https://doi.org/10.17221/430/2023-PSE

Effects of biogas residue addition, as cultivation substrate, on ginseng growth

Yan Gui[1,2], Meng Suo[1,2], Zhijie Qiu[1,2], Hao Wu[1,2], Zongjun Cui[3], Hongyan Yang[1,2]**

[1]*College of Life Sciences, Northeast Forestry University, Harbin, P.R. China*
[2]*Key Laboratory for Enzyme and Enzyme-like Material Engineering of Heilongjiang, Harbin, P.R. China*
[3]*College of Agronomy and Biotechnology, China Agricultural University, Beijing, P.R. China*
**Corresponding authors: acuizj@cau.edu.cn; yanghy@nefu.edu.cn*

Citation: Gui Y., Suo M., Qiu Z.J., Wu H., Cui Z.J., Yang H.Y. (2024): Effects of biogas residue addition, as ginseng cultivation substrate, on ginseng growth. Plant Soil Environ., 70: 101–110.

Abstract: The effects of biogas residue as a substrate on ginseng growth and its feasibility for ginseng cultivation are unclear. The present study used biogas residue at different concentrations and maturity levels to cultivate ginseng. The biological characteristics of ginseng, soil physiochemical indices, and ginseng and soil microbial communities were investigated. The results showed that with increasing ginseng content and maturity, the total fresh weight, total length and saponin content significantly increased. The enzyme activities of soil, NO_3^--N, and available phosphorus also increased. The microbiome analysis revealed that with the addition of biogas residue, microorganisms related to plant growth promotion, such as Chloroflexi, Gemmatimonadota and Mortierellomycota, were more common in the plant or rhizosphere soil. The results based on the co-occurrence network showed that the structure of the bacterial community was more stable than that of the fungal community with increasing biogas residue content. Our results indicated that biogas residue could be used as a ginseng cultivation substrate and promote growth.

Keywords: compost; medicinal plant; soil improvement; *Panax ginseng* C.A. Meyer.; soil properties; microbial community

Energy shortages have become a major concern for people. With the advancements in science and technology, crop yields are increasing, and more crop straw is being produced. The annual straw production in China is approximately 700 million tons (Lin et al. 2022). In addition to being used as a substrate, fertiliser, fuel, animal feed and raw material, approximately 15% of the water is still unused (Zhou et al. 2023). The problem of environmental pollution caused by excess straw poses a serious threat to human health and has become a global problem that urgently needs to be solved. Straw energy conversion, such as converting straw into biogas through anaerobic fermentation, is one of the most effective ways to address residual straw pollution.

During convergence, the production continuity determines that the emitted biogas residue is characterised by continuous, large amounts and incomplete material decomposition. Therefore, searching for efficient, convenient and low-cost methods is necessary to eliminate the subsequent pollution pressure caused by a large amount of excess biogas residue. Composting is a way to convert biogas residues into usable resources effectively. During the composting process, the undecomposed materials in the digestate are further degraded by microorganisms, and potential threats such as parasites, pathogens and weeds are significantly reduced (Bernal et al. 2009). Mature biogas residue compost has been used as a growing substrate for a variety of crops, such as zucchini, pepper, eggplant, lettuce, and tomato plants (Jara Samaniego et al. 2017, Meng et al. 2018, Liu et al. 2023a). However, perennial plants, particularly perennial medicinal plants, have rarely been reported.

Supported by the Joint Funds of the Natural Science Foundation of Heilongjiang Province, Project No. LH2022C013.

https://doi.org/10.17221/430/2023-PSE

Ginseng is an important perennial medicinal plant, and global demand is increasing. Chinese ginseng accounts for 76.9% of global ginseng production (Jiang et al. 2021), and additional ginseng needs to be cultivated to meet the large market demand. However, due to the severe ecological damage to forest areas caused by the original planting method, namely, cutting down trees and planting ginseng and because old ginseng land has not been resolved, the problem of acreage restriction has become a bottleneck in the development of the ginseng industry. Ginseng cultivation on agricultural land is one of the most effective ways to address the land limitation problem associated with ginseng cultivation (Jin et al. 2022). However, for ginseng cultivation, there are strict soil requirements. Soil enhancement must be carried out to grow ginseng on arable land. Composting is one of the necessary soil enhancement measures. Therefore, whether biogas residue can be used in ginseng cultivation must be systematically investigated.

In this study, we carried out a pot experiment to test whether biogas residues could be used as substrates for growing *Panax ginseng* and to determine suitable amounts of biogas and maturity levels. The effects of different treatment substrates on the biological properties, soil physicochemical properties and enzymatic properties of ginseng and the changes in the soil microbial communities of ginseng and soil were investigated. The results can contribute to a comprehensive understanding of the influence of biogas residues as substrates on ginseng growth and to developing solutions for enhancing soil quality during ginseng cultivation and high-value utilisation of biogas residues.

MATERIAL AND METHODS

Materials. Biogas residues were obtained from Sanhe Yingsheng Bioenergy Technology Co., Ltd. (39°97'N, 117°01'E). The original materials for biogas fermentation were corn straw after lactic acid fermentation (Zhang et al. 2023), and cow manure with a volatile solid ratio of 4:1. Biogas residues were treated and used as the substrate in this study. Two-year-old plant (*Panax ginseng* C.A. Meyer) seedlings were planted in the greenhouse of Northeast Forestry University (Harbin, China) from May 1st to June 15th, 2022, according to the World Reference Base (WRB), flower soil classified as Chernozem was used as the control (CK). The original physiochemical properties of the flower soil and biogas residues are shown in Table 1.

