Syringa reticulata subsp. amurensis growth influencing the microbial diversity and heavy metal contents in mixed substrate of municipal sludge and construction waste

Xueya Liu\textsuperscript{a,b}, Lijuan Liu\textsuperscript{a,b}, Pingsheng Leng\textsuperscript{a,b} and Zenghui Hu\textsuperscript{a,b,c}

\textsuperscript{a}Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing University of Agriculture, Changping, Beijing, China; \textsuperscript{b}College of Landscape Architecture, Beijing University of Agriculture, Changping, Beijing, China; \textsuperscript{c}Beijing Laboratory of Urban and Rural Ecological Environment, Beijing University of Agriculture, Changping, Beijing, China

**ABSTRACT**

The municipal sludge and construction waste can be used in cultivating substrate for plants, but the effect of plant growth on the microbial diversity and heavy metal contents is largely unknown. In this study, municipal sludge and construction waste residue were mixed to cultivate Syringa reticulata subsp. amurensis. After sowing seeds on the mixed substrate, the microbial diversity and heavy metal contents of the substrate were measured after 3-month and 7-month growth of S. reticulata subsp. amurensis. The results showed that S. reticulata subsp. amurensis seedlings grew well in the mixed substrate. Moreover, the substrate revealed a different microbial diversity. The relative abundance of the top microbial phylum, Proteobacteria, decreased with the plant growth, as well as Bacteroidetes and Actinobacteria. Gemmatimonadetes presented increasing relative abundance. In addition, the heavy metal contents decreased with plant growth, suggesting that S. reticulata subsp. amurensis removed heavy metals from the mixed substrate.

**INTRODUCTION**

Sewage sludge and construction waste residue are the main solid wastes in cities, which cause serious environmental problems worldwide [1]. Landfill and dumping are the dominant treatment modes for sewage sludge and construction waste residue, particularly in China, which result in secondary pollution and land encroachment [2,3]. Thus, ecological and recycling treatment modes are sought. Sewage sludge is the organic byproduct of the wastewater treatment process. A considerable amount of sewage sludge is produced annually worldwide. The amount of sewage sludge has increased at a rate of 10% per year in China. Sewage sludge has been used as organic fertilizer owing to its rich nutrients and organic matter [4]. However, as wastewater contains a diversity of pollutants, including heavy metals, organic micro-pollutants, and pathogens, which are concentrated during the treatment process [4,5], sewage sludge contains a large number of pollutants. Sewage sludge is rarely used in farming systems, as pollutants can be transmitted into the food chain. As an alternate pathway, sewage sludge has been applied to landscape plants. Applications of sewage sludge significantly promote the growth of landscape plants, and soil physical and chemical properties improve [4]. As landscape plants are not contributors to the food chain, the potential hazards to human consumption are avoided. In addition, the hazardous substances in the substrate from sewage sludge can be removed gradually through plants [6]. Thus, sewage sludge maybe used for urban forest construction and greening on ecological degradation area such as some rock slope zones along with road, which is an economic and ecological disposal mode.

Construction waste is also a type of main solid waste in cities. China is already the world’s largest producer of construction waste, accounting for 40% of all municipal waste. In 2018, 2.1 billion tons of construction waste was produced in China, and the output represents the accelerated growth trend [7]. A total of 40 million tons of construction waste is produced every year in Beijing and landfills are the main disposal pathway for this waste, which is reused and recycled at a low ratio. Thus, the mass of construction waste needs to be dealt with safely and in a resourceful manner. In our previous study, construction waste residue and sewage sludge were mixed to plant Ailanthus altissima [8], which demonstrated the feasibility of using this mixture as cultivating substrate. Although there are no nutrients, as the auxiliary ingredient of sewage sludge, addition of construction waste residue into mixed substrate can increase the air and water permeability. But the influence of plant growth on the properties of this mixed substrate is little known.

Microbes play a key role in nutrient biogeochemical cycling [9], energy flow, and organic matter decomposition [10], plant growth [11], and plant resistance
ornamental

[12,13], which have been widely recognized as important component of substrate quality. Microbial community is sensitive to environmental change, so it is an important indicator of sustainable land use [14]. All related previous studies have focused on the effect of municipal sludge on plant growth and physicochemical properties of the soil, whereas changes in the soil microbes are largely unknown. In the previous study, the microbial diversity of a mixed substrate was investigated and a difference was observed before and after planting [8]. Plants [15] and root exudates [16] influence the soil microbial community, which also changes along with plant growth [17,18]. In particular, as a mixed substrate compounded with municipal sludge may contain a large number of harmful microbes, the changes in the microbes during plant growth deserve more attention.

