Bacterial Diversity and Community Structure of the Jujube Rhizosphere in Southern Xinjiang Uygur Autonomous Region, China

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Abstract
Jujube is an important economic crop in the Xinjiang Uygur Autonomous Region. Microbial diversity in the rhizosphere is essential for plant quality; however, soil bacterial diversity and community structure in the jujube rhizosphere have not been characterized in this region. In this study, we used pyrosequencing to analyze bacterial diversity and community structure at different growth stages in the jujube rhizosphere in Hetian, Kashi, and Aksu prefectures. These results revealed a greater bacterial diversity in the 8-year jujube rhizosphere as compared with the 3-year-old rhizosphere taken from the same sampling area. Moreover, samples obtained from Kashi prefecture showed the largest diversity among the different areas. The most abundant phyla across all soil samples were Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Firmicutes. Dominant phyla in the 8-year jujube rhizosphere accounted for the increased observed diversity. Furthermore, comparative analysis of the bacterial communities with respect to rhizosphere age and sampling areas revealed a significant correlation between soil properties and phyla diversity. To the best of our knowledge, this is the first study of jujube rhizosphere bacterial diversity and community structure in the southern Xinjiang Uygur Autonomous Region, and we hope that our research provides a reference for future studies.

Introduction
The rhizosphere is a critical interface between plant roots and soil that supports nutrient exchange [Peiffer et al., 2013; Liu et al., 2015, 2021a] and is directly influenced by root secretions and associated microorganisms [Liu et al., 2021b]. Soil pH, soil structure, oxygen, and nutrient levels of the rhizosphere can differ across sampling sites, even for the same plant species [Singh et al., 2004], and thus plants create different rhizophere environments and select for those that contain specific rhizobacteria that are most beneficial for growth [Vessey 2003; Lugtenberg and Kamilova 2009; Ownley et al., 2009]. Composition of the rhizosphere bacterial community differs among plant species, soil type, root architecture, and growth stage [Berg and Smalla 2009; Bhattacharyya et al., 2021], and...
microorganisms that inhabit the rhizosphere play a vital role in the overall ecology of the plant/root system [Zilber-Rosenberg and Rosenberg, 2008]. Beneficial rhizosphere microbes can also assist with nutrient acquisition, provide protection against pathogens and stimulate immune responses [Rosenblueth and Martinez-Romero 2006; Van Wees et al., 2008; Berendsen et al., 2012]. Furthermore, symbiosis between microbes and plants confers a profound capacity for environmental adaptability and stress tolerance [Rodriguez and Redman, 2008; Humphreys et al., 2010].

The Xinjiang Uygur Autonomous Region is the largest province in China, with an area accounting for one-sixth of the entire country. Unfortunately, regional agricultural developments have lagged due to harsh climatic characteristics, including little rainfall, periodic drought, high salinization, sand storms, and hail [Zhang et al., 2013]; however, abundant sunshine and substantial differences between day and night temperatures are favorable to the fruit industry. Jujube is a functional food, as epidemiological evidence suggests that high consumption of jujube and its products is associated with a reduced risk of some cancers [Gao et al., 2014]. Moreover, jujube grown in Xinjiang has become popular with consumers due to its high nutritional value and is, therefore, considered an important agroeconomic asset. Owing to their ecological and economic importance, jujube dates have attracted substantial attention from researchers focusing on the nutritional value and enhanced preservation/processing techniques associated with the fruit [Lu et al., 1993; Jie et al., 2003]; however, no studies have addressed the bacterial rhizosphere of Xinjiang jujube to date.

Here, we used DNA pyrosequencing to assess the bacterial diversity and community structure of the jujube rhizosphere found in Hetian, Kashi, and Aksu prefectures of the southern Xinjiang Uygur Autonomous Region. Soil samples were collected from 3- and 8-year-old jujube plants from the same area, and community structure data

Fig. 1. Location of soil sampling sites across the southern Xinjiang Uygur Autonomous Region of China. The image was generated in Photoshop version 6.0 (Adobe; San Jose, CA, USA).
were used to determine differences in bacterial biodiversity among the three sampling areas and two ages of plants. Finally, the relationship between bacterial phyla and soil conditions was tested by redundancy analysis.

