Effects of planting Melia azedarach L. on soil properties and microbial community in saline-alkali soil

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Abstract

Saline-alkali soils are widely distributed in China, affecting plant growth and sustainable development of ecosystems. This study characterized the effects of planting Melia azedarach L. on chemical properties and microbial communities in saline-alkali soils [bare (CK), bulk (BS) and rhizosphere soil (RS)]. Compared with the bare soil, planting Melia azedarach L. lowered salt content and concentrations of extractable Na, K, Ca, Mg and Cl-, but significantly increased organic matter, total nitrogen, total phosphorus, available phosphorus, soil urease activity and alkaline phosphatase activity in the rhizosphere soil. High-throughput sequencing results indicated that bacterial richness and diversity decreased in the order RS>BS>CK. The richness of fungi was ranked RS>CK>BS, and their diversity decreased in the order CK>RS>BS. The three dominant bacterial phyla were Proteobacteria, Actinobacteria and Bacteroidetes, and the three dominant fungal phyla were Ascomycota, Basidiomycota and Glomeromycota. Redundancy analysis indicated that total phosphorus concentration and alkaline phosphatase activity significantly influenced bacterial diversity, whereas soil Ca and Mg concentrations were closely related to the fungal community diversity. In conclusion, planting Melia azedarach L. improved soil properties, increased the diversity and richness of soil microbial communities, and thus ameliorated the saline-alkali soil.

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Abstract: Saline-alkali soils are widely distributed in China, affecting plant growth and sustainable development of ecosystems. This study characterized the effects of planting *Melia azedarach L.* on chemical properties and microbial communities in saline-alkali soils [bare (CK), bulk (BS) and rhizosphere soil

(RS)]. Compared with the bare soil, planting *Melia azedarach L.* lowered salt content and concentrations of extractable Na, K, Ca, Mg and Cl^-, but significantly increased organic matter, total nitrogen, total phosphorus, available phosphorus, soil urease activity and alkaline phosphatase activity in the rhizosphere soil. High-throughput sequencing results indicated that bacterial richness and diversity decreased in the order RS>BS>CK. The richness of fungi was ranked RS>CK>BS, and their diversity decreased in the order CK>RS>BS. The three dominant bacterial phyla were Proteobacteria, Actinobacteria and Bacteroidetes, and the three dominant fungal phyla were Ascomycota, Basidiomycota and Glomeromycota. Redundancy analysis indicated that total phosphorus concentration and alkaline phosphatase activity significantly influenced bacterial diversity, whereas soil Ca and Mg concentrations were closely related to the fungal community diversity. In conclusion, planting*Melia azedarach L.* improved soil properties, increased the diversity and richness of soil microbial communities, and thus ameliorated the saline-alkali soil.

KEYWORDS

saline-alkali soils, amelioration, *Melia azedarach L.* , high-throughput sequencing, microbial communities

1 INTRODUCTION

Saline-alkali soils are widespread globally, occupying about 9.5×10^8 ha at present (Wang *et al.* , 2003). In China, the total area of saline-alkaline soils is about 3.6×10^7 ha, accounting for 4.88% of the available land area in the country; importantly, nearly 7% of cultivated soils contain excessive salt (Mao *et al.* , 2016). Soil salinization has become a global concern, and it is one of the main reasons for poor crop growth, soil desertification and ecological degradation (Pan *et al.* , 2011; Singh, 2016). Saline-alkali soils are characterized by high soil salinity, poor soil structure, sparse (if any) vegetation coverage, and low land productivity (Wang *et al.* , 2011a; Zhao *et al.* , 2018).

Various techniques have been used for amelioration of saline-alkali soil, including chemical, physical, biological and engineering improvements to increase fertility and crop yield (Liu *et al.* , 2015). Deep ploughing to 30-40 cm could break the hardened soil layers and reduce soil salinity (Riley & Ekeberg, 1998). Phytoremediation is a biological technique that uses plants to improve saline-alkali soils (Imadi *et al.* , 2016; Mishra *et al.* , 2002; Qadir & Oster, 2002). Using plants can effectively improve the physical and chemical properties of saline-alkali soils, increase the stability of the ecosystem, and enhance the carrying capacity of the environment; notably, these phytoremediation-related improvements of saline-alkali soils are stable and durable (Shahbaz & Ashraf, 2013).

Melia azedarach L. is an important deciduous tree species. It has ornamental value and is widely used in making furniture, plywood and toys as well as for firewood. In addition to the direct economic benefits, it may be used in re-vegetation of degraded areas (Husain & Anis, 2009). *Melia azedarach L.* has high medicinal value. Flowers, leaves, fruits and root bark can be used as medicine. Studies have shown that the extract of *Melia azedarach L.* leaves has anti-cancer effects (Ervina *et al.* , 2020). *Melia azedarach L.* has the advantages of rapid growth, strong adaptability, capacity to grow of low-fertility soil, and strong salt resistance (Dias *et al.* , 2014).

Amelioration of saline-alkali soils is contingent on improving basic soil properties as well as enhancing the diversity of soil microbes (Peng *et al.* , 2017). However, there is little research about a role of *Melia azedarach L.* in improving saline-alkali soils. We explored the potential of *Melia azedarach L.* to improve saline-alkali soil by characterizing its influence on the soil chemical properties, enzyme activities and microbial diversity to provide a theoretical basis for saline-alkali soil remediation.

2 MATERIALS AND METHODS

2.1 Field set-up

The experimental area was located in Xu Wei New District (34°37'N, 119deg29'E), located in the warm humid monsoon climate zone, with 14degC average annual temperature, 901 mm annual average rainfall and 855 mm annual average evaporation. Before planting *Melia azedarach L.* , field was ploughed to 30-40 cm.