The amounts of added biogas residue were set up as follows: 0 (CK), 30% (CM30), 70% (CM70), and 100% (CM100) mature biogas residue compost. The maturity stage was established as follows: fresh biogas residue (CF30), hemi-decomposed biogas residue (CH30), and mature biogas residue compost (CM30). The details are shown in Table 2. The cultivation substrate was mixed according to the proportions in Table 2 and loaded into pots with a 19 cm top diameter, 16 cm height and 14 cm bottom diameter, after which the two-year-old ginseng plants were transplanted into the pots. Each pot contained 1.5 kg

Table 1. Original physiochemical properties of the substrate materials

Item	Fresh biogas residue	Hemi-decomposed biogas residue	Mature decomposed biogas residue	Flower soil
Germination index (%)	18.00 ± 0.32^{c}	56.63 ± 0.10^{b}	145.10 ± 0.22^{a}	not detected
C/N ratio	87.63 ± 7.41^{a}	32.18 ± 0.30^{b}	22.66 ± 1.52^{b}	9.99 ± 0.25^{c}
Total N (g/kg)	2.90 ± 0.35^{c}	8.86 ± 0.41^{b}	12.58 ± 0.12^{a}	7.98 ± 0.36^{b}
NH_4^+-N (mg/kg)	0.56 ± 0.15^{a}	0.25 ± 0.07^{c}	0.21 ± 0.13^{d}	0.39 ± 0.09^{b}
NO_3^--N (mg/kg)	13.14 ± 0.62^{b}	18.93 ± 1.44^{a}	20.16 ± 1.97^{a}	7.39 ± 0.47^{c}
Total C (g/kg)	252.78 ± 10.54^{b}	277.53 ± 3.53^{a}	282.91 ± 5.71^{a}	75.65 ± 0.91^{c}
Available P (mg/kg)	0.35 ± 0.21^{a}	0.27 ± 0.14^{b}	0.20 ± 0.22^{d}	0.30 ± 0.03^{c}
Total P (mg/g)	4.87 ± 0.25^{cd}	5.88 ± 0.39^{ab}	6.21 ± 0.52^{a}	4.98 ± 0.06^{bc}
Available K (mg/kg)	676.83 ± 1.42^{a}	93.90 ± 7.75^{d}	219.57 ± 31.85^{b}	170.37 ± 12.96^{c}
Total K (g/kg)	10.98 ± 1.45^{a}	4.06 ± 0.72^{c}	3.56 ± 0.53^{c}	7.37 ± 0.17^{b}
Organic matter (g/kg)	435.78 ± 18.11^{b}	478.45 ± 6.09^{a}	487.74 ± 1.84^{a}	130.41 ± 1.56^{c}
pH	6.68 ± 0.04^{d}	6.82 ± 0.09^{c}	7.40 ± 0.08^{a}	6.98 ± 0.28^{b}
Moisture (%)	81.67 ± 1.14^{a}	71.43 ± 1.00^{b}	67.68 ± 1.15^{c}	28.19 ± 1.01^{d}

https://doi.org/10.17221/430/2023-PSE

Table 2. Treatments in this study

Treatment	Biogas residue (%)	Flower soil (%)
CK	0	100
CM30	30 (mature decomposed)	70
CM70	70 (mature decomposed)	30
CM100	100 (mature decomposed)	0
CF30	30 (fresh)	70
CH30	30 (hemi-decomposed)	70

of substrate, and one ginseng plant was cultivated. A total of 5 ginseng plants were used per treatment. Shade cultivation was performed. The average temperature was 22 °C, and physicochemical and biological analyses of the plants and soil were performed after 45 days of potting. Irrigation was carried out every two days with 20 mL of tap water until harvest. Each treatment was repeated three times.

Sampling and analysis of the biological properties of ginseng. Rhizosphere soils from all the treatment groups were collected and divided into two parts. One part was immediately stored in a refrigerator at –80 °C for soil microbial diversity detection. After passing through a 2 mm sieve, the other air-dried part was used to determine various physical and chemical properties. For plant samples, after measuring biological characteristics, the aerial and underground parts of the ginseng plants were collected separately, surface disinfected and frozen at –80 °C after surface disinfection for subsequent analysis of the endogenous microbiome.

Analysis and data processing of physical and chemical indices. The pH of the soil was determined with a pH meter. The NH_4^+-N content was determined *via* 2 mol/L KCl leaching spectrophotometry. The NO_3^--N content was determined by 1 mol/L NaCl extraction-zinc reduction spectrophotometry. Available P was determined by the double acid extraction method. Total N was determined using the Kjeldahl method. Total P was determined by sodium hydroxide-alkaline fusion molybdenum antimony spectrophotometry. The total K was determined spectrophotometrically by flame atomic absorption. The soil organic matter content was determined using the ignition loss method, and the total saponin content was determined using the perchloric acid-vanillin method (Wang et al. 2022b).

For the germination index (GI), 10 mL of the aqueous extract from the biogas residue (1:5, *w*/*v*) was added to cucumber seeds (10 seeds per dish, replicated three times). After 48 h in the dark at 25 °C, the number of germinated seeds and the length of the root radicals in each Petri dish were determined. GI (%) = 100 (mean number of germinated seeds per dish × mean root length per dish)/(mean number of germinated seeds in the control × average root length of the control) (Kong et al. 2023).

One-way ANOVA and significant difference analysis were performed for all the treatments. The significance level was set at $P < 0.05$.

High-throughput sequencing and data analysis of the microbiome. High-throughput Illumina sequencing characterised the aboveground, root and rhizosphere microbiomes (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China). The V3–V4 region of soil bacterial 16S rRNA was amplified using the primers 338F (5'-ACTCCTACGGGAGGCAGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and the V5–V7 region of plant endophytic bacterial 16S rRNA was amplified using the primers 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3') (Gan et al. 2019). The primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTCCATCGATGC-3') were used to amplify the soil fungi (Ramirez et al. 2018). Specific primers with barcodes were synthesised according to the indicated sequencing region, and the samples were subsequently amplified with a thermal cycler (GeneAmp® 9700, ABI, Foster City, USA).

Bacterial PCRs were performed in triplicate with 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mmol/L dNTPs, 0.8 μL of 5 μmol/L forwards primer, 0.8 μL of 5 μmol/L reverse primer, 0.4 μL of Fastpfu Polymerase, 0.2 μL of bovine serum albumin (BSA), and 10 ng of template DNA in a 20 μL reaction volume. The thermal cycling conditions were described previously (Wang et al. 2022a). The PCR products were identified by 2% agarose gel electrophoresis, purified using an AxyPrep DNA gel extraction kit (Axygen, Corning, USA) and quantified using a QuantiFluor™-ST Blue Fluorescence Quantification System (Promega, Madison, USA). The amplified sublibrary was sequenced on an Illumina PE250 platform (Biozeron, Shanghai, China). After machine sequencing, the data were filtered and spliced, after which database comparison and species annotation were conducted. The raw reads were deposited into the NCBI sequence read archive (SRA) with accession number SRR25297989~SRR25298096.

