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Characteristics of inter-root soil bacterial community structure and diversity of different sand-fixing shrubs at the southeastern edge of the Mu Us Desert, China

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Abstract

Purpose This study aimed to investigate the effects of different shrub plants on the structure and diversity of interroot soil bacterial communities, in order to provide scientific support for ecological restoration and revegetation of the Mu Us Desert.

Methods Three major shrub plants (*Artemisia ordosica*, *Salix psammophila*, *Caragana microphylla*) in the Mu Us Desert were selected for this study. Using high-throughput sequencing technology, the bacterial community structure and diversity in the inter-root soils of these plants were analysed in depth, and combined with the determination of soil physicochemical and microbiological properties, the response characteristics of the bacterial diversity in the inter-root soils of the different plants were assessed comprehensively.

Results It was found that although the soil pH did not show significant differences among different plant growths, the SOC, TN and TP contents were higher in Salix psammophila sample plot and Artemisia ordosica sample plot, which indicated that the plant growths had a positive effect on the soil nutrient contents. Through Venn diagram analysis, it was observed that the number of OTUs of bacteria in the soils of different shrubland sites varied, and all of them were higher than those in the soils of the sample sites where no plants grew, which indicated that plants had an effect on soil bacterial diversity. The bacterial Chao1 index were higher in the Artemisia ordosica sample plot sample site, suggesting that the growth of Artemisia ordosica contributes to the enhancement of soil bacterial richness. Soil bacterial communities showed compositional differences among different sample plots, especially the higher relative abundance of Betaproteobacteria in the Artemisia ordosica sample plot, which may be related to the increase of soil organic matter content.

Conclusion The results of the study revealed that specific plants, such as *Artemisia ordosica*, can significantly improve the soil nutrient status of windy sandy soils, increase soil organic matter and nitrogen content, and thus enhance the diversity and abundance of soil microorganisms. The bacterial community structure in the inter-root soils of different plants differed significantly, with changes in the relative abundance of the dominant phyla, such as *Alphaproteobacteria*, *Betaproteobacteria* and *Actinobacteria*, reflecting the differences in soil nutrient status. These findings emphasise the important role of plants on soil chemical properties and microbial community structure, providing an important basis for soil management and ecological restoration.

Keywords Shrubland, Bacterial community, High-throughput sequencing, The Mu Us Desert

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Introduction

Desertification is one of the major ecological and environmental problems facing the world, especially in the arid and semi-arid regions of northern China. (Zhang et al. 2015a, b; Chen et al. 2019). The Mu Us Desert, as one of the four major sandlands in China, has significant ecological vulnerability and serious desertification problems (Li et al. 2016; Gao et al. 2020). The area is seriously desertified, with a single tree species, sparse biological populations, fragile ecological environment and sensitive to change, which is a typical ecologically fragile area and one of the key areas for wind and sand prevention and control in China (Li and Li 2018; Gao et al. 2024). In order to improve the ecological environment in this area, the Chinese Government and the relevant departments have implemented a reforestation project, selecting drought-resistant, sand-resistant and fast-growing tree species, such as lemonade, salal, sea buckthorn, etc., and, through a combination of artificial planting and natural regeneration, have increased the cover of the vegetation, increased the content of the organic matter in the soil, and improved the structure of the soil so as to enhance the water-retaining and fertilizer-retaining capacity of the soil (Wang et al. 2015; Li et al. 2017; Zhou et al. 2020a, b). In addition, physical measures such as the establishment of windbreaks and grass-square sand barriers have effectively reduced wind erosion and guicksand, and lowered the frequency of sand and dust storms (Bardgett et al. 2013; Liu et al. 2018). Microorganisms are an important component of soil ecosystems, which are important drivers of soil organic matter and nutrient transformation (Hartmann and Witter 2008; Gao et al. 2024) and play a key role in ecosystem stability and sustainability (Phillips et al. 2013; Wang et al. 2016). By analysing the inter-root soil microbial community structure of different plants, the interactions between plants and soil microbes can be revealed, which plays an important role in understanding the function of desert ecosystems and improving the efficiency of vegetation restoration (Schlesinger et al. 2014; Wu et al. 2019).

