SOIL MICROBIAL CARBON SOURCE UTILIZATION UNDER DIFFERENT LAND USE PATTERNS ALONG THE YELLOW RIVER, CHINA

LI, W. S. 1 – Zhuang, J. J. 2 – Xu, P. 2 – Xu, X. B. 2* – Bibi, Z. 3

¹Medical College, Xinxiang University, Xinxiang 453000, China

²School of Biological Engineering, Xinxiang University, Xinxiang 453000, China

³Faculty of Agriculture, Department of Soil Science, the University of Agriculture, Dera Ismail Khan 29050, Khyber Pakhtunkhwa, Pakistan

> *Corresponding author e-mail: 421690071@qq.com

(Received 28th Oct 2023; accepted 19th Jan 2024)

Abstract. In this study biology-eco microplate culture technology was used to study the differences in carbon source utilization of soil microorganisms in land use patterns along the Yellow River in China. At the same time, physical and chemical indices of soil such as soil organic carbon and alkali-hydrolyzable nitrogen contents, three soil enzyme activities and two secondary metabolites contents were measured to explore the relationship between soil microbial carbon source utilization and soil characteristics. The results showed that farmland was the best carbon source utilization land use pattern, followed by poplar forest land, *Ligustrum lucidum* land and grassland. The consumption of carbon sources by the soil microorganisms in the four land use patterns were mainly carbohydrates and amino acids. The farmland showed the highest diversity of microorganisms. Furthermore, redundancy analysis (RDA) analysis showed that the metabolic functions and activities of four land use patterns microbial were mostly affected by the contents of organic carbon, alkali-hydrolyzed nitrogen and sucrase activity. Significant differences were observed among the physicochemical properties and microbial carbon source utilization of the four land use patterns along the Yellow River. Organic carbon, phosphatase and sucrase activities were the main factors affecting microbial diversity. This study provided a theoretical basis for future research on planning land use and helping soil ecological restoration along the Yellow River.

Keywords: Yellow River, land use patterns, soil microbes, carbon source utilization, soil enzyme activity

Introduction

Soil microorganisms, as an important component of soil ecosystems, participate in the formation of soil structure, nutrient cycling, and energy flow through the decomposition of animal and plant residues, which is of great significance for the maintenance of normal soil functions. Therefore, the diversity of soil microbial functions is one of the main criteria for evaluating soil quality (Zhong et al., 2010; Fei et al., 2010). Carbon source metabolic diversity, as an important indicator of functional microbial community changes, provides a reliable basis for understanding microbial metabolic functional diversity (Fan et al., 2014; Gorka et al., 2023). Among them, the Biolog-Eco microplate method can reflect microbial metabolic functional diversity based on the utilization ability of different microbial communities to different single carbon sources (Tian et al., 2011). This method has the advantages of high sensitivity and strong resolution. It can maximize the preservation of the original metabolic characteristics of microbial communities, playing an important role in revealing changes in soil microbial structure and functional diversity (Xi et al., 2003; Denier et al., 2022).

Land use patterns are the main manifestation of human agricultural activities. Changes in land use patterns can affect the circulation and supply of soil nutrients, directly leading to changes in soil texture and underground microbial community structure (Ji et al., 2019; Liu et al., 2022), thereby causing changes in soil microbial diversity. Deng et al. (2018) used Biolog Eco technology to study the impact of different land use methods on the diversity of soil microbial communities in the Liao-dong mountainous area. The results showed that the soil microbial diversity in forest land was higher than that in cultivated land. Zhu et al. (2018) also found that the soil microbial metabolic activity in arable land is the lowest, while the soil microbial metabolic activity in grassland is the strongest, and the microbial biomass carbon is also the highest, followed by forest land. Moreover, the relative utilization efficiency of soil microorganisms for six types of carbon sources in these three land use methods is highest in sugars, amino acids, and carboxylic acids. Qin et al. (2017) pointed out that forest land can fix, retain, and retain more soil nutrients, with high bacterial and fungal diversity.