Results

Soil Physicochemical Characteristics

The locations of soil sampling sites across the southern Xinjiang Uygur Autonomous Region of China were shown in Figure 1. Table 1 lists the geographic sites and physicochemical properties of the analyzed soil samples collected from Hetian (He), Kashi (Ka), and Aksu (Ak) prefectures from 3- and 8-year-old jujube plants. Our analyses showed that all soil samples were alkaline (pH 8.58–8.71) with a relatively low organic matter content (16.52–21.75 g·kg⁻¹) and moisture (11.38–16.83%). Additionally, soil samples collected from He prefecture showed a higher content of available nitrogen (N), phosphorous (P), and potassium (K) as compared with samples collected from the other two prefectures. Soil samples collected from different sites within the same area also showed different mineral content. For instance, the available K was higher in sample He-3 than in He-8, and the available P was higher in Ka-3 than in Ka-8. These apparent differences might be a consequence of differences in the climate of Xinjiang, land management practices, and/or the biochemical activities of microorganisms that varied between samples.

Table 1. Physical and geochemical characteristics of the soil samples from different areas

| Sites  | Location      | Sample ID | pH       | Moisture content, % | Available K, mg·kg⁻¹ | Available P, mg·kg⁻¹ | Available N, mg·kg⁻¹ | Organic matter, g·kg⁻¹ |
|--------|---------------|-----------|----------|---------------------|----------------------|----------------------|----------------------|------------------------|
| Hetian | 37°07′N       | He-3      | 8.61±0.01a | 16.83±0.03a         | 328.24±0.15a         | 45.64±0.01a          | 40.59±0.21a          | 20.89±0.02a            |
|        | 79°56′E       | He-8      | 8.71±0.06b | 15.52±0.02b         | 128.74±0.23a         | 34.95±0.03a          | 30.89±0.05b          | 20.29±0.06b            |
| Kashi  | 38°11′N       | Ka-3      | 8.58±0.05a | 12.29±0.04b         | 128.74±0.22a         | 12.32±0.05c          | 37.46±0.11a          | 21.75±0.05a            |
|        | 77°16′E       | Ka-8      | 8.61±0.02a | 12.74±0.02b         | 161.19±0.13a         | 5.72±0.08c           | 22.44±0.06c          | 16.52±0.03a            |
| Aksu   | 80°11′N       | Ak-3      | 8.65±0.04a | 11.38±0.01c         | 201.42±0.35b         | 20.02±0.06b          | 32.51±0.07b          | 18.13±0.04b            |
|        | 40°47′E       | Ak-8      | 8.67±0.01a | 12.37±0.05b         | 182.46±0.21b         | 24.38±0.01b          | 39.72±0.31a          | 19.35±0.01b            |

Statistically significant differences (p < 0.05) between 6 different soil samples from 3 areas. Different letters (a, b, c, d) in each column indicate a significant difference (p < 0.05) between samples according to Duncan’s multiple comparison test.

Estimators for the Diversity of Bacterial Communities

For the six soil samples, filtering out low-quality and short sequence reads yielded 63,820 reads for bacterial 16S rRNA gene sequences spanning the hypervariable regions V3 and V4, with each sample averaging 10,637 reads. Reads of an average length (414–419 bp) were used for subsequent analyses. Using a 3% dissimilarity cut-off for clustering, the reads were grouped into different OTUs (online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000525000). The results showed that the number of unique bacterial OTUs was generally higher in soil samples collected from 8-year-old rhizospheres in the same area, with the greatest number of OTUs (2,337) detected in sample Ak-8 and the least (1,513) in sample He-3. Consistently, the number of actinomycete OTUs detected in the 8-year-old rhizospheres was greater than that detected in 3-year-old rhizospheres. However, the greatest number of actinomycetes OTUs (771) was detected in sample Ka-8.