A co-occurrence network based on Spearman's correlation coefficient was constructed with NetworkX to

https://doi.org/10.17221/430/2023-PSE

study the interactions between soil bacteria and fungi in the different substrate treatments. The families with relative abundances greater than 0.01% in each treatment were screened, and related networks were constructed. The Spearman correlation threshold was 0.7, $P < 0.05$. Each node represents a family, and each edge represents a strong and significant correlation between different nodes. The network was visualised using the Gephi platform (http://gephi.github.io/). On the Majorbio Cloud platform (http://www.majorbio.com), NetworkX was used to calculate the network topology characteristics (average degree and modularity).

RESULTS AND DISCUSSION

Effects of biogas residues on the biological properties of ginseng. After 45 days of cultivation, indices were measured, including fresh weight, length, crown diameter, stem diameter and ginsenoside content. As shown in Figure 1, adding biogas residues significantly affected the ginseng growth properties. Ginseng growth significantly accelerated with the increasing addition of mature biogas residue ($P < 0.05$). Additionally, the maturity of the biogas residue also significantly influenced ginseng growth. A comparison of CM30, CF30 and CH30 revealed that the greatest fresh weight, length, and ginsenoside content occurred in the CM30 treatment group, while the lowest values occurred in the CF30 treatment group ($P < 0.05$). The results showed that adding mature biogas residues could promote plant growth and metabolite accumulation (Meng et al. 2017).

Effects of biogas residue content and maturity on the physicochemical and enzymatic properties of the soil. The analyses of substrate physicochemical properties showed that NO_3^--N, total N, total C,

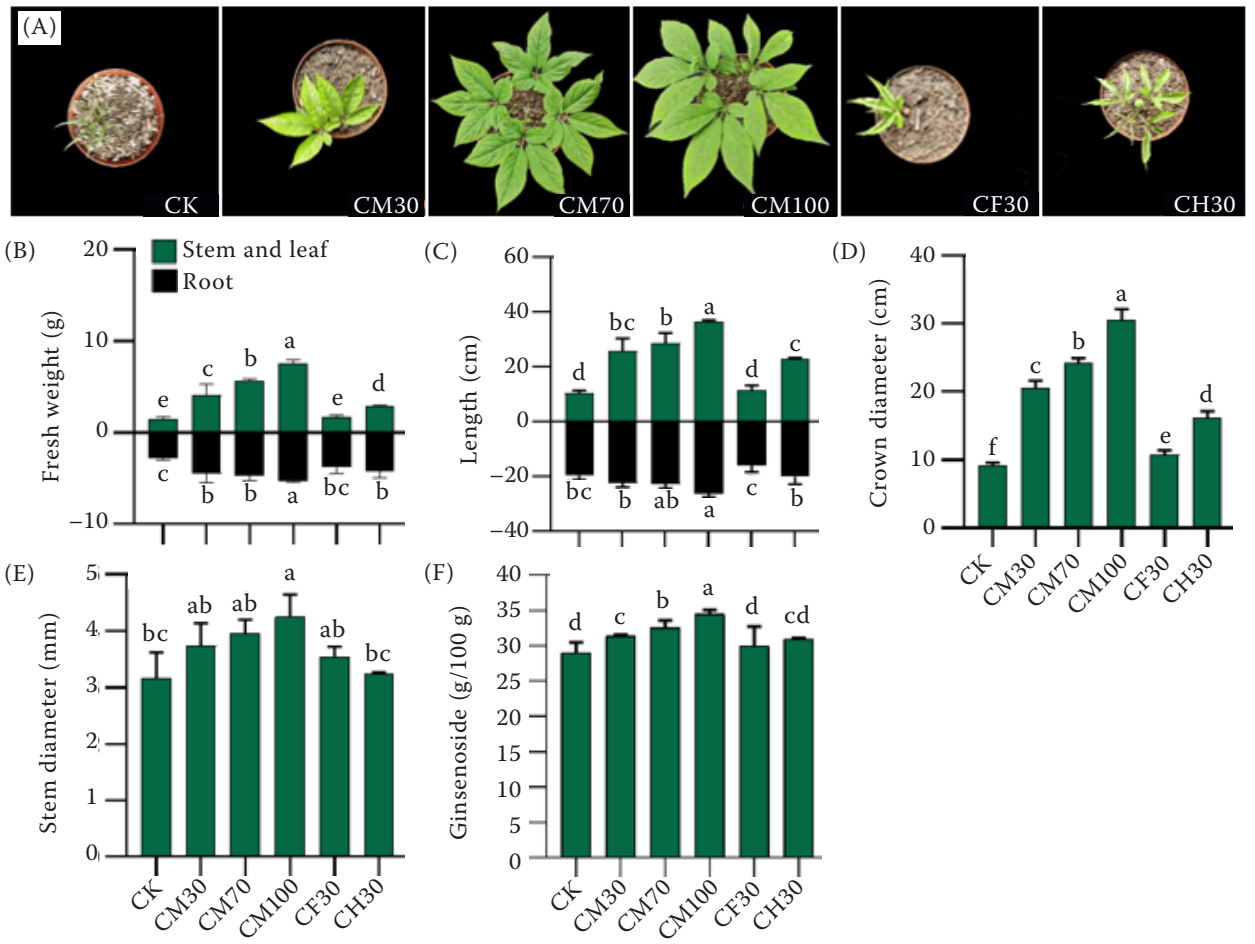

Figure 1. Biological properties of ginseng with the addition of biogas residues. The significance level was set at $P < 0.05$. CK – 100% flower soil; CM30 – 30% mature biogas residue + 70% flower soil; CM70 – 70% mature biogas residue + 30% flower soil; CM100 – 100% mature biogas residue; CF30 – 30% fresh biogas residue + 70% flower soil; CH30 – 30% hemi-decomposed biogas residue + 70% flower soil

https://doi.org/10.17221/430/2023-PSE

total P, and available P significantly increased with the addition of mature biogas residue (Table 3) and were the highest in the CM100 treatment ($P < 0.05$). Among the maturity treatments (CF30, CH30 and CM30), the NH_4^+-N concentration, available P concentration, and moisture content increased significantly, while the carbon-to-nitrogen ratio decreased significantly. These results showed that biogas residue could provide more nutrients for ginseng growth and better water-sustaining ability (Meng et al. 2022). In the present study, the growth of premier ginseng plants in the CM100 treatment group also confirmed this finding.