The Yellow River floodplain is a typical ecotone of water land interfaces, which can affect local climate, water resource balance, and biodiversity (Verones et al., 2013). With population growth and increased agricultural activities, nearly half of the world's floodplains have been transformed (Field et al., 2014). Different land uses in flood plain ecosystems may have many ecological consequences, such as changes in soil microbial community structure and carbon, nitrogen, and phosphorus cycling in the soil (Guo et al., 2012). At present, there are few reports on the structure and diversity of soil microbial communities in the Yellow River floodplain under different land use patterns. Therefore, this study focused on four patterns of soil utilization methods in the Yellow River floodplain, and Biolog Eco was used to study the impact of land utilization methods on the structure and diversity of soil microbial communities. In this experiment, Biolog-Eco microplate culture technology was used to study the differences in carbon source utilization of soil microorganisms in land use patterns along the Yellow River in China. At the same time, physical and chemical indices of soil such as soil organic carbon and alkali-hydrolyzable nitrogen contents, three soil enzyme activities and two secondary metabolites contents were measured. This study aimed to explore the relationship between soil microbial carbon source utilization and soil characteristics. Therefore, the findings of this study will a theoretical basis for future research on planning land use and helping soil ecological restoration along the Yellow River in China.

Materials and methods

Overview of the experimental community

The area along the Yellow River ($114^{\circ}14' - 114^{\circ}46'$ E, $34^{\circ}53' - 35^{\circ}14'$ N) belongs to a warm temperate continental climate with distinct four seasons and an average temperature of 14 °C over the years. The precipitation in August is the highest, with an average annual precipitation of 573.4 mm, a frost-free period of 220 days, and approximately 2400 hours of sunshine throughout the year. The surface is sandy loam soil. The area is based on farmland, with production and living land distributed along the embankment, with a large proportion of farmland and sparse distribution of forest, wetlands, and grasslands (Xie et al., 2023; Guo et al., 2024) (*Fig. 1*).

Li et al.: Soil microbial carbon source utilization under different land use patterns along the Yellow River, China - 1341 -



Figure 1. A GIS map of the Yellow River floodplain showing the distribution of sampled stations in the study area

Collection of soil samples

At the research site, four land use patterns (farmland, poplar forest land, *Ligustrum lucidum* land, grassland) were sampled in November 2021. Five plots were extracted from each land use pattern. The area of each plot is 20 meters \times 20 meters, with a buffer zone of at least 200 meters between any two plots to avoid pseudo duplication (Li et al., 2020). In each plot, according to the cross-sampling method, soil samples at a depth of 0-20 cm were collected using a soil auger, including the rock cores in the four corners and middle of each plot. A total of 25 soil samples were obtained from each land use pattern and sub samples from each plot were collected. Each plot is treated as a duplicate sample, and the five duplicate plot samples are separated. In total, each of the four patterns of land use has five replicates. Each sample is divided into two sub samples, placed in a self-sealing plastic bag in the refrigerator, and then transported to the laboratory for chemical and microbiological analysis. After removing all visible roots and stones, one sub sample was air dried and passed through a 0.25 mm sieve to measure the chemical composition of the soil, while the other sub sample group was stored at -20 °C for Phospholipid Fatty Acid Analysis (PLFA) measurement (Qu et al., 2016; Xue et al., 2021).

Determination of physical and chemical properties of soil

The pH values of soil were measured by pH meter (Leici PHS-3G, China), and the water to soil ratio were kept as 5:1. Soil moisture contents were determined by drying method at 65 °C. The alkali-hydrolyzed nitrogen contents of soil were determined by alkali-hydrolyzed diffusion method. The organic carbon contents of soil were measured using elementar total organic carbon analyzer (Elementar Vario TOC, Germany) (Cotrufo et al., 2022).

Determination of flavonoids and total phenols in soil

The total soil flavonoids and total phenol contents were determined by NaNO₂-Al(NO₃)₃ colorimetric method and Folinol colorimetric method, respectively. colorimetric method with rutin as the reference. The linear regression equation of absorbance and concentration of standard samples were established. The preparation and reaction of samples were carried out according to the reference method, and the detection was performed on Spectrophotometer (Persee T700, China) with 510 nm wavelength. The total flavonoids contents in soil samples were calculated by standard curve. The Folin-phenol colorimetric method was used to determine the total phenolic content in soil with gallic acid used as a control. The standard curve was established according to the reference method, and the preparation and determination reaction of phenolic extract in soil were carried out. The detection wavelength was set at 760 nm, and the total phenolic content in the extract was calculated according to the working curve (Li et al., 2015).