The size of a *Melia azedarach L.*planting hole was 70x70x70 cm, and the row spacing was 2x3 m. Three replicate plots were used to sample bare soil (CK), bulk soil (BS, far away from *Melia azedarach L.* roots) and rhizosphere soil of*Melia azedarach L.* (RS).

2.2 Soil sample collection

In August 2019, bare soil was taken randomly in places without vegetation to a depth of 0–20 cm using a soil auger (6 cm diameter). After excavating the 2.5-year-old *Melia azedarach* roots, bulk soil was collected far away from *Melia azedarach* roots, whereas rhizosphere soil (about 1-mm-thick layer around rots) was collected using a brush. Each sample had three biological replicates. A portion of each soil sample was snap-frozen in liquid nitrogen and kept at -80 for molecular analyses, whereas the other portion was transferred to the lab on ice and stored at 4 for analysing physical and chemical properties.

2.3 Measurement of soil properties

To determine soil salinity, pH, Na, K, Ca, Mg and Cl^-, we mixed 20.0 g of soil sample with 100 mL of carbon dioxide-free water, passed the suspension through qualitative filter paper, and collected the filtrate for analyses. Soil salinity was determined by a conductivity meter (FE38-Standard, Mettler Toledo, China). The soil pH was determined by a calibrated pH meter (FE28-Standard, Mettler Toledo, China). In the same supernatant filtrate, concentrations of soil Na, K, Ca and Mg were determined by ICP-AES(Optima 2100DV, Pekin-Elmer, USA) (Xin *et al.* , 2010). Soil Cl^- was measured by the silver nitrate titration method (Asakai, 2018).

To determine total nitrogen, total phosphorus and total potassium, we weighed 0.25 g of soil, added 4 mL of aqua regia, digested at 180degC for 20 min, then added 2 mL perchloric acid, and digested at 280degC until the brown smoke disappeared. Ultrapure water was used to make up the volume to 100 mL, followed by filtration. The filtrates were measured by ICP-AES (Optima 2100DV, Pekin Elmer, USA) (Semenkov & Koroleva, 2019).

Soil organic matter was measured using the potassium dichromate volumetric method (Osman *et al.* , 2013). Available phosphorus was determined by $NaHCO_3$ extraction followed by the molybdenum-antimony colorimetric method (Wang *et al.* , 2011b). Available potassium was determined by flame photometry (AP1200, Aopu, China) (Bilias & Barbayiannis, 2019).

2.4 Soil enzyme activities

Soil alkaline phosphatase was determined by a Soil Alkaline Phosphatase (S-AKP) Assay Kit (Cat. No. BC0280, Solarbio, Beijing, China) by measuring phenol produced during substrate hydrolysis (Ma *et al.* , 2011). For soil catalase activity, we used a Soil Catalase (S-CAT) Assay Kit (Cat. No. BC0105, Solarbio, Beijing, China) (Johnson & Temple, 1964). Soil urease activity was measured by a Soil Urease (S-UE) Assay Kit (Cat. No. BC0120, Solarbio, Beijing, China). The indophenol blue colorimetric method was used to measure the NH_3-N produced in urea hydrolysis by urease (Vlek *et al.* , 1980).

2.5 Microbial analyses

2.5.1 Soil DNA extraction

Soil DNA was extracted from 0.3 g of sieved (1 mm) soil using a Power Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The extracted genomic DNA was assessed by 1% w/w agarose gel electrophoresis and stored at -80degC (Rodrigues *et al.* , 2013).

2.5.2 PCR amplification

PCR amplification of the V3-V4 region of bacterial 16S rDNA was conducted using the universal primers 341F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR amplification of the fungal ITS1-ITS2 region was performed using the universal primers ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-TGCGTTCTTCATCGATGC-3'). Amplification reactions were performed in 25 μL volume containing 12.5 μL 2xTaq Plus Master Mix, 5 μM of each primer,

and 30 ng of template. PCR was carried out under the following conditions: 94 for 5 min, 28 denaturation cycles (bacteria) or 34 denaturation cycles (fungi) at 94 for 30 s, annealing at 55 for 30 s, extension at 72 for 60 s, extension at 72 for 7 minutes, and then cooling to 4°C.

2.5.3 MiSeq sequencing

PCR products were recovered by 2% w/w agarose gel electrophoresis, purified using an Axy Prep DNA Gel Recovery Kit (AXYGEN), eluted by Tris_HCl, and detected by 2% w/w agarose electrophoresis. High-throughput sequencing was performed using Illumina MiSeq PE300 sequencing technology. QIIME (Quantitative Insights Into Microbial Ecology) quality filters were used to filter the reads. The CD-HIT pipeline was used for picking operational taxonomic units (OTUs) with similarity of 97%.

Based on the 16S rDNA PCR amplification, a linear discriminant (LDA) effect size (LEfSe) analysis was conducted to identify species with significant differences in richness among treatment groups and to construct cladograms (Segata *et al.* , 2011).

In order to estimate alpha diversity, the OTU table was rarified, and three metrics were calculated: Chao 1 index to estimate the richness, the observed OTUs, and Shannon index to estimate diversity (Vishnivetskaya *et al.* , 2011).