Currently, domestic and international research on soil microbial diversity focuses on soil suitable for farming and remediation of contaminated soil (Zheng et al. 2021; Dong 2020). Microorganisms play a crucial role in soil ecosystems, and they are the key drivers of soil organic matter conversion and nutrient cycling, which play a central role in ecosystem stability and sustainability (Phillips et al. 2013; Zhang et al. 2018). Therefore, an in-depth understanding of the structure of plant and soil microbial communities and their diversity in desert areas is essential for developing effective desertification control strategies (Zhao et al. 2015; Iyobosa et al. 2021; Xiao et al. 2023a, b). Recent studies have focussed on vegetation

restoration, soil improvement and microbial diversity in the Mu Us Desert and its surrounding areas in an attempt to find effective ways to restore and rebuild damaged ecosystems (Fierer et al. 2007; Li et al. 2024a, b). Xiao et al. (2023a, b) studied the soil microbial community structure of three types of sand-fixing shrub forests in the Mu Us Desert and their effects on soil multifunctionality, and found that there were significant differences in soil organic carbon, total nitrogen, and total phosphorus among different shrub forests, and that soil multifunctionality was correlated with microbial amount of carbon, microbial amount of nitrogen, sucrase activity, and alkaline phosphatase activity, among other indicators. Li et al. (Li et al. 2024a, b) explored the inter-root soil bacterial community structure and diversity of four dominant plants in the Mu Us Desert, and found that Ascomycetes was the dominant bacterial phylum in the inter-root soil, and that soil pH and quick-acting phosphorus were the key environmental factors affecting the plant inter-root soil bacterial diversity indices and community structure (Schlesinger et al. 2011; Smith et al, 2016; Zhou and Li 2020). Through fieldwork and indoor analyses, the effects of different plant communities on soil physicochemical properties and changes in soil microbial diversity were assessed (Jones et al. 2012; Li and Zhang 2024; Xiao and Li 2023), so as to provide theoretical support and practical guidance for ecological restoration in desertified areas (Maestre and Delgado-Baquerizo 2015; Kong et al. 2021).

The plant inter-root is a key area where the soil microbial community interacts with the plant root system. Plant root secretions attract soil bacteria and form rich microbial communities in the inter-root soil, and these microbial communities play a crucial role in plant growth and healthy development (Wang et al. 2019; Xiao et al. 2023a, b). In this study, three dominant shrub species (Caragana microphylla, Salix psammophila, Artemisia ordosica) with strong adaptability in arid and wind erosion-affected areas of the Mu Us Desert were selected, and soil sampled and sequenced of 3 shrubs rhizospheresurrounding soil, in order to explore the structure of the inter-root soil bacterial communities of different plants with soil bacterial. The current status and differential change rule of community diversity provide scientific basis and theoretical support for desertification management and vegetation restoration in the region (Schlesinger et al. 2014).

Materials and methods

Design of the experiment

The study area is located at the southeastern edge of the Mu Us Desert (Fig. 1), in the wind-sand grassland area of northwestern Shenmu County, Yulin City, Shaanxi



Fig. 1 Location and distribution of sample plots (The red arrows indicate the dominant plants in the sample plots)

Province, China (38°40'-39°20'N, 109°20'-110°30'E), which lies between the Ordos Plateau and the Loess Plateau (Liu et al. 2016). Altitude between 718 and 1954 m (DEM, Digital Elevation Model). It has a temperate continental monsoon climate characterised by aridity and low rainfall, significant diurnal temperature differences, and a four-seasonal climate pattern (Wang et al. 2015). The mean annual temperature of the area is about 8.9 °C, the frost-free period is about 154 days, the annual precipitation is about 440.8 mm, and the mean annual sunshine hours are 2,879 h. The altitude of the study area ranges between 1206 and 1215 m above sea level, and the soil is dominated by wind-sand soils (Zhang et al. 2015a, b; Bardgett et al., 2013). Vegetation in the area consists mainly of herbaceous and shrub communities with deeprooted, drought-tolerant, and adaptive systems, which contribute to the fixation of sandy soils and improvement of soil structure (She et al. 2015; Fan et al. 2017). In the region, common shrub species include Artemisia ordosica, Salix psammophila, Caragana korshinskii, Hippophae rhamnoides, and Ammopiptanthus spp. while herbaceous plants are dominated by Stipa spp, Artemisia *spp.* and *Bromus inermis*, among others (Pei et al. 2019; ZiYang et al. 2021).