Rarefaction analysis indicated that the libraries represented the bacterial communities as the rarefaction curves almost approached plateaus (Fig. 2). By combining the rarefaction curves with the Shannon diversity index, the data were sufficient to analyze the differences among soil samples. The indices of diversity and richness for bacteria and actinomycetes in soil samples are shown in online supplementary Table S1. Notably, the coverage estimators for 8-year-old jujube soil samples were higher than in 3-year-old counterparts taken from the same sampling area, and both soil samples from Ka were greater than those from He and Ak. Additionally, similar trends were apparent according to both the Chao and Shannon diversity indices. Consistently, actinomycete diversity indices were higher in 8-year-old jujube rhizosphere soils than in those from the 3-year-old soil samples. Collectively, these
results indicated that 8-year-old jujube rhizosphere soil samples harbored greater bacterial diversity as compared to 3-year-old soil samples taken from the same area.

**Bacterial Community Structure Analysis**

All sequences were classified from the phylum down to the genus level according to the Mothur program. Proteobacteria and Actinobacteria were the predominant
bacterial phyla across all soil samples and accounted for 83.2% of the total bacterial sequences (Fig. 3a). Acidobacteria, Bacteroidetes, Firmicutes, and Gemmatimonadetes were present with low abundance in each sample (each phylum ∼3%). Many rare phyla such as Nitrospira, TM7, Euryarchaeota, Armatimonadetes, Planctomycetes, and Spirochaetes were identified but only accounted for <1% of the total microbial population. Additionally, unclassi-
fied bacteria comprised 4.8% of the population in all samples.

Bacterial phylum distribution varied among the soil samples. Proteobacteria accounted for 63.2% of the bacterial communities in sample Ak-8 but only 37.8% of those in Ka-3. By contrast, Actinobacteria comprised 42% of the bacteria in Ka-3 but only 21.5% in Ak-8. Moreover, there were also age-associated differences, even in the same sampling area. For example, Proteobacteria accounted for 37.8% of the bacterial communities in Ka-3 but 49.4% in Ka-8. The distributions of other phyla were also nonuniform. Gemmatimonadetes accounted for 5.82% of the bacterial communities in He-8, which was significantly higher than in Ka-8 (0.58%). The proportion of Firmicutes was equivalent in Ka-3 (5.81%) and Ka-8 (5.85%), but higher than that in other soil samples. Both He-3 and Ak-3 showed a relatively large percentage of unclassified bacteria (7.42% and 7.54%, respectively). Additionally, some phyla existed in only specific soil samples. For example, Euryarchaeota was found only in He-3, Armatimonadetes and Planctomycetes were found only in Ak-8, and Spirochaetes was found only in Ka-3.

Actinobacteria was a very important phylum that was widely distributed in all soil samples, although the sub-class distributions differed among the six soil samples (Fig. 3b). Actinobacteridae was the most abundant group in each sample, accounting for 81.8% in Ka-8 but only 46.8% in He-3. Unclassified bacteria (9.93–31.6%) formed the second most prevalent group, except in He-8, where Acidimicrobidae was the second highest. Rubrobacteridae was also prevalent in all soil samples (3.12–9.01%). Although Nitriliruptoridae was found in all samples, it was relatively infrequent (0.15–0.96%). Interestingly, Coriobacteridae was found only in Ak-8.

To further classify the most abundant bacteria in the soil rhizospheres, we subsequently identified the 25 most abundant OTUs among the samples (Fig. 4). The top abundant OTUs for He-3 were OTU002 Pseudomonas (3.1%), OTU004 Pseudomonas (2.9%), and OTU001 Arthrobacters (2.9%). He-8 was dominated by OTU002 Pseudomonas (2.8%), OTU003 Pseudomonas (2.6%), and OTU001 Arthrobacters (2.1%). Sample Ka-3 was dominated by OTU001 Arthrobacters (8.1%), OTU007 Pseudomonas (2.3%), and OTU009 Micrococcaceae (1.4%).
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Sample Ka-8 was dominated by OTU001 Arthrobacters (10.2%), OTU007 Pseudomonas (7.2%), and OTU004 Microccoccaceae (4.6%). The top abundant sequences in sample Ak-3 were related to OTU001 Arthrobacters (5.2%), OTU002 Pseudomonas (4.8%), and OTU003 Microccoccaceae (4.1%). The top abundant OTUs associated with sample Ak-8 were OTU003 Pseudomonas (10.9%), OTU002 Pseudomonas (9.1%), and OTU005 Pseudomonas (4.1%). Compared with the genus from the six soil samples, it was found that the top abundant OTU was different among different regions, and also different among sampling points with different growth years.