2.6 Statistical analysis

Each type of soil was measured in three independent replicate samples. The mean values and standard error of the means were calculated. Statistical analyzes were performed using IBM SPSS Statistics 20.0 (IBM, Armonk, New York, USA) and the mean differences were compared using Duncan's new multiple range test (p[?]0.05). The software used to draw figures was GraphPad Prism 8.0.1 and R package ggplot2 (version 3.2.0). The Venn diagrams of the OTUs were generated using the R package venn diagram (version 1.6.20). The PCA and redundancy analysis were created using the R package vegan (version 2.5–5). The correlations among soil physico-chemical and enzymatic properties were generated using the R package corrplot (version 0.84).

3 RESULTS

3.1 Basic soil properties

3.1.1 Soil salinity and pH

Soil salinity decreased significantly in the following order: CK>BS>RS. Compared with CK and BS, the salinity of RS decreased by 87% and 76%, respectively (Figure 1a). Soil pH was a little higher in RS than CK and BS (Figure 1b).

3.1.2 Concentrations of extractable soil elements

Concentrations of extractable Na, Mg, K, Ca and Cl^- all decreased in the order: CK>BS>RS, and there were significant differences between CK and RS (Figure 2). Compared with CK and BS, Na concentration in RS decreased by 76% and 64%, respectively (Figure 2a). The concentration of Mg in soil was very low, only 0.027 g/kg in RS (Figure 2b). The Cl^- concentration in soil changed the most in the process of phytoremediation, with CK being 9.6 times higher than RS (Figure 2e).

3.1.3 Soil nitrogen, phosphorus, potassium and organic matter

Total nitrogen and organic matter in soil increased in the order of CK<BS<RS (Table 1). Total phosphorus and available phosphorus were significantly improved only in RS. Total potassium and available potassium did not differ significantly among the soil samples.

3.1.4 Soil enzyme activities

The activity of soil alkaline phosphatase increased in the order: CK=BS<RS, and was higher in RS by 85% and 74% compared with CK and BS, respectively (Figure 3a). The urease activity in the soil showed the

same trend (Figure 3b). Catalase activity did not differ significantly among soils (Figure 3c).

3.2 Soil microorganisms

3.2.1 Analysis of soil microbial diversity

The sequencing coverage rate was greater than 0.986 (Table 2), indicating that the sequencing information properly reflected a vast majority of microbial diversity in soil. Bacterial Shannon and Chao1 indices were both in the order of CK<BS<RS, with significant differences, indicating that *Melia azedarach L.*increased the richness and diversity of bacterial communities. There was no significant difference in fungal Chao1 index, whereas the fungal Shannon index was significantly lower in BS and RS compared with CK, indicating that *Melia azedarach L* . enhanced fungal richness, but had no significant effect on fungal diversity.

The bacterial OTU values in CK, BS and RS were 2242, 2636 and 3388, respectively (Figure 4a). The fungal OTU values in CK, BS and RS were 1107, 782 and 910, respectively (Figure 4b). For both bacteria and fungi, the OTU values were greater in RS than BS, indicating *Melia azedarach L.* roots played an important role in enriching soil microbial populations.

PCA (Principal Component Analysis) was performed at the OTU level. The results showed discrete groupings of the three soil sample types, indicating relatively large differences among them (Figure 5).

3.2.2 Analysis of microbial community structure

Bacterial community composition included 11 identified phyla, with Proteobacteria, Actinobacteria and Bacteroidetes being the three dominant phyla (Figure 6a). Proteobacteria was the most abundant phylum in soil; the relative abundance in CK, BS and RS was 40-45%, 31-44% and 40-46%, respectively. The relative abundance of Actinobacteria in CK, BS and RS was 27-31%, 17-21% and 11-16 %, and that of Bacteroidetes was 6.3-8.4%, 13-19% and 11-12%, respectively. The proportion of other bacterial phyla was relatively low.

A total of nine fungal phyla were identified (Figure 6b). Ascomycota, Basidiomycota and Glomeromycota were the three dominant fungal phyla. The relative abundance of Ascomycota in CK, BS and RS was 27-37%, 82-87% and 44-69%, respectively. The relative abundance of Basidiomycota was 3.6-12%, 3.2-5.0% and 23-35%, and that of Glomeromycota was 9.5-12%, 0.09-0.15% and 0.01-0.28%, respectively. The abundance of the three dominant fungal phyla differed widely among the soils. Ascomycota had the highest abundance in BS, Basidiomycota had the highest abundance in RS, and Glomeromycota were found mainly in CK.

The cladograms showed the taxa (highlighted by small circles) that played an important role in the microbial community. The taxonomic tree of bacteria (Figure 7a) showed that, at the class level, Acidimicrobiia, Nitriliruptoria, Cytophagia, Longimicrobia were abundant in the bulk soil, Chloroplast, S0134_terrestrial_group, Betaproteobacteria, Deltaproteobacteria were abundant in the rhizosphere soil. At the order level, Chromatiales was abundant in the bare soil, Acidimicrobiales, Nitriliruptorales, Cytophagales, Longimicrobiales, Erythrobacteraceae were riched in the bulk soil, Rhizobiales, Myxococcales, Chromatiales were riched in the rhizosphere soil. At the family level, OM1_clade, Nitriliruptoraceae, Longimicrobiaceae, Erythrobacteraceae were abundant in the bulk soil. The taxonomic tree of fungi (Figure 7c) showed that at the class level Rhizophlyctidomycetes was abundant in the rhizosphere soil. At the order level, Microascales were abundant in the bulk soil, Hypocreales, Agaricales, Rhizophlyctidales were abundant in the rhizosphere soil. At the family level, Didymosphaeriaceae was abundant in the bare soil, Chaetomiaceae were abundant in the bulk soil, Rhizophlyctidaceae was abundant in the rhizosphere soil.