Syringa reticulata subsp. amurensis is an important ornamental flowering tree with strong stress resistance in the north of China, which has been widely used in urban greenings and urban forest construction. In this study, seeds of S. reticulata subsp. amurensis were sowed on the mixed substrate composed of municipal sludge and construction waste residue at a volume ratio of 1:1. Then, substrate heavy metal contents, microbial diversity, and growth of S. reticulata subsp. amurensis were investigated to evaluate the effect of tree growth on the mixed substrate.

Materials and methods

Experimental design

The experiment was carried out at the Science and Technology Park of Beijing University of Agriculture. The sewage sludge used in this study was obtained from the Xiaotangshan sewage treatment plant, Changping district, Beijing, and was dried in the air naturally. The construction waste residue originated from a construction site near Tingzhuang village, Changping district, Beijing, and the large bricks and stones were removed. The municipal sludge was mixed with the construction waste residue at a volume ratio of 1:1 to prepare the culture substrate. Three experimental plots (5 × 1 m) were poured with a mixed substrate (50 cm thickness) respectively. Three hundred S. reticulata subsp. amurensis seeds (China National Tree Seed Corp., Beijing, China) were sowed uniformly on each experiment plot. The S. reticulata subsp. amurensis seedlings grew under natural condition.

Soil sampling

The mixed substrate without S. reticulata subsp. amurensis was sampled before sowing. After growth for 3 months and 7 months, the substrates within 2 cm close to the roots of 10 medium-sized S. reticulata subsp. amurensis seedlings at a depth of 10–15 cm were collected and divided into two parts. One part of the substrate was used to determine heavy metal content, while the other part was frozen in liquid N2 immediately for subsequent DNA extraction. The samples are listed in Table 1.

| Soil samples | Meanings |
|--------------|----------|
| CK           | the mixed substrate before sowing |
| M            | the rhizosphere soil of Syringa plants in mixed substrate after 3-month growth |
| O            | the rhizosphere soil of Syringa plants in mixed substrate after 7-month growth |

Plant growth investigation

The survival rate of S. reticulata subsp. amurensis was calculated after 3-month and 7-month growth. The heights of 30 medium-sized seedlings were measured with a meter stick. Coverage was tested using the canopy projection method.

Determination of heavy metals

Heavy metal contents, including As, Cd, Cr, Cu, Pb, and Zn were determined by inductively coupled plasma atomic emission spectroscopy (6,300; Thermo Fisher Scientific Inc., Waltham, MA, USA) after microwave digestion. The Multi-Element Calibration Standard 3 (PerkinElmer inc., Waltham, MA, USA) containing As, Cd, Cr, Cu, Pb, and Zn with the concentration of 10 μg/mL was used as standard reference material.

DNA extraction

Total genomic DNA was extracted from the soil samples using the CTAB/SDS method. DNA concentration and purity were monitored by 1% agarose gel electrophoresis. Each sample extraction was carried out three times. The DNA was diluted to 1 ng·L⁻¹ using sterile water. The extracted DNA was used as template DNA for high-throughput sequencing analysis by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

Microbial 16S rRNA gene amplification

We used the protocol of Caporaso et al. [19] to determine the diversity and composition of the microbial communities in the soil samples. Polymerase chain reaction (PCR) amplifications were conducted with the 515 F-806 R primer set, which amplifies the V4 region of the 16S rDNA gene. All PCR reactions were carried out in 30 μL reaction volumes with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA); 0.2 μM of the forward and
reverse primers, and 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, elongation at 72°C for 30 s, and 72°C for 5 min.

**Quantification and qualification of the PCR products**

The PCR products were mixed with the same volume of 1× loading buffer (containing SYB Green) and 2% agarose gel electrophoresis was performed for detection. Samples with a bright main band between 400 and 450 bp were chosen for further experiments. The PCR products were mixed in equally dense ratios. The mixture of PCR products was purified with the GeneJET Gel Extraction Kit (Thermo Scientific).