Analysis of soil enzyme activities showed that catalase, sucrase, urease and acid phosphatase activities significantly ($P < 0.05$) increased with increasing biogas residue content (Table 3). With CM100 treatment, the activities of catalase, sucrase, urease and acid phosphatase increased by 102.04, 4 574.40, 1 404.49 and 364.33%, respectively, compared to those in the CK treatment. A similar trend occurred among the maturity treatments. Compared to the CF30 treatment, the catalase, sucrase, urease and acid phosphatase activities in the CM30 treatment were 75.25, 662.69, 61.41 and 168.29%, respectively. Enzyme activities are closely related to C, N, and P in soil, and the increase in enzymes is related to the rapid cycling of these nutrient elements (Wu et al. 2020), thus promoting ginseng growth.

Microbial community structure in plant and rhizosphere soils. At the phylum level, several phyla, such as Proteobacteria, Firmicutes and Actinobacteriota, are generally found in plants and soil (Kou et al. 2023) and were detected in both ginseng and rhizosphere soil (Figures 2A–C). Bacteroidota

Table 3. Physicochemical properties of the substrates after ginseng cultivation

	CK	CM30	CM70	CM100	CF30	CH30
NH_4^+-N (mg/kg)	0.12 ± 0.01^{d}	0.31 ± 0.10^{a}	0.20 ± 0.13^{b}	0.08 ± 0.03^{e}	0.12 ± 0.26^{d}	0.15 ± 0.23^{c}
NO_3^--N (mg/kg)	58.48 ± 0.42^{e}	159.16 ± 2.17^{c}	180.45 ± 2.29^{b}	237.79 ± 3.19^{a}	69.22 ± 1.97^{d}	70.00 ± 1.92^{d}
Total N (g/kg)	3.92 ± 0.07^{cd}	4.83 ± 0.14^{c}	8.65 ± 0.21^{b}	13.72 ± 0.25^{a}	2.72 ± 0.28^{d}	4.01 ± 0.57^{cd}
Total C (g/kg)	72.58 ± 1.03^{cd}	85.48 ± 4.77^{c}	142.29 ± 1.84^{b}	220.41 ± 11.56^{a}	85.91 ± 4.41^{c}	97.28 ± 1.06^{c}
C/N	18.89 ± 3.29^{bc}	17.69 ± 0.52^{c}	16.47 ± 0.25^{c}	15.98 ± 0.84^{c}	31.84 ± 3.41^{a}	24.62 ± 3.78^{b}
Total P (mg/g)	1.26 ± 0.10^{d}	3.65 ± 0.97^{c}	6.67 ± 0.23^{b}	9.45 ± 0.32^{a}	1.44 ± 0.28^{d}	0.93 ± 0.37^{d}
Available P (mg/kg)	1.69 ± 0.04^{d}	2.80 ± 0.01^{c}	7.32 ± 0.05^{b}	10.13 ± 0.05^{a}	0.22 ± 0.05^{f}	0.30 ± 0.07^{e}
Total K (g/kg)	6.62 ± 0.38^{a}	5.59 ± 0.18^{b}	5.01 ± 0.03^{c}	4.32 ± 0.07^{d}	5.93 ± 0.14^{b}	6.03 ± 0.02^{b}
Available K (mg/kg)	279.30 ± 63.85^{a}	236.50 ± 63.63^{ab}	144.13 ± 12.12^{b}	264.70 ± 30.86^{a}	195.97 ± 22.47^{ab}	175.27 ± 22.32^{ab}
pH	7.02 ± 0.02^{c}	7.06 ± 0.25^{bc}	7.13 ± 0.02^{b}	7.25 ± 0.17^{a}	6.70 ± 0.02^{d}	6.73 ± 0.35^{d}
Moisture (%)	48.34 ± 0.71^{d}	64.42 ± 0.91^{b}	66.43 ± 2.21^{b}	74.68 ± 1.28^{a}	48.34 ± 0.71^{d}	57.33 ± 2.18^{c}
Organic matter (g/kg)	125.13 ± 1.78^{d}	147.36 ± 8.21^{cd}	245.31 ± 3.19^{b}	379.99 ± 1.99^{a}	148.10 ± 7.61^{cd}	167.72 ± 1.83^{c}
Catalase [mg/(g.min)]	6.61 ± 0.58^{e}	10.69 ± 0.31^{c}	11.63 ± 0.12^{b}	13.39 ± 0.09^{a}	6.24 ± 002^{e}	7.18 ± 0.18^{d}
Sucrase (mg/g)	2.11 ± 0.08^{f}	32.24 ± 0.26^{c}	65.31 ± 1.22^{b}	98.63 ± 3.34^{a}	4.30 ± 0.08^{e}	12.17 ± 0.20^{d}
Urease (mg/g)	0.05 ± 0.01^{f}	0.50 ± 0.85^{c}	0.67 ± 1.22^{b}	0.73 ± 2.30^{a}	0.31 ± 0.31^{e}	0.38 ± 1.36^{d}
Acid phosphatase (mg/g)	0.05 ± 0.03^{e}	0.11 ± 0.06^{c}	0.22 ± 0.07^{b}	0.24 ± 0.14^{a}	0.04 ± 0.02^{e}	0.08 ± 0.11^{d}

CK – 100% flower soil; CM30 – 30% mature biogas residue + 70% flower soil; CM70 – 70% mature biogas residue + 30% flower soil; CM100 – 100% mature biogas residue; CF30 – 30% fresh biogas residue + 70% flower soil; CH30 – 30% hemi-decomposed biogas residue + 70% flower soil