The LDA scores distribution histograms showed significant differences in richness among the soils, with the length of the bars representing the magnitude of the microbial influence. Regarding bacterial genera,*Thiohalophilus* was abundant in the bare soil, *Cesiribacter*was abundant in the bulk soil (Figure 7b). At the level of fungal genera, *Malbranchea* was abundant in the bulk soil, and*Rhizophlyctis* was enriched in the rhizosphere soil (Figure 7d).

3.3 Association analysis between microorganisms and soil properties

Microbial data were combined with soil physico-chemical and enzymatic properties to explore the correlations between the environmental factors and microbial abundance and diversity. Salinity, Na, K, Ca and Cl^- showed significant negative correlations with bacterial Chao1 index and Shannon index. Phosphorus, organic matter, urease and total nitrogen were significantly and positively correlated with bacterial Chao1 index and Shannon index (Figure 8).

The effects of soil environmental factors on microbial phyla were analyzed by RDA (Figure 9). Total phosphorus and alkaline phosphatase in soil had significant influence on bacterial diversity. Available potassium and catalase had a positive correlation with Proteobacteria, and Mg and Ca correlated positively with Actinomycetes. Total potassium had a positive correlation with Bacteroidetes (Figure 9a).

The RDA analysis of fungi showed that Ca and Mg were closely related to fungal diversity. Total potassium had a positive correlation with Ascomycota, pH and total phosphorus correlated positively with Basidiomycota, and Mg and Ca had a positive correlation with Glomeromycota (Figure 9b).

4 DISCUSSION

Saline-alkali soils have poor productivity unless improved by appropriate ameliorative measures (Singh *et al.* , 2016). Phytoremediation has attracted increasing attention in amelioration of saline-alkali soils because of its low economic cost and environmentally friendly characteristics (Qadir *et al.* , 2003). In this study, the positive effect of planting *Melia azedarach L.* on properties of saline-alkali soil was obvious. *Melia azedarach L.* reduced soil salinity and the content of extractable elements, increased soil total nitrogen, total phosphorus, available phosphorus, organic matter and enzyme activities, indicating it could improve the physical and chemical properties of saline-alkali soils.

Soil salinity is an important factor restricting global sustainable development, aggravating soil water and nutrient losses (Nouri *et al.* , 2017). Excessive salinity reduces soil productivity, affects the metabolism of soil organisms, interferes with ionic balance, and induces osmotic stress in plants (Pan *et al.* , 2011; Yue *et al.* , 2020). In the study presented here, soil salinity reduced the diversity and abundance of bacteria (Figure 8); however, planted *Melia azedarach L.* significantly reduced soil salinity, changing the soil from highly saline to slightly saline (Figure 1a). However, as salinity decreased, the soil pH showed a slight upward trend (Figure 1b), which may be related to the litter of *Melia azedarach L.* being rich in base cations.

Saline-alkali soils usually contain high concentrations of salt elements. Excessive Na causes soil dispersion, plant toxicity, and may cause mineral nutrition problems; other elements, such as K,Ca,Mg and Cl^- can also harm plants by reducing osmotic potential of soil solution (Sparks, 1995). In the present study, planting of*Melia azedarach L.* decreased Na, K, Ca, Mg and Cl^- concentrations in soil (Figure 2), showing this plant species could remove excess elements to improve the properties of saline-alkali soil.

Total nitrogen, total phosphorus, available phosphorus and organic matter were highest in the rhizosphere soil of *Melia azedarach L.*and lowest in the bare soil (Table 1). These findings may be due to the root exudates and litter of *Melia azedarach L.* being decomposed by soil organisms, thereby increasing soil nutrients.

Enzymes regulate many soil biological processes and are secreted by soil microorganisms, plants and animals (Marx *et al.* , 2001). Phosphatase activity is an indicator of soil phosphorus cycling; it can catalyze the hydrolysis of the ester-phosphate bond and release phosphate (Nannipieri *et al.* , 2011). Soil urease activity is a factor influencing soil nitrogen content (Albiach *et al.* , 2000). The activity of soil catalase is related to soil respiration intensity and the activity of soil microbial communities (Cheng *et al.* , 2013). In the study presented here, *Melia azedarach L.* increased the activities of alkaline phosphatase and urease in saline-alkali soil (Figure 3a, Figure 3b), indicating the capacity of this plant species to promote soil phosphorus and nitrogen cycling. Catalase activity was higher in RS than BS (Figure 3c), indicating that *Melia azedarach L.* root system had positive effect on the activity of microorganisms.

Soil microorganisms play an important role in maintaining soil ecosystem functions, including organic matter decomposition, nutrient cycling, bioremediation, soil organic matter stabilization, and soil aggregate formation (Dangi *et al.* , 2018). The MiSeq sequencing results showed that planting of *Melia azedarach L.*

increased the abundance and diversity of bacteria, but reduced the abundance and diversity of fungi (Table 2), which may be related to a decrease in salinity. Other studies have shown that low-salt soils have lower fungal biomass than high-salt soils (Muhammad *et al.* , 2006).

Proteobacteria is the largest phylum of soil bacteria, including many bacterial taxa with agricultural importance, e.g. those involved in N_2 fixation and soil nitrogen cycle (Spain *et al.* , 2009). In the present study, the abundance of Proteobacteria was higher in the *Melia azedarach L.* rhizosphere than bulk and bare soil, indicating that the planting of *Melia azedarach L.* can improve the availability of nitrogen. In addition to a role in N availability, Proteobacteria can serve as biocontrol agents in the soil-plant ecosystem (Lueders *et al.* , 2006).