**Library preparation and sequencing**

Sequencing libraries were generated using the TruSeq DNA PCR-free Library Prep Kit for Illumina (New England Biolabs) following the manufacturer’s recommendations, and index codes were added. Library quality was assessed by the Qubit® 2.0 Fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system (Agilent Technologies, Palo Alto, CA, USA). The library was sequenced on an Illumina HiSeq platform and 250 bp paired-end reads were generated.

**Data analysis**

Paired-end reads from the original DNA fragments were merged using FLASH [20], which is a very fast and accurate analytical tool designed to merge paired-end reads when there are overlaps between reads1 and reads2. Paired-end reads were assigned to each sample according to unique barcodes. The sequences were analyzed using QIIME software [19], and in-house Perl scripts were used to analyze alpha- (within samples) and beta- (among samples) diversity. First, the reads were filtered with QIIME quality filters. Then, we used pick_de_novo_otus.py to choose the operational taxonomic units (OTUs). Sequences with ≥ 97% similarity were assigned to the same OTU. We picked a representative sequence for each OTU and used the RDP classifier to annotate taxonomic information for each representative sequence [21]. QIIME calculates both weighted and unweighted unifrac values, which are phylogenetic measures of beta diversity. We used weighted unifrac values for the principal components analysis (PCA).

**Results**

The survival rate, height, and coverage of *S. reticulata* subsp. *amurensis* were investigated after 3-month and 7-month growth (Figure 1). As results, the survival rate was about 70% after 3-month growth, which decreased to about 60% after 7-month growth (Figure 1A). Height and coverage were significantly higher after 7-month growth than those after 3-month growth (Figure 1B,C). Height after 7-month growth was 21.00 cm, which was 3.43-fold that after 3-month growth. Coverage increased significantly ($P < 0.05$). Coverage after 7-month growth was about 1.50-fold higher than that after 3-month growth.

The heavy metal contents in the substrates were also examined (Table 2). CK had higher heavy metal contents than those in M and O. Heavy metal contents decreased gradually from CK to M and to O. Cr (7.93%) and Pb (11.33%) contents in CK were significantly higher than those in M. The contents of all heavy metals decreased in O compared to M, particularly Cd, Cr, Cu, and Zn, whose contents changed significantly ($P < 0.05$). The contents of As, Cd, Cr, Cu, Pb, and Zn were significantly lower in O than those in CK by 14.35%, 34.04%, 24.01%, 20.78%, 19.54%, and 12.47%, respectively ($P < 0.05$).

Moreover, compared to the Environmental Quality Standard for Soils of China (GB15618-1995), the concentrations of heavy metals arrived at values of the second-degree soil.

![Figure 1](image-url)

**Figure 1.** The survival rate (A), height (B), and coverage (C) of *S. reticulata* subsp. *amurensis*. The value is the average, and standard errors are shown. Statistical significance (least significant difference (LSD)) of the difference in height of seedlings on substrates is indicated by different small letters ($P < 0.05$).
Table 2. Contents of heavy metals in substrates.

| Heavy metal | CK | M | O | Standard |
|-------------|----|---|---|----------|
| As          | 10.20 ± 0.62 a | 9.87 ± 0.62 ab | 8.92 ± 0.43 b | 20.00 |
| Cd          | 0.47 ± 0.07 a  | 0.43 ± 0.06 a  | 0.31 ± 0.04 b  | 1.00 |
| Cr          | 46.28 ± 1.29 a | 42.61 ± 1.42 b | 35.17 ± 0.92 c | 250.00 |
| Cu          | 68.76 ± 2.86 a | 66.34 ± 1.84 a | 54.47 ± 0.73 b | 100.00 |
| Pb          | 27.54 ± 1.71 a | 24.42 ± 1.19 b | 22.16 ± 1.01 b | 350.00 |
| Zn          | 252.78 ± 6.45 a| 246.39 ± 6.26 a| 221.25 ± 5.18 b| 300.00 |

Note: The average and standard errors are shown. Statistical significance (least significant difference (LSD)) of the difference in contents of heavy metals of substrates is indicated by different lower-case letters ($P < 0.05$). 'Standard' indicates the values of second-degree soil in the Environmental Quality Standard for Soils of China (GB15618-1995).