https://doi.org/10.17221/430/2023-PSE

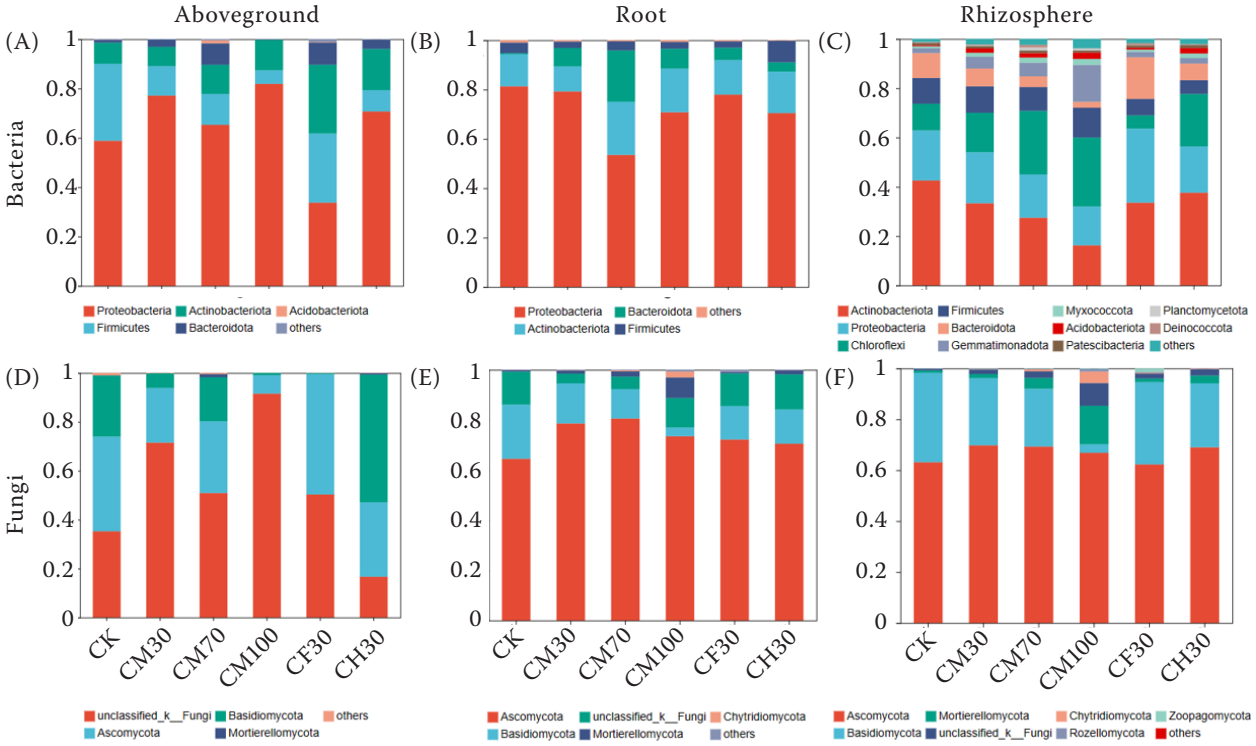

Figure 2. Microbial community structure at the phylum level from different treatments with biogas residue addition. CK – 100% flower soil; CM30 – 30% mature biogas residue + 70% flower soil; CM70 – 70% mature biogas residue + 30% flower soil; CM100 – 100% mature biogas residue; CF30 – 30% fresh biogas residue + 70% flower soil; CH30 – 30% hemi-decomposed biogas residue + 70% flower soil

and Chloroflexi were detected in the ginseng roots (Figure 2B) and rhizosphere soil (Figure 2C). Among the treatments involving biogas residue (CK, CM30, CM70 and CM100), the abundance of Firmicutes decreased, while the abundance of Actinobacteriota increased in ginseng plants (Figures 2A, B). In the rhizosphere soil, the abundances of Actinobacteriota, Proteobacteria and Bacteroidota significantly decreased ($P < 0.05$), while the abundances of Chloroflexi and Gemmatimonadota distinctly increased ($P < 0.05$) (Figure 2C). With the increase in maturity of the biogas residue (CF30, CH30 and CM30), the differences in the microbial community from the ginseng plants mainly occurred aboveground. The abundance of Proteobacteria significantly increased, while the abundances of Actinobateriota, Firmicutes and Bacteroidota decreased significantly ($P < 0.05$) (Figure 2A). In the rhizosphere soil, higher abundances of Chloroflexi, Firmicutes and Gemmatimonadota were detected (Figure 2C). In this study, Chloroflexi and Gemmatimonadota increased with increasing content and maturity of the biogas residue in the substrate, which indicated that these two bacterial phyla played important roles in plant growth promotion (Hao et al. 2023).

Fungi, Ascomycota, Basidiomycota, Mortierellomycota, and Chytridiomycota were detected in all the treatments (Figures 2D–F). These phyla have been detected in perennial medicinal plants such as *Panax ginseng* C.A. Meyer and *Astragalus membranaceus* (Fisch.) Bge (Leng et al. 2023, Wang et al. 2023). Compared with aboveground root and rhizosphere, more unclassified_k_Fungi were detected (Figures 2D–F), which indicated that the fungal community structure aboveground of ginseng needs to be explored in detail (Han et al. 2023). With the increase in biogas residue content (CK, CM30, CM70 and CM100), similar changes occurred between the roots and rhizosphere (Figures 2E, F). The abundances of Mortierellomycota and unclassified_k_Fungi increased. In this study, the changes in fungal communities in plants and soil were not as significant as in bacterial communities. Recently, the function of Mortierellomycota was investigated (Ozimek and Hanaka 2020). As an indicator of soil health, many genera were confirmed to promote plant growth

https://doi.org/10.17221/430/2023-PSE

or significantly inhibit soil-borne pathogenic fungi (Wang et al. 2022b, Liu et al. 2023b).

Correlation analyses of the soil microbiome and physicochemical characteristics and enzyme activities. The results from the distance-based redundancy analysis (db-RDA) showed that bacteria negatively correlated with some physicochemical indices in the CK treatment were positively correlated with those in the CM70 and CM100 treatments. For example, microorganisms, including bacteria (Chloroflexi, Gemmatimonadota, Firmicutes, Planctomycetota, Acidobacteriota, and Patescibacteria) and (Ascomycota, Mortierellomycota, Chytridiomycota, Zoopagomycota, and Rozellomycota), were positively correlated with the activities of sucrase, catalase, and urease in CM100 plants. In contrast, they were negatively correlated with those in CK plants (Figures 3A, C). These microorganisms are mostly involved in organic matter decomposition and the nitrogen cycle, accelerating the material cycle and promoting ginseng growth (Liu et al. 2023b, Peng et al. 2023). Similarly, the microorganisms positively correlated