Ascomycota play an important role in most terrestrial ecosystems. Ascomycota can decompose organic matter, thus playing an important role in nutrient cycling. The fruiting bodies of Ascomycota provide food for many animals, and many Ascomycota also form symbiotic relationships with other organisms, including plants. In this study, Ascomycota was the dominant fungal phylum, which was most abundant in the bulk soil, whereas one of Acomycota genera (*Rhizophlyctis*) was significantly enriched in the rhizosphere soil. Other studies have shown that*Rhizophlyctis* may play an important role in decomposition of organic matter in many soil-based ecosystems (Weber & Webster, 2000).

Microbial diversity is affected by biotic as well as abiotic factors (Singh *et al.* , 2009). High salinity, Na, K, Ca and Cl^- concentrations reduced the diversity and abundance of bacteria, whereas increased phosphorus, organic matter, urease and total nitrogen were associated with increased diversity and abundance of bacteria (Figure 8). The results of RDA showed that total phosphorus and alkaline phosphatase were the most important factors influencing diversity of bacterial communities in soil, whereas Ca and Mg were related closely to the fungal diversity (Figure 9). Planting *Melia azedarach L.* improved the properties of the saline-alkali soil, thereby increasing the diversity of the soil microbial communities.

5 CONCLUSIONS

This study explored a phytoremediation potential of *Melia azedarach L.* to improve saline-alkali soil. *Melia azedarach L.*not only reduced soil salinity and the content of extractable elements, but also increased (i) nitrogen, phosphorus and organic matter content as well as enzyme activities, and (ii) the diversity and abundance of soil microbial communities to improve the properties of coastal saline-alkali soil. This study provided a scientific basis for (i) using*Melia azedarach L.* in remediation of coastal saline-alkali soils and (ii) the understanding of the underlying ameliorative mechanisms.

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CONFLICT OF INTEREST

There are no competing financial interests associated with the publication of this article.

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Figures and Tables Legends

FIGURE 1 Soil salinity (a) and pH (b) in the saline-alkali soil. Different lowercase letters represent significant differences (p [?]0.05). Means +- standard error (n = 3). CK, bare soil; BS, bulk soil of Melia azedarach L.; RS, rhizosphere soil of Melia azedarach L.

FIGURE 2 Concentrations of extractable soil elements, including Na (a), Mg (b), K (c), Ca (d) and Cl^- (e) in the saline-alkali soil. Different lowercase letters represent significant differences (P [?]0.05). Means +- standard error (n = 3). CK, bare soil; BS, bulk soil of*Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.*

FIGURE 3 Soil enzyme activities, including alkaline phosphatase (a), urease (b) and catalase (c) in the saline-alkali soil. Different lowercase letters represent significant differences (P [?]0.05). Means +- standard

error (n = 3). CK, bare soil; BS, bulk soil of *Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.*

FIGURE 4 Venn diagram of the OTUs of bacteria (a) and fungi (b) in different soil samples. CK, bare soil; BS, bulk soil of *Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.*

FIGURE 5 PCA analysis of bacteria (a) and fungi (b) based on OTUs. CK, bare soil; BS, bulk soil of *Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.*

FIGURE 6 Comparison of relative abundance of bacterial (a) and fungal phyla (b). CK, bare soil; BS, bulk soil of *Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.*

FIGURE 7 The LDA scores distribution histograms (b, d) showing taxa with significantly different abundance. The differences are mapped to cladograms (taxonomic trees) (a, c), with bacteria in the top part and fungi in the bottom part. Note: CK, bare soil; BS, bulk soil of*Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.* In the cladograms, the taxa associated with small circles and the shading in the colour of a specific soil played an important part in the microbial community in that soil (significantly different from other soils). The diameter of the small circle represents relative abundance of the taxa. The taxa without a significant difference are coloured yellow.

FIGURE 8 Correlations among soil physico-chemical and enzymatic properties. The pairwise comparisons are shown, with a color gradient denoting Spearman correlation coefficients. The non-significant correlations ($P > 0.05$) are not shown.

FIGURE 9 Redundancy analysis of soil physico-chemical properties with bacterial (a) and fungal taxa (b). The arrows represent environmental factors, and the arrow length indicates a degree of correlation between environmental factors and microbial communities. CK, bare soil; BS, bulk soil of *Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.*

TABLE 1 Soil nitrogen, phosphorus, potassium and organic matter

TABLE 2 Alpha Diversity Indices

TABLE 1 Soil nitrogen, phosphorus, potassium and organic matter

	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Available potassium(mg/kg)	Available
CK	0.51±0.03c	0.57±0.06b	17±1.1a	68±3.5a	16±1.0b
BS	0.68±0.02b	0.65±0.01b	18±2.4a	67±2.2a	27±3.4b
RS	0.98±0.05a	0.90±0.17a	18±4.2a	68±2.7a	54±8.7a

CK, bare soil; BS, bulk soil of *Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.*

Means ± standard error (n = 3); means followed by different letters are significantly different at P [?]0.05.