Site selection and sample collection

When a particular plant cover is higher than the other plants in the sample plot, that plant species is the dominant species (Chen et al. 2002). In September 2022, three representative sand-fixing shrub plots (Table 1), C1 (Caragana microphylla sample plot), C2 (Salix psammophila sample plot) and C3 (Artemisia ordosica sample plot), were selected within the fixed sandy area (Li et al. 2016). Three three 30 $m \times 30$ m sample plots were randomly established for each shrubland species, with 40 m between plots, and the surface layer of dead plant residues was removed before sampled. In each sample plot, five dominant shrubs were randomly selected and 100 g of of soil was collected from the rhizosphere-surrounding soil of the shrubs for soil sampling (bacteria loosely attached to the roots; hereinafter inter-root soil. and mixed into one sample, which was screened through a 2-mm sieve to remove plant and animal debris and gravel(Lee et al. 2020). Screened soil samples were placed

Treatment	Shrub dominant	Heights(m)	Vegetation coverage(%)	Companion plant
C1	Caragana microphylla	1.64	64	Calamagrostis epigeios
				Aster alfaics
				Cynanchum chinense
				Psammochloa villosa
C2	Salix psammophila	1.95	69	lxeridium gracile
				Heteropappus altaicus
				Leymus secalinus
				Setaria viridis
C3	Artemisia ordosica	0.58	51	Stipa breviflora
				Cynanchum thesioides
				Lespedeza davurica
				Leymus secalinus
				lxeridium gracile
				Calamagrostis epigeios

in self-sealing bags and placed in sampling boxes containing ice packs, transported to the laboratory within one hour, and stored in a refrigerator at -80 °C for subsequent analyses. A total of nine soil mixture samples were collected (Kong et al. 2021).

In addition, each shrub sample site was surveyed for vegetation conditions during the active plant growth period, including vegetation cover and height, as well as other plants growing along with it. Herbaceous plant species were recorded through five 1 m² herbaceous survey sample plots set up near the soil sampling sites. The survey showed that the C3 sample site was relatively rich in associated plants (Xiao et al. 2023a, b).

Determination of soil chemical properties

Soil-distilled water slurry was prepared at a ratio of 1:1 (15 g/15 ml) and soil pH was determined using a pH meter (Wang et al. 2015).Soil organic carbon, (SOC) was determined using the potassium dichromate volumetric method (external heating method).Total nitrogen (TN) was determined using the Kjeldahl method (Phillips et al. 2013; Schlesinger et al. 2014). Soil total nitrogen (TN) was determined by Kjeldahl method. Total phosphorus (TP) was determined using UV spectrophotometer (Bardgett et al. 2013).

Extraction, PCR amplification and sequencing of DNA from soil microorganisms

Microbial diversity of nine soil samples was analyzed by DNA extraction and high-throughput sequencing (Zhang et al. 2015a, b). Firstly, genomic DNA was extracted from the samples using the CTAB method and the purity and concentration of the DNA was examined by 1% agarose

gel electrophoresis. Subsequently, an appropriate amount of sample was selected and diluted to a concentration of 1 ng/µl using sterile water. PCR amplification of the V4 variable region was carried out using 341F (5'-CCTAYG GGRBGCASCAG-3') and 806R (5'-GGACTACNNGGG GTATCTAAT-3') primers. Diluted genomic DNA was used as a template for the PCR reaction in conjunction with the selection of the sequencing region using special primers with Barcode, as well as Phusion® High-Fidelity PCR Master Mix with GC Buffer and High-Fidelity Enzymes from New England Biolabs to ensure amplification efficiency and accuracy (Lusk et al. 2021). Libraries were constructed using the NEB Next® Ultra DNA Library Prep Kit, and the constructed libraries were tested and Q-PCR quantified by Agilent 5400. After confirming the quality of the library, sequencing was performed using the Illumina sequencing platform (Zhang and Xu 2022).

Statistical analysis of data

Data were analysed by one-way ANOVA using SPSS software and multiple comparisons between treatments were made using Duncan's multiple range test (P < 0.05). Data were analysed and graphs were plotted using R software version Shannon's index was used to assess the diversity of species while Chao1 index was used to calculate and estimate species richness. UPGMA (Unweighted Pair-Group Method with Arithmetic means) cluster analysis, which takes into account both species diversity and abundance, and principal coordinate analysis (PCoA) based on Bray–Curtis distances were used to assess over-all differences in microbial community structure (Schlesinger et al. 2014).