Comparison of Bacterial Communities among the Different Sampling Areas

The bacterial OTU shared among the three sampling areas were visualized with a Venn diagram. At a 3% dissimilarity level, 3,003, 3,200, and 3,140 bacterial OTUs were identified in samples from He, Ka, and Ak, respectively, of which 711 were shared among all three sites (Fig. 5a). He and Ka prefectures shared 1,069 OTUs, He and Ak shared 1,074 OTUs, and Ka and Ak shared 1,185 OTUs. A similar analysis was performed for actinomycetes (Fig. 5b). Seven primary phyla were shared among the three areas: Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes, and Nitrospirae. Euryarchaeota and Spirochaetes were found specifically in He and Ka, respectively, whereas Armatimonadetes and Planctomycetes were only found in Ak. Additionally, five subclasses were shared among the three areas, whereas Coriobacteridae was identified only in Ak.

Comparison of Bacterial Communities with Respect to the Age of the Jujube Rhizosphere

A metastats analysis was used to compare jujube rhizosphere biodiversity with respect to age in samples obtained from the same area. For the soil samples from He prefecture, the dominant phyla were the same for samples He-3 and He-8, except for Euryarchaeota, which was identified only in the He-3 sample. However, the dominant phyla had an unbalanced distribution between the 3 and 8-year samples, with analysis of variance revealing that the proportion of each of the microbial phyla (Acidobacteria, Actinobacteria, Firmicutes, Nitrospirae, Gemmatimonadetes, Proteobacteria, and unclassified bacteria) varied significantly between these samples. Proteobacteria accounted for 60.3% of the bacteria in 3-year-old jujube soil, whereas they accounted for 51.8% in 8-year-old soil. Furthermore, Gemmatimonadetes and Nitrospi-
ra comprised 5.82% and 0.51% of the bacteria in 8-year-old soil samples, but only accounted for 1.15% and 0.11% in the 3-year-old soil samples, respectively. For actinomycetes, similar bacterial subclasses were observed in the two different growth years, albeit in different proportions. Specifically, Actinobacteridae was the most predominant group in both 3- and 8-year-old soil samples, accounting for 46.8% and 61.7% of all species, respectively. Acidimicrobidae was the second most abundant group in both the 3-year-old (14.1%) and 8-year-old soils (20%). Additionally, the 8-year-old soils accounted for a higher proportion of bacterial communities than the 3-year-old soils, as illustrated by community analyses in the Kashi and Aksu prefectures.

**Linking Bacterial Communities to Soil Properties**

Redundancy analysis revealed a significant correlation between soil properties and phylum abundance (Fig. 6a). In particular, the prevalent phyla in He-3 correlated with available K and N (p < 0.05). Additionally, bacterial communities in He-8 were related to available P and moisture, whereas those in Ak-3 were related to moisture, organic matter, and available P (p < 0.05). No such connections were found in the other samples. Moreover, the relative abundance of Acidobacteria correlated positively with available P and pH, whereas Nitrospira, Gemmatimonadetes, Chiorobi, and unclassified bacteria correlated positively with moisture and organic matter and negatively with available K and N. In addition, the relative abundance of TM7, Chlamydiae, Proteobacteria, Bacteroidetes, Planctomycetes, and Verrucomicrobia correlated positively with available N and K and negatively with organic matter. We also found that Actinobacteria, Firmicutes, Elusimicrobia, WS3, Euryarchaeota, and Cyanobacteria had no significant correlation with any soil property. Further, we analyzed the effect of soil properties on actinomycetes (Fig. 6b). At the subclass level, He-3 subclasses correlated with increased available K and moisture, whereas there was no significant correlation with other soil properties (p < 0.05). The relative abundance of Acidimicrobidae correlated positively with available N and organic matter, while Coriobacteridae correlated positively with pH. Actinobacteridae and Nitirimuruptoridae showed no significant correlation with any soil property.