Determination of soil enzyme activities

Urease activity was determined by indophenol blue colorimetry. 5 g soil sample volume was selected, and the sample processing and reaction were carried out according to the method of reference (Guo et al., 2012; Kim et al., 2022), and the detection wavelength was 578 nm. The experiment control group was set at the same time. The standard curve was established before sample detection, and the concentration of NH₃-N was calculated using the standard curve, and the urine enzyme activity was expressed as the number of milligrams of NH₃-N per gram of soil in 24 hours (unit is mg g⁻¹·24 h⁻¹). The sucrase activity was processed and reacted by 3, 5-dinitrosalicylic acid colorimetric method according to the reference method. The detection wavelength was 508 nm and was expressed as mg g⁻¹ 24 h⁻¹ of glucose produced by 1.0 g of soil after 24 h. The phosphatase activity of soil was determined by the colorimetric method of benzene disodium phosphate, and the sample was processed and reacted according to the reference method. The detection method is phosphate, and the sample was processed and reacted according to the reference method. The detection wavelength was 508 nm and 1.0 g of soil after 24 h was expressed as mg g⁻¹·24 h⁻¹.

Culture of Biolog-ECO MicroPlate

The absorbance value of Biolog EcoPlate was measured by microplate reader (PerkinElmer EnSpire, United States) according to the method of reference (Yao et al., 2012; Kim et al., 2022). Soil samples were screened through a 1-mm sieve prior to the Biolog test. The water content of sample was measured, which was used to add the amount of fresh soil sample equivalent to 10 g of dried mass. Briefly, the soil samples were first mixed with 0.85% NaCl sterile water and were placed in a triangular flask in shaking incubator at a speed of 200 r/min for 30 min. Post 2 min of standing at 0°C, the supernatant (5 mL) was collected, mixed with 45 mL of sterile water, and poured in a

triangular flask. Soil extract (1:1000) was prepared by 3 times increase in its dilution, and enzyme-linked immunosorbnent assay (ELISA) was carried out immediately. For continuous culture at 28°C for 10 days, 150 μ L of soil extract was preheated to a biologeco plate well at 25°C. After every 12 h, the plates were read on a microplate reader and the absorbability values were measured at 590 nm and 750 nm to obtain C₅₉₀ and C₇₅₀.

Each experiment was performed in triplicates with three replicas for each sample.

Statistics

Microsoft Excel.2010 and SPSS19.0 software were used for data and variance analysis, respectively. Analysis of variance (ANOVA) was carried out to determine the differences between the measured parameters for different treatments. The least significant difference (Duncan) at p = 0.05 was used to elucidate any significant differences. RDA was performed on software R-4.1.2.

Average well color development (AWCD) was used to check the soil microbial metabolic intensity of each well using formula as follow:

$$AWCD = \sum (C_{590} + C_{750}) /_{31}$$
(Eq.1)

C590 is the absorbance value of carbon source well in the reaction plate at 590 nm minus the control well, and C570 is the absorbance value of carbon source well in the reaction plate at 570 nm minus the control well.

To evaluate the diversity of soil microbial community, Shannon-wiener index (H), Pielou evenness index (EH) and Gini diversity index (D) were used, and the calculation formulas were followings (Yao et al., 2012; Kim et al., 2022):

$$H = -\sum (Pi \times \ln Pi)$$
 (Eq.2)

$$EH = {}^{H}/_{H_{max}} = {}^{H}/_{\ln S}$$
(Eq.3)

$$D = 1 - \sum Pi^2$$
 (Eq.4)

$$Pi = \frac{(C_{590} - C_{750})}{\sum (C_{590-} - C_{750})}$$
(Eq.5)

where, S is the number of color changing holes in the ECO plate.

Results

Comparison of soil physicochemical properties

Significant differences in the physiochemical properties of selected four land use patterns were showed in *Table 1*. The soil moisture content, organic carbon content, and alkaline nitrogen content in farmland soil were the highest, with soil moisture content and organic carbon content significantly higher than the other three land use patterns. The alkaline nitrogen content in farmland soil was not significantly different from that in poplar forest soil, but significantly higher than that in grassland and *Ligustrum lucidum*

forest soil. The pH values of the four land use patterns are all between 7.82 and 8.33, but there was no significant difference between them.