Results

Inter-root soil chemistry in different shrubland forests

Soil pH did not differ among C1, C2, and C3, all of which were alkaline. The SOC content of C2 and C3 soils was higher than that of C1. The TN and TP content of C2 and C3 soils was higher, and the content of C1 was relatively low (Table. 2). In the three sample soils, the growth of different plants did not change the pH of the wind-sand soil, while the growth of *Artemisia* ordosica effectively improved the soil nutrients (Zhang et al. 2015a, b).

Venn diagram analysis of inter-root soil bacterial composition in different shrubland sites

The number of OTUs shown in Fig. 2 is the average of the three sequence samples analyzed at each site. The number of OTUs in C1, C2 and C3 samples were 4887, 5069 and 5200, respectively, and the number of OTUs common to the three soils was 1699 (Fig. 2). The highest number of OTUs was found in C3 samples, and the number of OTUs was slightly higher than C1 under the C2 treatment. The number of OTUs was slightly higher that the growth of different plants affects the number of inter-root soil bacterial OTUs, and the number of the composition of the inter-root soil bacterial species of *Artemisia ordosica* was higher than that of other plants (Liu et al. 2016).

Inter-root soil bacterial richness and diversity in different shrublands

Soil bacterial α -diversity can reflect the distribution of bacteria in soils with different compounding ratios. Chao1 index showed that the richness indices of C2 and C3 samples were larger, which were 2805 and 3217 (Table. 3), respectively, and the bacterial richness of C3 samples was higher, which indicated that the growth of *Artemisia ordosica* had a contributing effect on the soil bacterial richness. The Shannon indexes in this study ranged from 9.17 to 9.96, with the C3 sample having a relatively high Shannon index, but the differences among the three treatments were not significant.

Table 2 Chemical properties of soils at different sites

Treatment	рН	SOC (g/kg)	TN (g/kg)	TP (g/kg)
C1	8.47±0.05 a	1.79±0.05 b	0.14±0.02 a	0.22±0.02 b
C2	8.53±0.03 a	2.99±0.14 b	0.23±0.02 b	0.27±0.01 a
C3	8.51±0.03 a	3.21±0.05 a	0.39±0.01 c	0.39±0.02 a

Different lowercase letters indicate significant differences among the treatments (P < 0.05)



Fig. 2 Venn diagram of the number of inter-root soil bacterial OTUs in different shrub forests

Differential analysis of inter-root soil bacterial community composition in different shrublands

The soil bacterial community composition shown in Fig. 3 is the average of the three sequence samples analyzed at each site. The community composition and species abundance at different taxonomic levels can be presented through histograms. In this study, community composition and species abundance were analysed based on the phylum level. The relative abundance of different plant inter-root soil bacterial communities at the phylum level (Fig. 3). The three dominant phyla in the C1 sample plot were Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria, followed by Actinobacteria and Deltaproteobacteria, respectively. The three dominant phyla in C2 were Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, followed by Betaproteobacteria and Deltaproteobacteria, and the three dominant phyla in C3 were Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria,

Table 3	Soil bacterial	richness ar	nd diversity	indices in	the inte	r-
root of d	ifferent shrub	forests				

Chao1	Shannon			
2487±29 a	9.17±0.04 a			
2805±30 b	9.20±0.11 a			
3217±26 a	9.96±0.06 a			
	Chao1 2487±29 a 2805±30 b 3217±26 a			

Differences between treatments with lowercase letters indicate statistical significance (p<0.05)



Fig. 3 Characteristics of community composition at the level of major phyla of inter-root soil bacteria in different shrubland forests

Actinobacteria, followed by Betaproteobacteria and Deltaproteobacteria, respectively. The three dominant phyla in C3 were Alphaproteobacteria, Betaproteobacteria, Actinobacteria, followed by Gammaproteobacteria and Deltaproteobacteria, respectively. The inter-root soils of C1 and C2 samples showed a high concentration of Gammaproteobacteria and Actinobacteria had high relative abundance, while Betaproteobacteria had low relative abundance. While Betaproteobacteria are eutrophic bacteria, they need more soil organic matter for their growth, indicating that the soil nutrients were higher in the C3 sample site, suggesting that the growth of *Artemisia ordosica* can effectively improve the soil nutrients in windy sandy soils.

