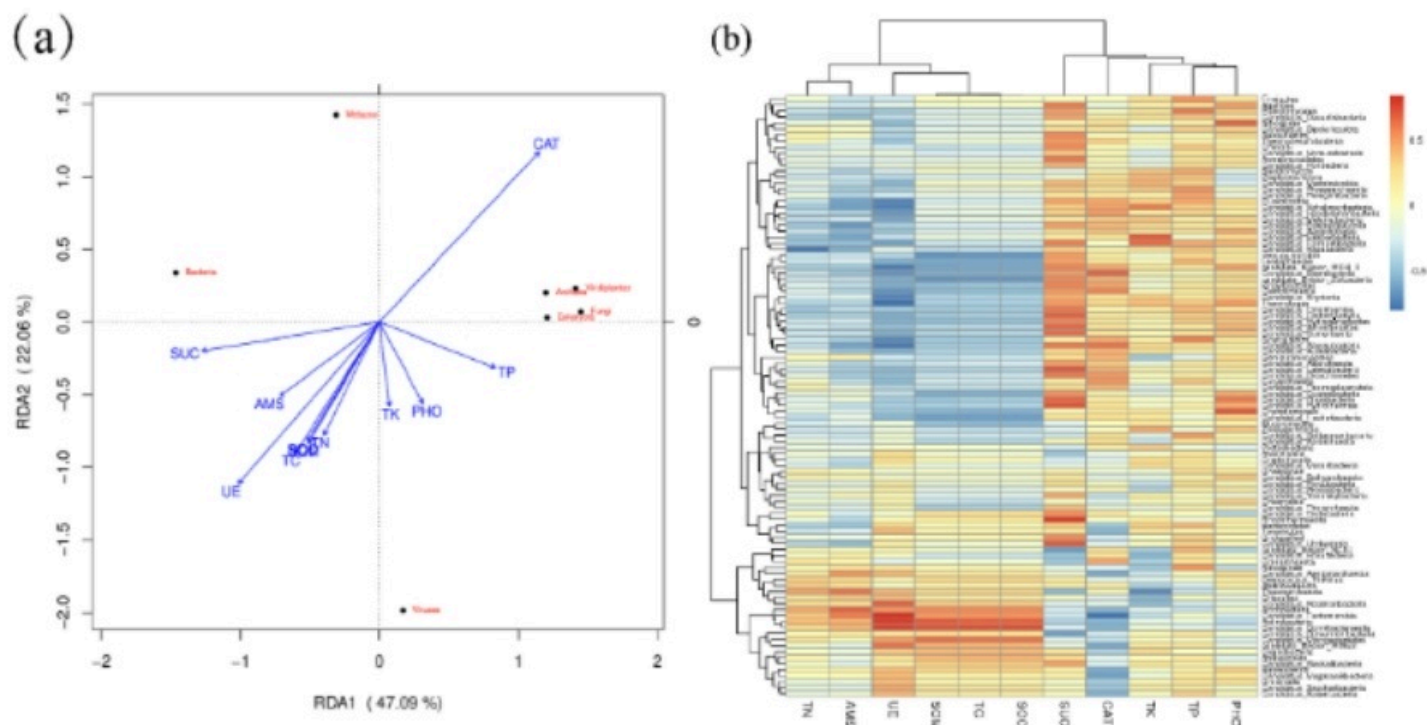


Figure 12

Species–environmental factor correlation analysis.