Land use patterns	pH of soil	Moisture content of soil (%)	Organic carbon (mg·L ⁻¹)	Alkaline hydrolysis nitrogen (mg·g ⁻¹)
<i>Ligustrum lucidum</i> land	7.82 ± 0.11 a	$15.26\pm1.34~b$	12.61±0.44 c	1.46±0.04 b
Grassland	$8.33 \pm 0.21 \ a$	$11.48\pm1.89~\text{c}$	6.64±0.26 e	0.53±0.03 c
Poplar forest land	7.98 ± 0.16 a	$16.00\pm1.27~b$	15.06±0.18 b	1.73±0.03 a
Farmland	$8.12\pm0.17~\mathrm{a}$	$24.62\pm1.49~a$	17.88 ± 0.33 a	1.82±0.11 a

Table 1. Comparison of soil physicochemical properties

Note: Data are "mean \pm standard error". Different lowercase letters in the same column indicate significant differences between different vegetation types at the P<0.05 level

Comparison of soil total phenol and flavonoid contents

As shown in *Table 2*, the highest total phenol was found in poplar forest $(0.057 \text{ mg} \cdot \text{g}^{-1})$, but no significant difference was observed between poplar forest, *Ligustrum lucidum* land and farmland. The content of flavonoids in the poplar forest was the highest (3.14 mg \cdot \text{g}^{-1}), which was not significantly different from farmland, but had significant differences from the fields of *Ligustrum lucidum* and grassland.

Land use patterns	The total phenol (mg·g ⁻¹)	Flavonoids (mg·g ⁻¹)
Ligustrum lucidum land	$0.051\pm0.002~ab$	$2.40\pm0.03~b$
Grassland	$0.047 \pm 0.003 \text{ b}$	$2.06\pm0.08~\text{b}$
Poplar forest land	0.057 ± 0.004 a	$3.14 \pm 0.10 \text{ a}$
Farmland	0.056 ± 0.009 a	$3.04 \pm 0.10 \text{ a}$

 Table 2. Comparison of total phenolic and flavonoid contents in soil

Note: Data are "mean \pm standard error". Different lowercase letters in the same column indicate significant differences between different land use patterns at the P<0.05 level

Comparison of enzyme activities

The phosphatase activity of farmland soil was 203.05 mg·g⁻¹·24h⁻¹, which was significantly higher than that of the other soil and 3.12 times that of grassland (*Table 3*). The sucrase activity of Farmland was the highest (17.88 mg·g⁻¹·24h⁻¹) but was not significantly different from that of the poplar forest land. The Sucrase activity of grassland soil was the lowest (6.64 mg·g⁻¹·24 h⁻¹). Compared with the other soil types, urease activity (0.58 mg·g⁻¹·24 h⁻¹) was the highest in the Farmland soil.

Comparison of average absorbance values of soil microorganisms

The AWCD values of soil microorganisms in the four land use patterns were increased with the increase of culture time (*Fig. 2*). Post 24 h of early culture, the AWCD values of soil microorganisms did not changed significantly. The metabolic activity and the rate of carbon consumption of soil microorganisms were increased rapidly from 96 h to 180 h. After that, consumption of carbon source was slow down and the metabolic function

became weakened. The AWCD values of soil microorganisms in the farmland and poplar forest land were increased rapidly, and the range was higher than that of the *Ligustrum lucidum* and grassland soil. Grassland soil showed the slowest increase, but the trend of increase was consistent. The AWCD value of soil microorganisms in the farmland was the highest, showing that soil microorganisms of farmland had better utilization of carbon source.

Land use patterns	Phosphatase (mg·g ⁻¹ ·24 h ⁻¹)	Sucrase (mg·g ⁻¹ ·24 h ⁻¹)	Urease (mg·g ⁻¹ ·24 h ⁻¹)
Ligustrum lucidum land	$177.14 \pm 6.71 \text{ b}$	$12.61\pm0.44\ b$	$0.30\pm0.005~\text{c}$
Grassland	$65.44 \pm 5.16 \text{ c}$	$6.64\pm0.26~c$	$0.12\pm0.007\;d$
Poplar forest land	$181.19 \pm 8.60 \; b$	$15.06 \pm 0.18 \text{ ab}$	$0.45\pm0.01\ ab$
Farmland	203.05 ± 8.04 a	17.88 ± 0.33 a	$0.58\pm0.02~a$