Analysis of the β -diversity and intergroup variability of inter-root soil bacterial communities in different shrubland forests

Based on Weighted Unifrac distance, PCoA analyses of soil bacterial communities in different proportions of compounding treatments were performed, and the principal coordinate combination with the largest contribution rate was used for graphing. The results of the analysis showed (Fig. 4) that PCoA1 was the principal



Fig. 4 PCoA analysis of inter-root soil bacterial communities in different shrub forests

coordinate component that explained as much as possible the variation of the data, with an explanation rate of 19.6%; PCoA2 was the next highest, with an explanation rate of 17.5%, and the 2 principal components explained 37.1% of the bacterial community. On PCoA1, soil bacterial communities under C2 and C3 treatments were close to each other in terms of distance and had some similarity in structure. Both were more distant and different from the soil bacterial community under C1 treatment.

Correlation between inter-root soil physico-chemical properties, bacterial community structure and diversity in different shrubland forests

The inter-root soil chemical factors (pH, SOC, TN and TP), and the top 5 bacterial phyla in terms of relative abundance (*Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*) were analysed separately for three different shrubland inter-root soil chemical factors, which were subjected to Spearman correlation analysis. The results showed (Fig. 5) that *Alphaproteobacteria* showed positive correlation with soil SOC, TN, and TP values and negative correlation with soil pH at the phylum classification level, while *Betaproteobacteria* showed positive correlation with all soil chemical factors and significant positive correlation with soil SOC, TN, and TP; *Actinobacteria* showed negative correlation with soil SOC, TN and TP values, and less correlation with soil pH; *Gammaproteobacteria* showed negative correlation with soil SOC, TN and TP values, and positive correlation with soil pH; *Deltaproteobacteria* showed negative correlation with soil chemical factors, and significant negative correlation with soil pH. *Deltaproteobacteria* were negatively correlated with soil chemical factors and significantly negatively correlated with soil pH. Combined with the proportions of bacterial abundance in the bacterial composition analyses, soil SOC, TN and TP had a greater influence on the composition of inter-root soil bacterial communities in the three different shrubland species.

The inter-root soil chemical factors (pH, SOC, TN and TP) were analysed by Spearman's correlation with bacterial community composition relationships (Chao1, Shannon) for three different shrubland sites, respectively. The results showed (Fig. 6) that soil pH, SOC, TN and TP values were significantly positively correlated with Chao1 index; soil SOC, TN and TP values were significantly positively correlated with Shannon index, and soil pH was positively correlated with Shannon index; in general, soil pH, SOC, TN and TP had a positive effect on the diversity of inter-root soil bacterial communities in the three different shrubland species. The effects of soil pH,



Fig. 5 Correlation analysis of inter-root soil chemical factors (pH, SOC, TN and TP) and dominant bacterial phyla in different shrubland forests



Fig. 6 Correlation analysis of the relationship between inter-root soil chemical factors (pH, SOC, TN and TP) and bacterial community composition in different shrubland forests

SOC, TN and TP on the diversity of inter-root soil bacterial communities in the three different shrubland species were more significant.

Discussions

The results of this study coincide with current findings in the literature on the effects of plants on soil chemical properties and microbial communities (Bardgett et al. 2013; Zhang et al. 2015a, b; Smith et al. 2019). For example, previous studies have shown that plant root secretions can significantly alter the chemical properties and microbial composition of inter-root soils (Liu et al. 2016; Chen et al. 2018; Wang et al. 2019).

In Sun 's study, the dominant flora in the bacterial community of different shrubby plants inter-root soil in the desert were *Proteobacteria* · *Actinobacteria* · *B acteroidetes*, which is in agreement with the results of the present study (Sun et al. 2020; Zhou et al. 2020a, b). Soil nutrient indexes, such as total nitrogen, significantly increased with the restoration period and were significantly positively correlated with soil fungal and bacterial diversity(Huang et al. 2019; Ma et al. 2022; Gong et al. 2024).