**Discussion**

The analysis of amplified and sequenced 16S rRNA genes was an important method for analysis of microbial community composition, and the increased length of se-
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The age of jujube rhizospheres also had a marked effect on bacterial community composition. Notably, richness and diversity indices showed that 8-year-old jujube rhizosphere bacterial communities were more diverse than those from 3-year-old plants. A similar trend was also observed with actinomycetes at the subclass level. The association between rhizosphere age and bacterial diversity may result from the increased quantity and quality of root exudates observed with older plants [Dunfield and Geremia, 2003; Houlden et al., 2011]. However, the Shannon index curves reached saturation with increasing numbers of sequences, indicating that nearly all species in the sample were covered and the data would be acceptable for use in our analysis.

The sequence analyses revealed that Proteobacteria, Actinobacteria, and Acidobacteria were the most abundant phyla in all samples, consistent with other studies demonstrating that these three bacterial phyla represent more than 75% of the total 16S rRNA gene clones in the libraries studied [Roesch et al., 2007]. In our study, the number of Proteobacteria and Actinobacteria accounted for 83.2% of the bacterial sequences. Moreover, Acidobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes, Nitrospira, TM7, and unclassified bacteria were frequently encountered in the jujube rhizospheres. Significant differences in soil bacterial composition were also observed in the three sampling areas. Proteobacteria were substantially more prevalent in all sites with the exception of Ka-8, in which Actinobacteria was the most abundant phylum. To explain why some bacterial phyla are more abundant in soil than others, some researchers have proposed the concept of copiotrophic and oligotrophic bacteria [Fierer et al., 2007]. Proteobacteria are considered copiotrophic that associate with large amounts of available nutrients. In the present work, sample Ka-8 contained the lowest available K, P, and N and organic matter content, which may explain the limited Proteobacteria diversity.

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Many studies have shown that environmental factors shape bacterial community structure [Lauber et al., 2008; Rousk et al., 2010; Brockett et al., 2012; Sui et al., 2019; Liu et al., 2021c]. The present study used redundancy analysis to determine the effect of soil properties on the abundance of bacterial phyla and actinomycetes subclasses. Soil pH is an important factor for bacterial community structure [Lauber et al., 2008], and showed little to no effect only on Acidobacteria and Coriobacteriidae diversity. In comparison, several other soil properties – such as moisture content, organic matter, and available N, P, and K – correlated significantly with the abundance of certain bacterial phyla and actinomycetes subclasses. For instance, the relative abundance of Nitrospira, Gemmatimonadetes, Chlorobi, and unclassified bacteria correlated positively with moisture and organic matter and negatively with available K. Moreover, Acidimicrobiales correlated positively with available N and organic matter, whereas Coriobacteriidae correlated positively with pH. Furthermore, Proteobacteria and Bacteroidetes correlated positively with available N. This finding may be a suitable explanation for the higher percentage of Proteobacteria and Bacteroidetes in Ak-8 than in Ak-3, in which the content of available N was lower than in Ak-8.

Materials and Methods

Sample Collection and Soil Characterization

Soil was collected from jujube (variety Junzao) rhizospheres across the southern Xinjiang Uygur Autonomous Region of China. Six soil samples were carried out in May 2021 and collected from each of the three prefectures: Kashi (39°07′ N, 79°56′ E), Hetian (38°11′ N, 77°16′ E), and Aksu (80°11′ N, 40°47′ E). The three areas from which soil samples were taken are separated by approximately 460–770 km. Jujube trees of 3 or 8 years of age were selected at each site, and three soil samples for each age were randomly collected by five-point sampling. For every point, jujube roots at a depth of 20–30 cm were gently shaken to remove loosely adhering soil with minimum injury, and the rhizosphere soil of about 1 kg was carefully collected from fine roots by gently scraping adhering soil using fine brushes, and soils of the same type collected in the same plot were thoroughly mixed as one sample. At the same time, soil samples were collected for measuring physical and chemical properties. All soil samples were placed into separate sterile plastic bags, immediately placed on ice, and then transported to the laboratory for storage at −20°C until DNA extraction. The physical/chemical parameters of soil samples were measured as follows. Soil moisture content was estimated with the oven dry-
weight method, and pH was determined using a glass electrode meter in a suspension of 1 g soil in 5 mL distilled water. Available P was extracted with sodium bicarbonate and then measured with the molybdenum blue method. Available K was determined by flame photometry [Zhao et al., 2014]. Available N was determined by potassium persulphate oxidation. Organic matter content was determined as previously described [Walkley and Black, 1934].