The tags of all samples were analyzed after high-throughput sequencing (Table 3). As listed in Table 2, the numbers of raw, total, and taxon tags of CK were higher than those of M and O. Every OTU represented one kind of microbial species. However, the number of CK OTUs was lower than that of the other two soil samples. The number of M OTUs exceeded 4,000, and the number of O OTUs was 3,849, but the number of CK OTUs was less than 3,800.

The rarefaction curves of all samples were generated using QIIME software (version 1.7.0, Figure 2). The curves of all samples gradually flattened after a sharp rise along with the increase in sequence number, indicating that the amount of sequencing data was large enough to reflect the vast majority of microbial species in the samples.

Venn diagrams of the OTUs are presented in Figure 3. In total, 1,583 similar OTUs were found among CK, M, and O. There were 556, 496, and 437 unique OTUs in CK, M, and O, respectively, indicating a gradual decrease. Similar OTUs were far more common than unique OTUs. The difference in microbial diversity was more apparent between CK and O than between M and O or between CK and M.

To analyze diversity among all samples, microbial species and their proportions at the phylum level are shown in Figure 4. Proteobacteria represented the largest proportion in the population, nearly reaching 50.00%, but a gradual decrease was found from CK to M, and to O. Bacteroidetes also accounted for a high proportion in CK, which exceeded 12.00% and was also higher than those in M and O, similar to Proteobacteria. Similar patterns were observed for the relative abundances of Actinobacteria and Firmicutes. However, the proportions of Acidobacteria, Gemmatimonadetes, Planctomycetes, and Verrucomicrobia were lower in CK than those in M and O. Gradual increases were found in the proportions of Gemmatimonadetes, Planctomycetes, and Verrucomicrobia from CK to M, and to O.

A hierarchical clustering analysis of the microbial communities at the genus level was performed to demonstrate the different compositions of the microbial community structures in these samples (Figure 5). The relative abundances of these 35 genera varied significantly in different soil samples. The abundances of Dokdonella, Aimnobacter, Psychrobacter, Streptomyces,

Table 3. Source data for the microbial community analysis.

| Samples | Base (nt) | Raw PE | Raw tags | Clean tags | Total tags | Taxon tags | Unclassified tags | OTUs |
|---------|-----------|--------|----------|------------|------------|------------|-------------------|------|
| CK      | 18,000,122| 76,798 | 74,990   | 73,864     | 70,977     | 66,894     | 4                 | 3,765|
| M       | 14,943,838| 63,281 | 61,621   | 60,915     | 58,984     | 54,953     | 0                 | 4,226|
| O       | 17,176,088| 71,913 | 71,285   | 69,890     | 67,894     | 64,437     | 1                 | 3,849|
| Mean    | 16,706,683| 70,665 | 69,299   | 68,223     | 65,952     | 62,095     | 2                 | 3,947|
Taibaiella, Rhodanobacter, Sporosarcina, Pseudomonas, Flavotalea, Pedobacter, Bacillus, Acinetobacter, Lysobacter, and Brevundimonas were relatively high in CK. H16, RB41, unidentified_Nitrospiraceae, Terrimonas, unidentified_Sub group_6, Sphingomonas, and Aquicella were found in M at high relative abundances. However, Rhizomicrobium, Steroidobacter, Filimonas, Planctomyces, unidentified_Gemmatimonadaceae, unidentified_Rhodospirillaceae, unidentified_Anaerolineaceae, Halangium, Flavobacterium, Chryseolinea, Gemmatimonas, Hirschia, and Bryobacter showed high relative abundances in O.

The difference in beta diversity among these three groups was analyzed in Figure 6 and significant differences were detected among them. CK had the largest value, which was significantly higher than M and O, and the O value was the lowest.

The soil microbial communities were subjected to a PCA (Figure 7). PC1 accounted for 25.25% and PC2 accounted for 15.86% of the total variation. Although CK and M exhibited relatively large variations within the group, these three soil samples were clearly distinguishable along the first and second PCs.