In addition, it has also been found that specific plant species affect soil microbial diversity by altering soil organic matter content (Phillips et al. 2013; Phillips et al. 2013). In this study, the enhancement of soil SOC and TN content by Artemisia ordosica was consistent with the previous findings, further validating the positive effects of plants on soil nutrients. In terms of microbial community structure, the results of the present study were in line with the studies of Phillips et al. Both pointed out that plant species were important influences on the structure of inter-root microbial communities (Phillips et al. 2013; Smith et al. 2016). Meanwhile, increased abundance of Betaproteobacteria may be associated with elevated soil organic matter content, which is consistent with Schlesinger et al. (Schlesinger et al. 2014) that soil organic matter is a key element in shaping the structure of microbial communities. The results of the PCoA analyses revealed that the interroot soil bacterial of different plants β -diversity of the communities, in agreement with Wang et al. (Wang et al. 2015), who similarly found significant differences in the inter-root soil microbial communities of different plants. In addition, the significant correlation between soil chemical properties and bacterial community composition in this study also fits with the findings of Bardgett et al. (Bardgett et al. 2013; García-Palacios et al. 2013), who emphasised the importance of soil nutrients for microbial community structure and function. Taken together, this study provides new perspectives for a deeper understanding of plant-soil interactions through the comprehensive analysis of inter-root soil chemical properties and microbial communities in different shrubland sites. These results not only improve our understanding of how plants affect soil nutrients and microbial communities, but also provide scientific support for soil management and ecological restoration efforts.

Conclusions

This study revealed the significant effect of plant growth on soil properties by analysing the chemical properties, bacterial composition, richness and diversity of interroot soils from different shrubland sites. It was found that although the growth of different plants did not change the pH of the windswept soil, the growth of *Artemisia ordosica* significantly increased the organic matter (SOC) and total nitrogen (TN) content of the soil, thus improving the soil nutrient status (Zhang et al. 2015a, b).

Through Venn diagram analysis, it was further found that the number of bacterial operational taxonomic units (OTUs) in the inter-root soils of different plants varied, with the number of bacterial species composition in the inter-root soils of *Artemisia ordosica* being higher than that of the other plants, indicating that the specific plants had a significant effect on the structure of the interroot microbial community (Liu et al. 2016). In addition, according to the α -diversity indicators of soil bacteria (e.g., Chao1 index), the number of bacterial species was significantly higher in C3 than in C2, which confirms that the inter-root soil microbial diversity of *Artemisia ordosica* is richer.

In terms of bacterial community composition, the dominant phyla in sample plots C1, C2 and C3 were *Alphaproteobacteria*, *Gammaproteobacteria* and *Betaproteobacteria*. Masuda et al. showed that *Betaproteobacteria* play an important role in soil nutrient cycling by participating in the processes of nitrogen fixation and nutrient mineralization in the soil, thus contributing to the nutrient status and fertility of the soil. Since the C3 site had the highest number of *Betaproteobacteria*, this suggests that the C3 site is relatively rich in soil nutrients. (Masuda et al. 2022).

Through PCoA analysis, the study also explored the β -diversity and intergroup variability of the inter-root soil bacterial communities of different plants. The results showed that the soil bacterial community structures under the C2 and C3 treatments had some similarities, while the differences with the C1 treatment were larger, which further confirmed the effect of plant species on soil microbial community structure (Schlesinger et al. 2014).

Finally, Spearman correlation analyses showed significant correlations between soil chemical factors (SOC, TN and TP) and bacterial community composition and diversity indices (e.g. Chao1 and Shannon index). These results emphasise the importance of soil nutrient status on the structure and function of inter-root microbial communities (Wang et al. 2015).

Ethical statements

I certify that this manuscript is original, has not been published anywhere, and will not be submitted elsewhere for publication while being considered by the Annals of Microbiology. The study is not split up into several parts to increase the number of submissions and submitted to various journals or to one journal over time. No data have been fabricated or manipulated (including images) to support the conclusions. No data, text, or theories by others are presented as if they were our own.

The submission has been received explicitly from all co-authors, and authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

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Authors' contributions

LS: guided the completion of this experiment, completed the first draft paper, and made revisions. LH: guided the experimental methods and thesis writing. LY and BP: performed experiments, recorded data, and data analysis. SZ and DH: data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The data were obtained by the authors.

Declarations

Ethics approval and consent to participate

The study did not violate ethics, and all participants agreed to publish the paper.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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