Table 3. Comparison of soil properties of different land use patterns

Note: Data are "mean \pm standard error". Different lowercase letters in the same column indicate significant differences between different land use patterns at the P < 0.05 level



Figure 2. Variation curve of AWCD of soil bacteria in different land use patterns

Fingerprint analysis of physiological carbon metabolism of soil microorganisms

In terms of the types and quantity of carbon sources used, performance of farmland soil was better than the other three patterns, followed by poplar forest land (*Fig. 3*). The results showed that soil microorganisms of farmland used all 29 kinds of carbon sources, except α -cyclodextrin and L-threonine, while Poplar forest land used eight carbon sources, such as L-asparagine, L-serine and Ttwain, but almost not used the three carbon sources, such as D-glucosaminic acid, L-threonine and α -buketoic acid. Soil microorganisms in grassland utilized 4 carbon sources, including Ttwain and Lasparagine, but hardly utilized 7 carbon sources, such as D-glucosamine, D-galactolactate and 2-hydroxybenzoic acid. The soil microorganisms of ligustrum lucidum land had better utilized 7 carbon sources, such as D-fibrinose, D-galacturonic acid and Lasparagine, but hardly utilized 6 carbon sources, such as β -methyl-D-glucoside, I-alginol and α -butanoic acid.



Figure 3. Soil bacterial physiological carbon metabolism fingerprint. Note: G1. D-cellobiose;
H1. a-D-lactose; A2. β-Methyl-D-glucoside; B2. D-Xylose; C2. I-Algitol; D2. D - Mannitol; E2. N-acetyl-D-glucamine; F2. D-glucosamine acid; G2. α-D-glucose-1-phosphate; H2. D, L-α-Glycerol phosphate; A3. D-Galactonic acid lactone; B3. D-Galacturonic acid; A4-F4 are amino acids, including A4. L-arginine; B4. L-asparagine; C4. L-phenylalanine; D4. L-serine; E4. L-threonine; F4. Glycyl-L-glutamic acid; B1-H3 is carboxylic acid, including B1. methyl pyruvate; E3. γ - Hydroxybutyric acid; F3. itaconic acid; G3. α - Butanone acid; H3. D-Malic acid; C1-F1 is a polymer, including C1. Tween; D1. Tween 80; E1. α- Cyclodextrin; F1. Liver sugar; C3-D3 are phenolic acids, including G4. Phenylethylamine H4. Putrescine

Utilization of different carbon sources

The carbon sources of Biolog ECO microplate include sugars, amino acids, carboxylic acids, polymers, phenolic acids, and amines. The utilization of carbon sources by soil microorganisms is stable after 180 h. Based on the 180 h AWCD value, the utilization of these six carbon sources by soil microorganisms was evaluated. Among the six carbon sources, carbohydrates and amino acids are the most easily utilized sources by soil microorganisms, followed by polymeric acids and carboxylic acids, phenolic acids, and amines. There was no significant difference in the utilization of six carbon sources between farmland soil and poplar forest soil (*Fig. 4*). The utilization efficiency of grassland for six carbon sources is significantly lower than the other three land use methods. The utilization efficiency of carbohydrates, amino acids, polycarboxylic acids, and phenolic acids in farmland is higher than that of other land use methods, but there is no significant difference compared to poplar forest land. The poplar forest land has the highest utilization of amines, but there is no significant difference compared to farmland.



Figure 4. Comparison of the utilization of six types of carbon sources by soil bacteria in different land use patterns. Note: Different lowercase letters indicate differences in the use of six identical carbon sources by different land use methods (P < 0.05)

Diversity indices of soil microbial carbon source utilization

Based on AWCD value of 180 h, the diversity indices of soil microbial carbon source utilization were calculated. The results showed that the diversity indices of soil microbial community to carbon source utilization was different among different vegetation types (*Fig. 5*). Shannon-wiener index, Pielou evenness index and Gini diversity index of the farmland were the highest, which were 3.15, 0.91 and 2.77, respectively. However, no significant difference was found between the soil of ligustrum lucidum land and poplar forest land, and the three soil were significantly higher than that of the grassland. The diversity and uniformity of microbial carbon source utilization were better in the farmland, ligustrum lucidum land and poplar forest land, with higher microbial diversity. The diversity and evenness of grassland soil were the lowest.