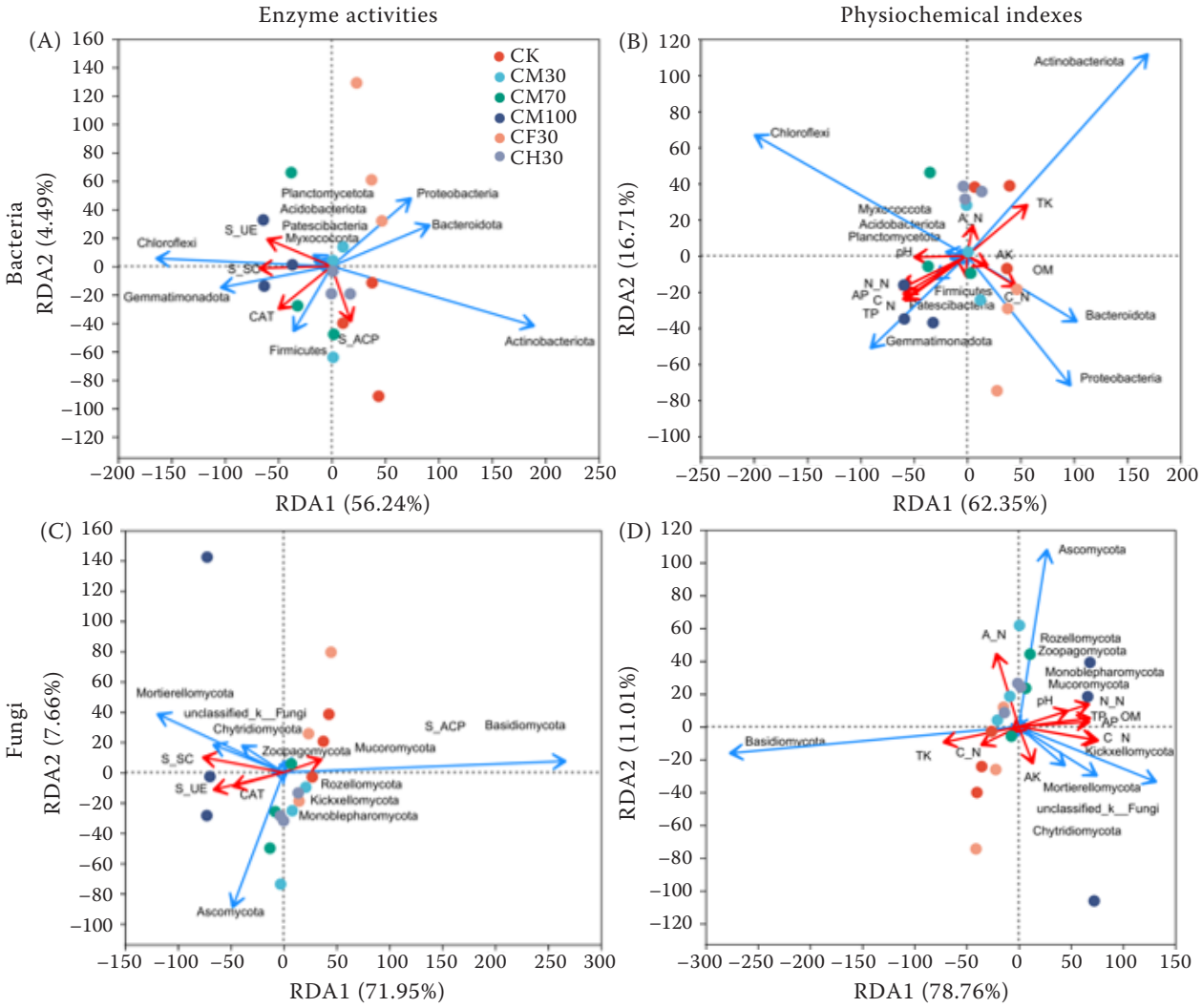

Figure 3. Distance-based redundancy analysis (db-RDA) of microbial communities and physiochemical characteristics in the rhizosphere soil. (A) Bacterial and enzymatic activities; (B) bacterial and physiochemical indices; (C) fungal and enzymatic activities; (D) fungal and physiochemical indices. S-UE – urease; S-SC – sucrase; CAT – catalase; S-ACP – acid phosphatase; A-N – NH_4^+-N; N-N – NO_3^--N; TN – total N; TC – total C; TP – total P; TK – total K; AP – available P; AK – available K; OM – organic matter; CK – 100% flower soil; CM30 – 30% mature biogas residue + 70% flower soil; CM70 – 70% mature biogas residue + 30% flower soil; CM100 – 100% mature biogas residue; CF30 – 30% fresh biogas residue + 70% flower soil; CH30 – 30% hemi-decomposed biogas residue + 70% flower soil

https://doi.org/10.17221/430/2023-PSE

with total C, total N, available P, NO_3^--N and total P in CM70 and CM100 were negatively correlated in CK (Figures 3B, D).

In the different maturity treatments, soil sucrase, catalase and urease activities were negatively correlated with the abundances of Actinobacteriota, Bacteroidota, Proteobacteroidota and Basidiomycota. There was a positive correlation for bacteria, such as Chloroflexi, Gemmatimonadota, Firmicutes, Planctomycetota, Acidobacteriota, and Patescibacteria, and fungi, such as Mortierellomycota, Chytridiomycota, Zoopagomycota, and Rozellomycota. The opposite was true for soil acid phosphatase (Figures 3A, C). In addition, bacteria, including Chloroflexi, Gemmatimonadota, Firmicutes, Planctomycetota, Acidobacteria, Planctomycota, Patescibacteria, Ascomycota, Rozellomycota, Mortierellomycota, and Chytridiomycota, and fungi, including Zoopagomycota, were positively correlated with total C, total N, available P, NO_3^--N, available K, pH and organic matter. The microorganisms negatively correlated with total C, total N, available P, NO_3^--N, available K, pH and organic matter included Actinobacteriota, Bacteroidota, Proteobacteroidota and Basidiomycota.

Analysis of the co-occurrence network of different substrate treatments. Based on the soil microbial co-occurrence network analysis, interrelationships within the same bacteria or fungi were determined in this study. As shown in Figure 4, the core bacteria in the CK, CM30 and CM70 treatments with increasing amounts of biogas residue were Actinobacteriota. In contrast, the core bacteria in the CM100 treatment were Acidobacteriota and Gemmatimonadota. The core fungus in the CK treatment was Basidiomycota, and the core fungus in the CM30, CM70 and CM100 treatments was Mortierellomycota. According to Table 4, the negative correlation ratio of bacteria improved with increasing biogas residue content, which indicated that the bacterial community structure was more stable. However, the negative correlation ratio of fungi decreased, indicating that the fungal structural communities were unstable (Vries et al. 2018).