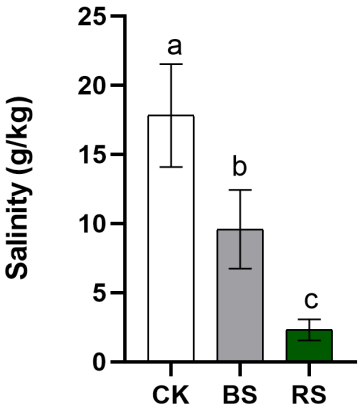
TABLE 2 Alpha Diversity Indices

Sample	Sequencing coverage	Sequencing coverage	Chao1 index	Chao1 index	Chao1 index	Shannon index	Shan
	Bacteria	Fungi	Bacteria	Bacteria	Fungi	Fungi	Bact
CK	0.989^{a}	0.996^{a}	1995^{c}	605^{a}	605^{a}	7.41^{c}	7.41
BS	0.989^{a}	0.995^{a}	2341^{b}	587^{a}	587^{a}	8.56^{b}	8.56
RS	0.986^{a}	0.994^{a}	3114^{a}	659^{a}	659^{a}	9.26^{a}	9.26

CK, bare soil; BS, bulk soil of *Melia azedarach L.* ; RS, rhizosphere soil of *Melia azedarach L.*

The data are means ± standard error (n = 3). Means followed by different letters in a column are significantly different at P [?]0.05.

(a)