Utilization of soil microbial carbon source and redundancy analysis of soil physicochemical indices

By RDA analysis of AWCD values at 180 h, the correlation between the degree of carbon source utilization by soil microbial community and soil physicochemical indices were evaluated. As shown in *Fig.* 6, different samples showed certain regularity, and the difference of carbon source utilization was mainly dependent on different plant communities. All soil test parameters explained that 82.69 % of the total variables of soil microbial community were changed (Monte Carlo displacement test, P = 0.01). Among them, first axis was regarded as the constraint axis, which explained various test index variables around 27.16%. In terms of arrow direction and length, soil organic carbon, alkali-hydrolyzed nitrogen, and soil sucrase activity were the most important factors affecting soil microbial carbon utilization.



Figure 5. Comparison of soil carbon source utilization diversity indices for different land use patterns



Figure 6. Redundancy analysis between the absorbance values of in 31 wells and soil physicochemical properties. Note: TP: total phenols, TF: total flavonoids, UR: Urease, PE: Phosphatase, Sucrase, OC: organic carbon, AN: Soil alkaline hydrolyzable nitrogen. Y-1: Ligustrum lucidum land, Y-2: Grassland, Y-3: Poplar forest land, Y-4: Farmland

Discussion

Properties of the four land use patterns along the Yellow River in Xinxiang were significantly different. Compared with the other three patterns, the soil water content, organic carbon, alkali-hydrolyzable nitrogen and phosphatase activities were significantly higher in farmland soil. The organic carbon, alkali-hydrolyzable nitrogen, total phenol, and flavonoids contents as well as the activities of phosphatase and urease in grassland soil were the lowest. The correlation analysis showed that soil properties and their enzyme activities were significantly correlated with the soil organic carbon and alkali-hydrolyzable nitrogen contents. The results of this study are consistent with that of Xu et al. (2009) in which the effects of wheat straw returning to field were evaluated on soil physicochemical properties and enzyme activities.

Hu et al. (2019) showed that characteristics including soil structure, physicochemical properties have significant influence on microbial biomass and different soil enzyme activities. RDA analysis showed that soil organic carbon content, alkali-hydrolyzed nitrogen content and sucrase content were the most important factors which affected soil microbial carbon source utilization in plant community. Qu et al. (2016) also obtained similar results in their study on the differences of metabolic fingerprints of soil microbial carbon sources in Songnen grassland with different grazing intensities. In short, soil properties are closely related to the utilization of carbon sources by soil microorganisms.

Biolog-eco micro-plate culture method is used to obtain a large amount of valuable data and information and is thus an important method to study the functional diversity of soil microbial community. It has various advantages including simplicity, high efficiency (Du et al., 2016), and can intuitively observe the trend of soil microbial utilization of carbon sources under different vegetation. The results showed that with the passage of time, the average color values of soil microorganisms in the four land use patterns covered by the Farmland were increased, which were consistent with the metabolic status of soil microorganisms. The AWCD value of soil microorganisms were increased slowly in 0-24 h, which might be due to the adaptation process of soil microorganisms to the microplate environment. Post 24 h, an exponential period of rapid growth was observed. At 180 h, the growth rate reached at its peak which then became stationary called the stationary period of soil microbial growth. These results are consistent with that of Dong et al. (2011) and Qu et al. (2016). The basic reason is that microbes have their phases of growth. At log phase, they start to grow, and the division rate of the population is close to zero. After adaptation, metabolic activities gradually become vigorous and the RNA contents in cells starts increasing. The population then enters the logarithmic phase (log or exponential), and the growth rate of microorganisms become rapidly increased with a steady geometric number. After that, a stationary phase is observed in which the growth rate becomes equal to the inflection point. In this experiment, the AWCD values of soil microorganisms in the four land use patterns were all in line with the growth rule over time.

Based on the diversity and evenness of soil microbial community, the composition and stability of soil microbial community can be further reflected (Chakraborty et al., 2019; Yu et al., 2020). The diversity indices of soil microbial community in the farmland were the highest, indicating that a number of (kinds and quantities) of microorganisms were found with high metabolic activity in this land use pattern. Zhang et al. (2002) studied the relationship between the number of soil microorganisms and soil fertility under land use, and found that the number of soil microorganisms were positively correlated with a number of tested nutrient indices. It could be considered that the number of

microorganisms depends on nutrient contents. Among the four kinds of soils, the soil organic carbon content, alkali-hydrolyzable nitrogen and other physicochemical properties of the farmland were the highest. The diversity index of farmland was also higher than that of the other four kinds of soil. The reason is that the farmland has external input of carbon and nitrogen sources, and its soil water contents were significantly higher than other land use patterns, which has a positive impact on the process of nitrogen and carbon transformation and is beneficial to soil microbial activity and community structure.