**DNA Extraction, PCR Amplification, and 454 Pyrosequencing**

Total genomic DNA was extracted from 0.4 g of soil using the soil DNA kit (OMEGA, Bio-Tek, Winooski, VT, USA) according to the manufacturer’s instructions. The concentration and quality of the DNA extracted were analyzed with a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) (A<sub>260</sub>/A<sub>280</sub> > 2.0). The bacterial 16S rRNA genes were amplified from genomic DNA by PCR using the following barcoded primers: V3-F, 5'-TACGGGRAGGCAGCAG-3'; V4-R, 5'-AGGGTACTAATATCCT-3'. PCR was performed in 20 μL reaction volumes containing 1 μL DNA template (10 ng·μL<sup>-1</sup>), 0.4 μL (10 pmol) of each primer, 0.15 μL Ex Taq (TaKaRa, Tokyo, Japan), 2 μL 10 × buffer, 1.6 μL 2.5 mmol·L<sup>-1</sup> dNTPs (TaKaRa, Tokyo, Japan), and 13.5 μL ddH<sub>2</sub>O. PCR was performed with a thermal cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: initial denaturation at 98°C for 2 min, followed by 35 cycles of 98°C for 15 s, 56°C for 30 s, and 72°C for 40 s, with a final extension at 72°C for 10 min. PCR products were purified using the TaKaRa agarose gel DNA purification kit (TaKaRa, Tokyo, Japan) and quantified with a Quant-iT PicoGreen double-stranded DNA assay (Invitrogen, Carlsbad, CA, USA). A mixture of the purified 16S rRNA gene amplifications from each soil sample was subjected to pyrosequencing on the 454 GS FLX Titanium platform (Roche, Basel, Switzerland) at the National Human Genome Center of China (Shanghai, China) according to the manufacturer’s instructions.

**Processing of Pyrosequencing Data**

Pyrosequencing data were processed using Mothur (version 1.25.1) following the Schloss standard operating procedure [Lee et al., 2011]. Pyrosequencing reads with ambiguous nucleotides, sequences <200 bp, one or more primer mismatches, or two or more barcode mismatches were denoised and excluded from further analysis. The filtered sequences were then assigned to soil samples according to the corresponding barcodes using a bespoken Java program. Operational taxonomic units (OTUs) at the 3% dissimilarity level were determined by comparing the sequences with those in the Silva database (http://www.arb-silva.de/) using Mothur, and the most abundant sequence in each OTU was selected as the representative sequence. Representative sequences were taxonomically classified using a Ribosomal Database Project naïve Bayesian rRNA classifier 2.2 (https://www.rdp.cme.msu.edu/classifier/classifier.jsp) with a confidence threshold of 0.8. The relative proportion of a given phylogenetic group with respect to the entire microbial community was defined as the number of sequences affiliated with that group divided by the total number of sequences per sample.

**Bioinformatics and Statistical Analysis**

An OTU-based analysis was performed to calculate the species richness, diversity, and coverage of each rhizosphere sample. The R software package (version 2.14.2) was used to calculate the Shannon-Wiener index, Simpson’s diversity index, and evenness index for each sample. Rarefaction curves generated in Mothur were used to compare the relative levels of bacterial OTU diversity across all soil samples. Community structure and distance coefficients were calculated and then used for the unweighted pair-group method with arithmetic mean cluster analysis. Venn diagrams were generated using custom Perl scripts [Zeng et al., 2013]. The relationship between bacterial phyla and soil conditions was analyzed by redundancy analysis in R. All chemical data are expressed as the mean value ± standard error. Significant differences (p < 0.05) in α-diversity and soil properties between samples were determined by analysis of variance in SAS 8.0 (SAS Institute, Cary, NC, USA).

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**Statement of Ethics**

This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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**Author Contributions**

Zhaoyang Liu designed the experiments; Bingcong Ji participated in sample collection and data analysis; Yaqing Zhang performed the experiments; Zhaoyang Liu and Xunli Liu wrote the manuscript. All the authors have read and agreed to the published version of the manuscript.

**Data Availability Statement**

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.