With increasing maturity, the core bacteria in the CF30 treatment were Actinobacteriota and Bacteroidota, while the core bacteria in the CH30 and CM30 treatments were Actinobacteriota. The core fungi in the CF30 treatment were Basidiomycota, while the core fungi in the CH30 and CM30 treatment groups were Basidiomycota and Mortierellomycota. The data from Table 4 show that the negative correlation ratio of bacteria and fungi first increased and then decreased, which could be related to the more diverse microbial communities involved in the maturation of biogas residues (He et al. 2022, Wang et al. 2022b).

In conclusion, in this study, biogas residue of different concentrations and maturities was used as a substrate for ginseng cultivation, and its effects

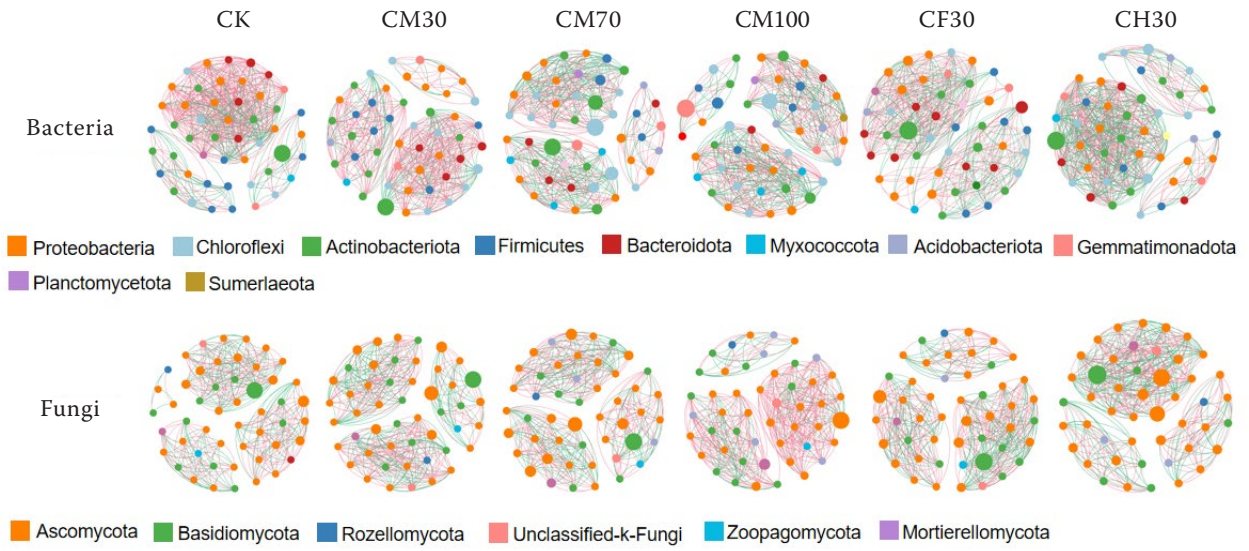

Figure 4. Soil bacterial and fungal co-occurrence network analysis. CK – 100% flower soil; CM30 – 30% mature biogas residue + 70% flower soil; CM70 – 70% mature biogas residue + 30% flower soil; CM100 – 100% mature biogas residue; CF30 – 30% fresh biogas residue + 70% flower soil; CH30 – 30% hemi-decomposed biogas residue + 70% flower soil

https://doi.org/10.17221/430/2023-PSE

Table 4. Coefficients for co-occurrence network analysis of rhizosphere soil fungi

Microorganism	Item	CK	CM30	CM70	CM100	CF30	CH30
Bacteria	nodes	48	49	50	49	50	49
	edges	469	434	426	430	448	497
	positive correlation ratio (%)	69.3	69.3	53.9	53.7	57.1	51.1
	negative correlation ratio (%)	30.7	30.7	46.1	46.3	42.9	48.9
	average degree	19.54	17.71	17.04	17.55	17.92	20.29
	modularity	0.33	0.53	0.59	0.54	0.52	0.36
Fungi	nodes	50	49	48	48	49	47
	edges	342	390	364	399	392	385
	positive correlation ratio (%)	51.7	56.1	55.7	74.2	56.4	50.1
	negative correlation ratio (%)	48.3	43.9	44.3	25.8	43.7	49.9
	average degree	13.68	15.92	15.17	16.63	16	16.39
	modularity	0.65	0.63	0.65	0.57	0.62	0.51

CK – 100% flower soil; CM30 – 30% mature biogas residue + 70% flower soil; CM70 – 70% mature biogas residue + 30% flower soil; CM100 – 100% mature biogas residue; CF30 – 30% fresh biogas residue + 70% flower soil; CH30 – 30% hemi-decomposed biogas residue + 70% flower soil

on ginseng growth and rhizosphere soil were investigated. The results showed that adding biogas residues could promote ginseng growth by directly supplying nutrients and indirectly enriching plant growth-promoting rhizobacteria. Therefore, based on our results, biogas residues can be used as a substrate for ginseng cultivation. By combining physiochemical indices with microbial community analysis, mature biogas residue compost could be the best choice for growing *Panax ginseng*.

REFERENCES

Bernal M., Alburquerque J., Moral R. (2009): Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresource Technology, 100: 5444–5453.

Gan Y., Zhao Q., Ye Z. (2019): Denitrification performance and microbial diversity of immobilized bacterial consortium treating nitrate micro-polluted water. Bioresource Technology, 281: 351–358.

Han S., Li H., Rengel Z., Du Z., Hu N., Wang Y., Zhang A. (2023): Biochar application promotes crops yield through regulating root development and the community structure of root endophytic fungi in wheat-maize rotation. Soil and Tillage Research, 234: 105827–105836.

Hao C., Du P., Ren J., Hu L., Zhang Z. (2023): Halophyte *Elymus dahuricus* colonization regulates microbial community succession by mediating saline-alkaline and biogenic organic matter in bauxite residue. Science of The Total Environment, 905: 167140–167153.

He J., Zhu N., Xu Y., Wang L., Zheng J., Li X. (2022): The microbial mechanisms of enhanced humification by inoculation with *Phanerochaete chrysosporium* and *Trichoderma longibrachiatum* during biogas residues composting. Bioresource Technology, 351: 126973–126983.

Jara Samaniego L., Murcia P., Bustamante M., Perez-Espinosa A., Paredes C., López M., López Lluch D., Gavilanes I., Moral R. (2017): Composting as sustainable strategy for municipal solid waste management in the Chimborazo Region, Ecuador: suitability of the obtained composts for seedling production. Journal of Cleaner Production, 141: 1349–1358.