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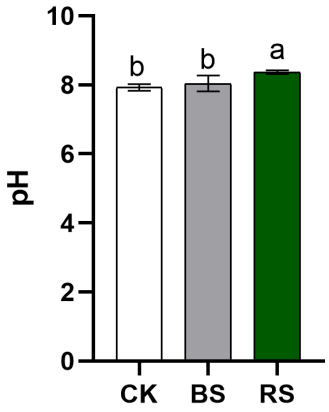
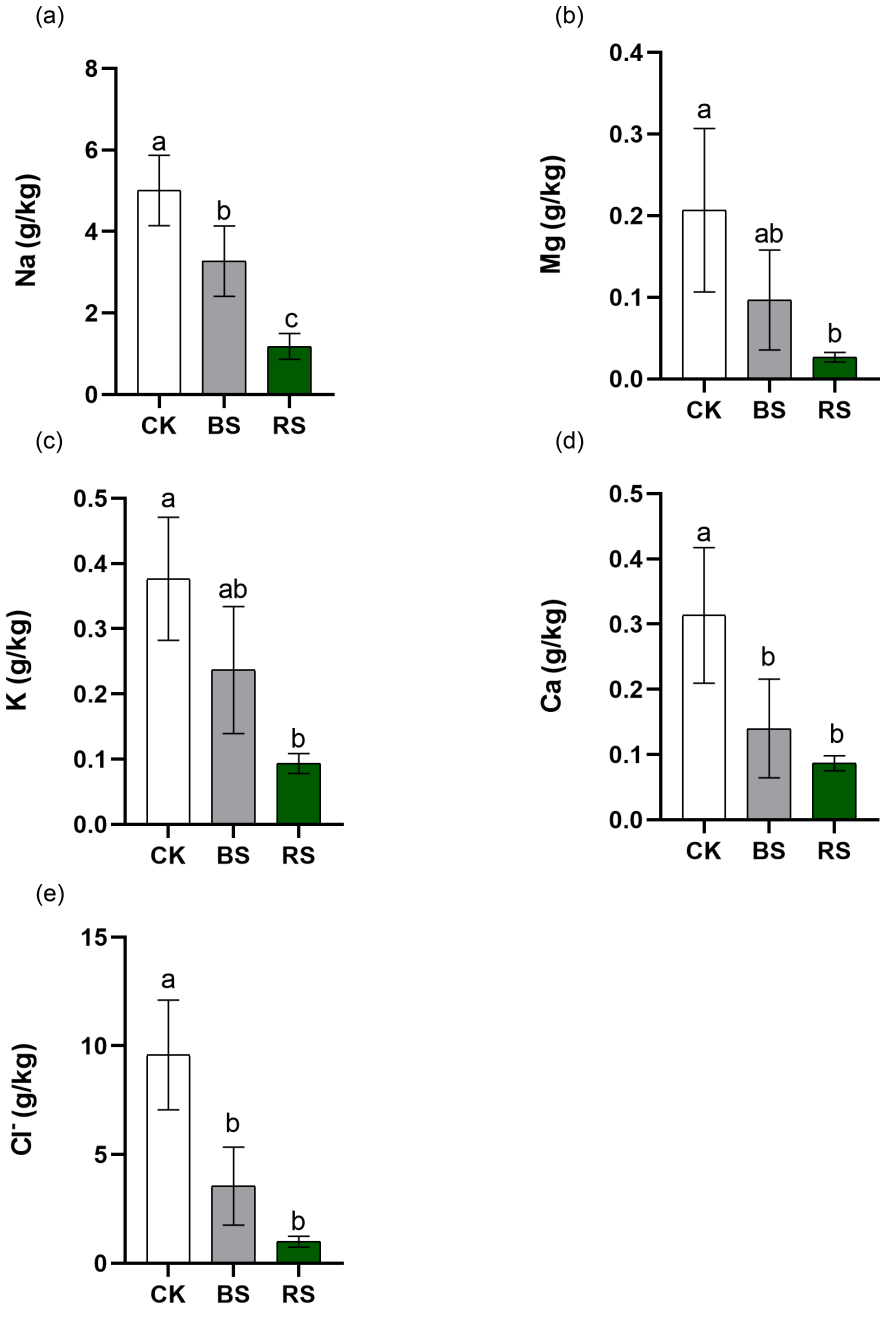
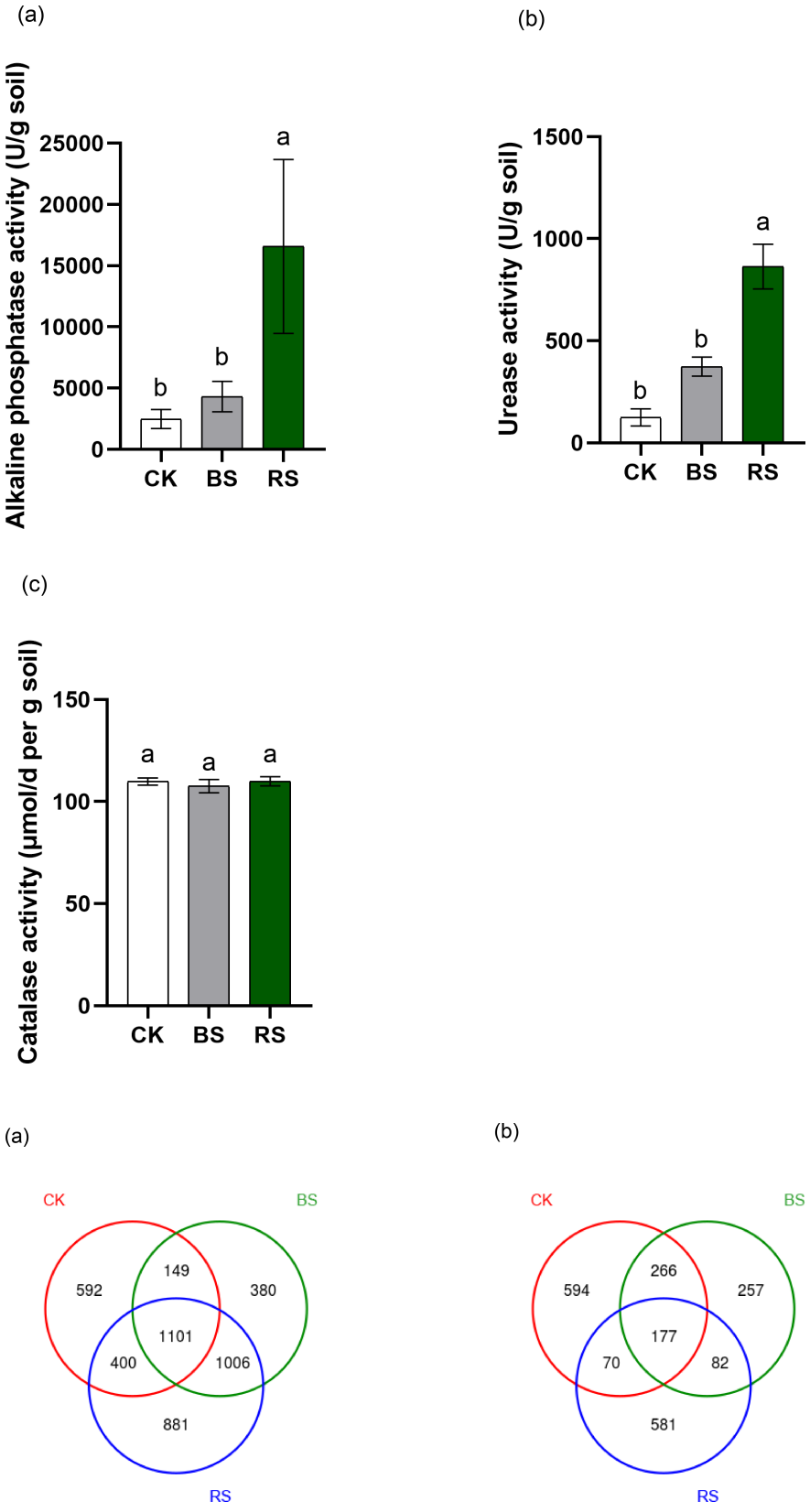
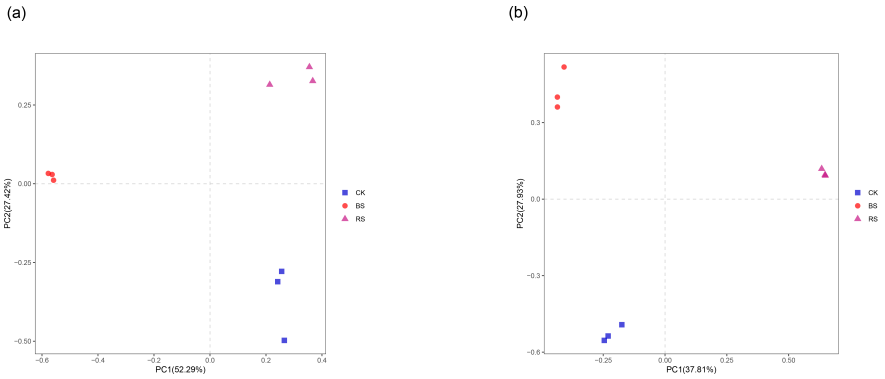
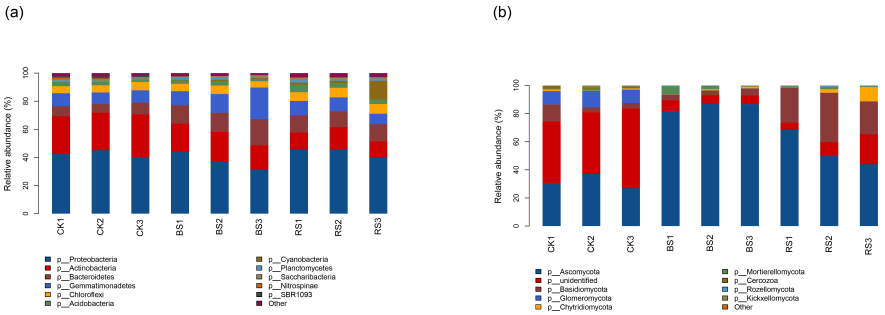
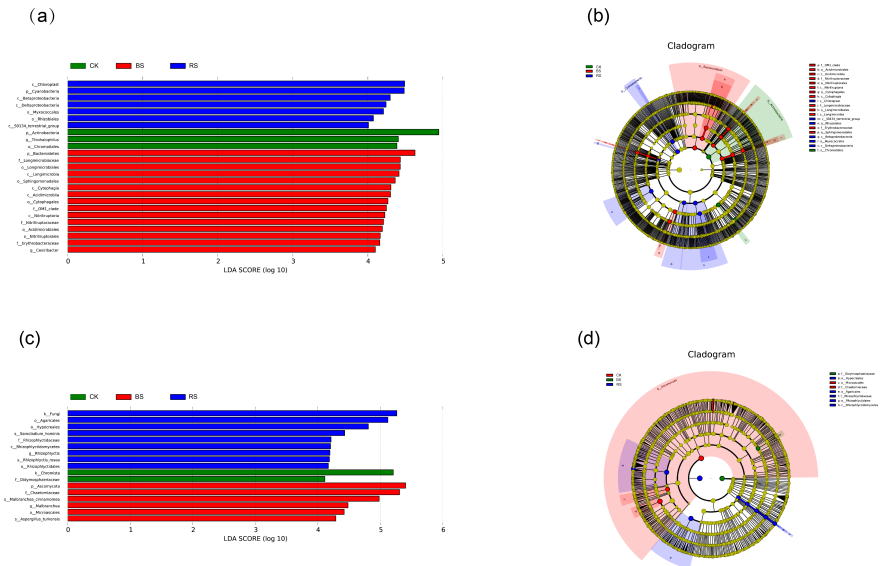