**Discussion**

Solid wastes, including sewage sludge and construction waste cause environmental problems, which has attracted more and more attention. Applying sewage sludge and construction waste for vegetation restoration in degraded ecosystems is a feasible recycling pathway, which avoids the potential risk of toxic contaminants entering the human food web [8]. In this study, the changes in microbial diversity of a mixed substrate of sewage sludge and construction waste residue were investigated after planting S. reticulata subsp. amurensis using high-throughput Illumina HiSeq platform sequencing. In addition, plant growth and heavy metal contents were surveyed.

Microbes play an important role in soil physicochemical properties and plant growth. Our experimental results demonstrate that the microbial communities in the mixed substrate significantly differed before and after planting S. reticulata subsp. amurensis, and also changed along with plant growth. Proteobacteria was the most common microbial phylum in all samples. Moreover, the relative abundance of Proteobacteria is about 50.50% in activated sludge from a municipal wastewater treatment plant in Northern China [22]. Proteobacteria include many important ammonia oxidizing bacteria involved in nitrogen fixation, such as Betaproteobacteria and Gammaproteobacteria [23]. Thus, the municipal sludge provided nitrogen for S. reticulata subsp. amurensis. Bacteroidetes was another main microbial phylum in the mixed substrate, which contains many pathogenic bacteria and is frequently observed in highly saline aquatic environments. Planting S. reticulata subsp. amurensis decreased the relative abundance of Bacteroidetes. Acidobacteria, Gemmatimonadetes, and Actinobacteria were the other main phyla in the samples. Acidobacteria inhabits a wide variety of terrestrial and aquatic habitats and is particularly abundant in acidic soils, peatlands, and mineral iron-rich environments. These bacteria possess a special driving effect and ecological function in systems. The increase in the relative abundance of Acidobacteria in the substrate suggested that it played an important role in the substrate ecosystem after planting S. reticulata subsp. amurensis. Gemmatimonadetes comprise approximately 2.00% of soil bacterial communities [24]. Gemmatimonadetes in soil are reported to be positively correlated with soil NO₃⁻N content. So, the high level in

*Figure 4. Relative abundance of the dominant microbial phyla observed in 16S rRNA gene sequences in different samples.*
Suggested that the growth of *S. reticulata* subsp. *amurensis* improved the nutrient structure of the substrate. Actinobacteria are widely distributed in terrestrial and aquatic ecosystems, and have many functions, such as decomposition of organic substances and production of antagonistic substances against plant pathogens [25]. However, members of Actinobacteria can infect plants and cause disease [26]. The heatmap analysis, beta diversity analysis, and PCA also showed differences among the different sample groups at the genus level. Similar to the phylum level, the microbial communities in O were significantly different from those in CK, and the microbial community composition of M was between that of CK and O. This result indicates that planting *S. reticulata* subsp. *amurensis* significantly changed microbial community composition. Sun et al. reported that planting apple seedlings has a large impact on the soil bacterial community [27]. Growth of *S. reticulata* subsp. *amurensis* resulted in the high relative abundances of *Rhizomicrobium*, *Steroidobacter*, *Filimonas*, and *Planctomyces* in the mixed substrate. The change in relative abundance of microbes in the mixed substrate along with growth of *S. reticulata* subsp. *amurensis* seedlings may have resulted from the change in nutrient composition and physicochemical properties, as well as the root exudates, which need to be investigated in another study.

*S. reticulata* subsp. *amurensis* seedlings exhibited a high survival rate in the mixed substrate, although there was a decrease in survival after 7-month growth. Moreover, height and coverage were also good. These

---

**Figure 5.** Hierarchically clustered heatmap analysis of microbial community at the genus level. The scale bar shows the standardized Z-value of the microbial relative percentages. The larger value in the scale bar shows the larger relative abundance of this genus in the sample.
findings indicate that this mixed substrate provided ample nutrients for *S. reticulata* subsp. *amurensis* seedlings. The growth of tree peony [28] and *Mangifera persiciforma* [29] are also promoted significantly by adding sewage sludge compost as fertilizer to the culture substrate. Our results illustrate that a mixture of municipal sludge and construction waste residue achieved the same promoting effect on plant growth as applying sludge to the soil. In a previous study, we tested the nutrient content of this mixed substrate, which was significantly higher than that of garden soil, so *S. reticulata* subsp. *amurensis* seedlings experienced ample nutritional conditions [8].