Jiang G., Feng X., Wu Z., Li S., Bai X., Zhao C., Ameer K. (2021): Development of wheat bread added with insoluble dietary fiber from ginseng residue and effects on physiochemical properties, *in vitro* adsorption capacities and starch digestibility. LWT – Food Science and Technology, 149: 111855–111864.

Jin Q., Zhang Y., Ma Y., Sun H., Guan Y., Liu Z., Ye Q., Zhang Y., Shao C., Mu P., Wang Q. (2022): The composition and function of the soil microbial community and its driving factors before and after cultivation of *Panax ginseng* in farmland of different ages. Ecological Indicators, 145: 109748–109761.

Kong Y., Zhang J., Yang Y., Liu Y., Zhang L., Wang G., Liu G., Dang R., Li G., Yuan J. (2023): Determining the extraction conditions and phytotoxicity threshold for compost maturity evaluation using the seed germination index method. Waste Management, 171: 502–511.

Kou B., He Y., Wang Y., Qu C., Tang J., Wu Y., Tan W., Yuan Y., Yu T. (2023): The relationships between heavy metals and bacterial communities in a coal gangue site. Environmental Pollution, 322: 121136–121148.

https://doi.org/10.17221/430/2023-PSE

Leng F., Cui X., Zhu N., Zhu X., Wang X., Wang Y. (2023): Characterization of root microbial communities associated with *Astragalus membranaceus* and their correlation with soil environmental factors. Rhizosphere, 25: 100656–100668.

Lin X., Wang N., Li F., Yan B., Pan J., Jiang S., Peng H., Chen A., Wu G., Zhang L., Huang H., Luo L. (2022): Evaluation of the synergistic effects of biochar and biogas residue on CO_2 and CH_4 emission, functional genes, and enzyme activity during straw composting. Bioresource Technology, 360: 127608–127616.

Liu L., Li C., Li H. (2023a): Long-term microbial community succession and mechanisms of regulation of dissolved organic matter derivation in livestock manure fermentation system. Chemosphere, 329: 138588–138597.

Liu Z., Li Y., Sun Y., Feng F., Tagawa K. (2023b): Preparation of biochar-based photothermal superhydrophobic coating based on corn straw biogas residue and blade anti-icing performance by wind tunnel test. Renewable Energy, 210: 618–626.

Meng X., Liu B., Xi C., Luo X., Yuan X., Wang X., Zhu W., Wang H., Cui Z. (2017): Effect of pig manure on the chemical composition and microbial diversity during co-composting with spent mushroom substrate and rice husks. Bioresource Technology, 251: 62–69.

Meng X., Liu B., Zhang H., Wu J., Yuan X., Cui Z. (2018): Co-composting of the biogas residues and spent mushroom substrate: physicochemical properties and maturity assessment. Bioresource Technology, 276: 281–287.

Meng X., Wang Q., Lv Z., Cai Y., Zhu M., Li J., Ma X., Cui Z., Ren L. (2022): Novel seedling substrate made by different types of biogas residues: feasibility, carbon emission reduction and economic benefit potential. Industrial Crops and Products, 184: 115028–115040.

Ozimek E., Hanaka A. (2020): Mortierella species as the plant growth-promoting fungi present in the agricultural soils. Agriculture, 11: 7–25.

Peng Y., Chen Q., Guan C.-Y., Xiao Y., Jiang X., Wei M., Tan J., Li X. (2023): Metal oxide modified biochars for fertile soil management: effects on soil phosphorus transformation, enzyme activity, microbe community, and plant growth. Environmental Research, 231: 116258–116269.

Ramirez K., Knight C., Hollander M., Brearley F., Constantinides B., Cotton A., Creer S., Crowther T., Davison J., Delgado-Baquerizo M., Dorrepaal E., Elliott D., Fox G., Griffiths R., Hale C., Hartman K., Houlden A., Jones D., Krab E., Vries F. (2018): Detecting macroecological patterns in bacterial communities across independent studies of global soils. Nature Microbiology, 3: 189–196.

Vries F., Griffiths R., Bailey M., Craig H., Girlanda M., Gweon H., Hallin S., Kaisermann A., Keith A., Kretzschmar M., Lemanceau P., Lumini E., Mason K., Oliver A., Ostle N., Prosser J., Thion C., Thomson B., Bardgett R. (2018): Soil bacterial networks are less stable under drought than fungal networks. Nature Communications, 9: 3033–3045.

Wang J., Wang J., Liu T., Li X., Gao J., Jiang Y., Chen C.-Q. (2023): *Bacillus amyloliquefaciens* FG14 as a potential biocontrol strain against rusty root rot of *Panax ginseng*, and its impact on the rhizosphere microbial community. Biological Control, 182: 163323–163334.

Wang L., Zhang Y., Wang Y., Suo M., Wu H., Zhao M., Yang H. (2022a): Inoculation with *Penicillium citrinum* aids ginseng in resisting *Fusarium oxysporum* by regulating the root and rhizosphere microbial communities. Rhizosphere, 22: 100535–100549.

Wang Y., Wang L., Suo M., Qiu Z., Wu H., Zhao M., Yang H. (2022b): Regulating root fungal community using *Mortierella alpina* for *Fusarium oxysporum* resistance in *Panax ginseng*. Frontiers in Microbiology, 13: 850917–850934.

Wu Y., Chen W., Li Q., Guo Z., Li Y., Zhao Z., Zhai J., Liu G., Xue S. (2020): Ecoenzymatic stoichiometry and nutrient limitation under a natural secondary succession of vegetation on the Loess Plateau, China. Land Degradation and Development, 32: 163323–163334.

Zhang H., Wu J., Zhao X., Yan P., Yang R., Yan J., Yuan X., Cui Z. (2023): Improving aerobic stability and methane production of maize stover silage with lactic acid bacteria inoculants: focus on pentose-fermentation. Industrial Crops and Products, 201: 116861–116874.

Zhou J., Wu H., Shi L., Wang X., Shen Y., Tian S., Hou L.A. (2023): Sustainable on-farm strategy for the disposal of antibiotic fermentation residue: co-benefits for resource recovery and resistance mitigation. Journal of Hazardous Materials, 446: 130705–130715.

Received: October 30, 2023
Accepted: January 22, 2024
Published online: February 9, 2024