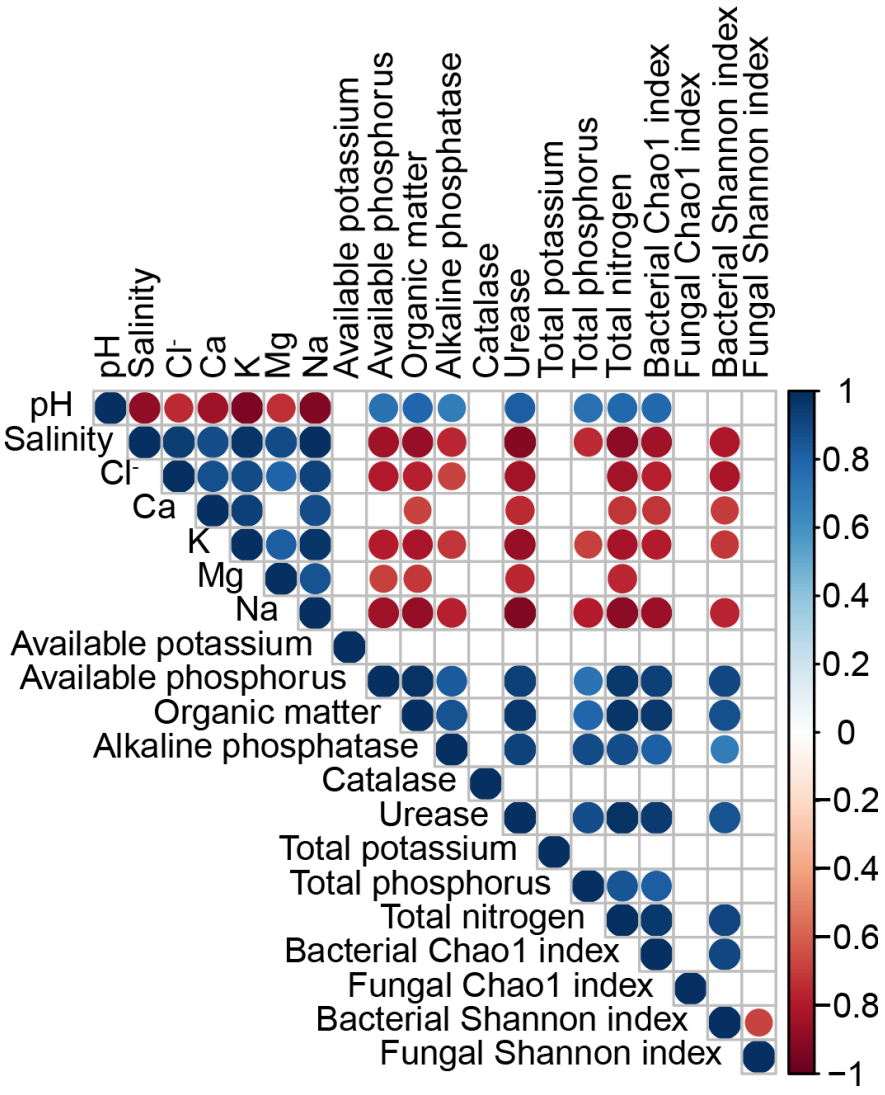




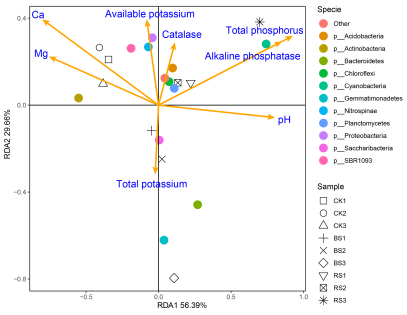








(a)



(b)