Furthermore, these two solid waste types can be reused. Heavy metals are the main harmful ingredients in land used of sewage sludge [30,31]. However, the contents of heavy metals measured in the mixed substrate matched the level of second-degree soil referring to the Environmental Quality Standard for Soils of China (GB15618-1995), which was also found in our previous study [8]. Adding construction waste residue to the mixed substrate decreased the heavy metal contents in the municipal sludge. Moreover, the contents of the main heavy metals and Zn decreased in the mixed sludge along with growth of the *S. reticulata* subsp. *amurensis* seedlings, indicating that *S. reticulata* subsp. *amurensis* seedlings absorbed heavy metals to decrease their contents in the mixed substrate. Many previous studies focused on heavy metals during land applications of municipal sludge, and reported that as long as the application amount was controlled within a reasonable level, the pollution and hazard of heavy metals were avoided [32–34]. We reported similar results in a previous study. *Forsythia suspensa*, *Sophora japonica*, *Cotinus coggyria*, and *Ailanthus altissima* absorbed large amounts of heavy metal elements from a mixed substrate prepared of raw sludge and fly ash, which makes the environment safe and has great

![Figure 6. Difference analysis of beta diversity among three sample groups. Turkey test was performed on the beta diversity statistical significance.](image)

![Figure 7. PCA of microbial communities among three sample groups.](image)
prospects for recovery [6]. In a recent study, Antonkiewicz et al. found that the ash-sludge mixtures had a more favorable effect on the soil in terms of pH, TOC, total nitrogen, and total exchangeable bases than the waste used separately, and plants also took up more heavy metals to reduce the accumulation in the soil during a long-term use [35].

Conclusions

In this study, we used municipal sludge mixed with construction waste residue as a substrate to grow healthy S. reticulata subsp. amurensis plants. Moreover, along with plant growth, the substrate revealed different microbial diversities, indicating that plant growth influenced the microbial community. In addition, the heavy metal contents decreased in the mixed substrate along with plant growth, suggesting that S. reticulata subsp. amurensis transferred the heavy metals from the mixed substrate. The results demonstrated that the mixed substrate can be applied for urban forest construction and greening, which was also be improved by plant growth.

Acknowledgments

We also gratefully acknowledge the Beijing Municipal Education Commission for their financial support through Innovative Transdisciplinary Program of Ecological Restoration Engineering.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Beijing Municipal Natural Science Foundation under Grant [number 8132005].

References

[1] Brodersen J, Juul J, Jacobsen H. Review of selected waste streams: sewage sludge, construction and demolition wastes, waste oils, wastes from coal-fired power plants and biodegradable municipal waste. Technical Reports, European Topic Centre on Waste. European Environment Agency; 2002.
[2] Li J, Hu Y, Gan J. Present situation on resources utilization of sewage sludge and its potential problems in China. Appl Mech Mater. 2014;448-453:756–760.
[3] Ding Z, Yi G, Tam VWVWY, et al. A system dynamics-based environmental performance simulation of construction waste reduction management in China. Waste Manage. 2016;51:130–141.
[4] Singh RP, Agrawal M. Potential benefits and risks of land application of sewage sludge. Waste Manage. 2008;28:347–358.
[5] Wei Y, Liu Y. Effects of sewage sludge compost application on crops and cropland in a 3-year field study. Chemosphere. 2005;59:1257–1265.
[6] Ma B, Liu L, Liu X, et al. Evaluation of the environmental and plant growth effectiveness of a new substrate consisting of municipal sludge and fly ash. Waste Manage. 2019;99:163–171.
[7] Wang Z, Ran C. Decoupling relationship between construction waste production and economic growth from a spatiotemporal perspective. J Eng Manage. 2020;34(3):39–44.
[8] Liu X, Liu L, Leng P, et al. Feasible and effective reuse of municipal sludge for vegetation restoration: physiochemical characteristics and microbial diversity. Sci Rep. 2019;9:879.
[9] Jiang X, Liu W, Liu Q, et al. Soil N mineralization, nitrification and dynamic changes in abundance of ammonia-oxidizing bacteria and archaea along a 2000 year chronosequence of rice cultivation. Plant Soil. 2013;365:59–68.
[10] Bastida F, Kandeler E, Moreno JL, et al. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Appl Soil Ecol. 2008;40:318–329.
[11] Jacoby R, Peukert M, Succurro A, et al. The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. Front Plant Sci. 2017;8:1617.
[12] Khan AG. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol. 2005;18(4):355–364.
[13] Pineda A, Zheng S, van Loon JJA, et al. Helping plants to deal with insects: the role of beneficial soil-borne microbes. Trends Plant Sci. 2010;15:507–514.
[14] Xu Q, Jiang P, Xu Z. Soil microbial functional diversity under intensively managed bamboo plantations in southern China. J Soil Sediments. 2008;8:177.
[15] Yang R, Tang J, Chen X, et al. Effects of coexisting plant species on soil microbes and soil enzymes in metal lead contaminated soils. Appl Soil Ecol. 2007;37:240–246.
[16] Wu J, Yu S. Effect of root exudates of Eucalyptus urophylla and Acacia mearnsii on soil microbes under simulated warming climate conditions. BMC Microbiol. 2019;19:224.
[17] Jiao S, Chen W, Wang J, et al. Plant growth and oil contamination alter the diversity and composition of bacterial communities in agricultural soils across China. Land Degrad Dev. 2018;29:1660–1671.
[18] Jiao S, Chen W, Wei G. Resilience and assemblage of soil microbiome in response to chemical contamination combined with plant growth. Appl Environ Microbiol. 2019;85(6):e02523–18.
[19] Caporaso JG, Kuczynski J, Stombaugh J, et al. QLIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335–336.
[20] Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957–2963.
[21] Wang Q, Garrity GM, Tiedje JM, et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261–5267.
[22] Wang Z, Miao M, Kong Q, et al. Evaluation of microbial diversity of activated sludge in a municipal wastewater treatment plant of northern China by high-throughput sequencing technology. Desalin Water Treat. 2016;57:1–6.

[23] Juretschko S, Timmermann G, Schmid M, et al. Combined molecular and conventional analyses of nitrifying bacterial diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira-like* bacteria as dominant populations. Appl Environ Microbiol. 1998;64:3042–3051.

[24] Janssen PH. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl Environ Microbiol. 2006;72:1719–1728.

[25] Ventura M, Canchaya C, Tauch A, et al. Genomics of *Actinobacteria*: tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev. 2007;71:495–548.

[26] Hogenhout SA, Loria R. Virulence mechanisms of gram-positive plant pathogenic bacteria. Curr Opin Plant Biol. 2008;11:449–456.

[27] Sun J, Zhang Q, Zhou J, et al. Illumina amplicon sequencing of 16s rRNA tag reveals bacterial community development in the rhizosphere of apple nursery at a replant disease site and a new planting site. PloS One. 2014;9:e111744.

[28] Xue D, Huang X. The impact of sewage sludge compost on tree peony growth and soil microbiological, and biochemical properties. Chemosphere. 2013;93:583–589.

[29] Chu S, Wu D, Liang L, et al. Municipal sewage sludge compost promotes *Mangifera persiciforma* tree growth with no risk of heavy metal contamination of soil. Sci Rep. 2017;7:13408.

[30] Shamuyarira KK, Gumbo JR. Assessment of heavy metals in municipal sewage sludge: a case study of Limpopo province, South Africa. Int J Environ Res Public Health. 2014;11(3):2569–2579.

[31] Feng J, Jia L, Liu Q, et al. Source identification of heavy metals in sewage sludge and the effect of influent characteristics: a case study from China. Urban Water J. 2018;15(4):381–387.

[32] Morera MT, Echeverria J, Garrido J. Bioavailability of heavy metals in soils amended with sewage sludge. Can J Soil Sci. 2002;82:433–438.

[33] Huang X, Xue D, Xue L. Changes in soil microbial functional diversity and biochemical characteristics of tree peony with amendment of sewage sludge compost. Environ Sci Pollut R. 2015;22:11617–11625.

[34] Mohamed B, Mounia K, Aziz A, et al. Sewage sludge used as organic manure in Moroccan sunflower culture: effects on certain soil properties, growth and yield components. Sci Total Environ. 2018;627:681–688.

[35] Antonkiewicz J, Kowalewska A, Mikołajczak S, et al. Phytoextraction of heavy metals after application of bottom ash and municipal sewage sludge considering the risk of environmental pollution. J Environ Manage. 2022;306:114517.