The analysis of difference in soil microbial community's ability to use different carbon sources plays an important role in discovering the function of microbial community (Cheng et al., 2021). In this study, it was found that soil microorganisms of ligustrum lucidum land, poplar forest land and the farmland used the most carbon sources, especially carbohydrates, amino acids, and polymers, but the utilization rate of phenolic acids was the lowest. The number and types of carbon sources used by grassland were less. These results indicated that the types and numbers of microorganisms that metabolize different carbon sources are more in the soil of farmland. It is speculated that the types and quantities of soil carbon sources depends on the difference of land use patterns, the number and distribution of microorganisms metabolizing different carbon sources in soil. The leading factors to such differences include ecological factors such as plant species composition, soil physical and chemical properties, and may also be related to plant residues and root exudates (Tang et al., 2021). Studies (Hawes et al., 2012; Huang et al., 2013) have shown that composition of root exudates can be used as carbon sources by microorganisms and as mediators of interactions between plants and microorganisms. Therefore, it can be speculated that soil microorganisms of different plant communities have different degrees of carbon source utilization.

Conclusions

This study indicated that farmland was the best carbon source utilization land use pattern, followed by poplar forest land, Ligustrum lucidum land and grassland. The farmland showed the highest diversity of microorganisms. Furthermore, RDA analysis showed that the metabolic functions and activities of four land use patterns microbial were mostly affected by the contents of organic carbon, alkali-hydrolyzed nitrogen and sucrase activity. Significant differences were observed among the physicochemical properties and microbial carbon source utilization of the four land use patterns along the Yellow River. Organic carbon, phosphatase and sucrase activities were the main factors affecting microbial diversity. This study provided a theoretical basis for future research on planning land use and helping soil ecological restoration along the Yellow River.

Acknowledgements. This study was supported by Henan International Science and Technology Cooperation Project (Grant No.232102520014), Training program for young backbone teachers in colleges and universities of Henan Province (Grant No. 2021GGJS162) and the Key Scientific and Technological Research Projects of Henan Province (Grant No.222102110403).

Author contributions. Li Wensheng: Writing - Original draft, Methodology, Investigation, Visualization, Funding acquisition. Zhuang Jingjing: Formal analysis, Investigation, Writing - review & editing. Xu Ping: Formal analysis, Investigation, Writing - review & editing. Xu Xiaobo: Experimental design, Methodology, Writing- review & editing. Zarina Bibi: Writing - review & editing, Funding acquisition.

Competing interest. The authors declare no competing financial interests.

Ethical approval. All methods were carried out in accordance with relevant guidelines and regulations of the institutional review board at Xinxiang University.

REFERENCES

- [1] Chakraborty, P., Tribedi, P. (2019): Functional diversity performs a key role in the isolation of nitrogen-fixing and phosphate-solubilizing bacteria from soil. Folia Microbiologica 64(3): 461-470.
- [2] Cheng, K. K., Tang, H. M., Li, C., Tang, W. G., Xiao, X. P., Yi, Z. X. (2021): Impact of long-term tillage management on utilization of microbial carbon sources in rhizosphere and non-rhizosphere soils under a double-cropping rice paddy field. – Environmental science and pollution research international 29(10): 15205-15214.
- [3] Cotrufo, M. F., Haddix, M. L., Kroeger, M. E., Stewart, C. E. (2022): The role of plant input physical-chemical properties, and microbial and soil chemical diversity on the formation of particulate and mineral-associated organic matter. Soil Biology & Biochemistry 168: 108648-108648.
- [4] Deng, J. J., Zhu, W. X., Zhou, Y. B., Yin, Y., Bai, X. J, Zhang, H. Z., Zhang, Y. M., Qin, S. J. (2018): Effects of different land use patterns on the soil microbial community diversity in montane region of eastern Liaoning Province, China. – Chinese Journal of Applied Ecology 29(7): 2269-2276.
- [5] Denier, J., Faucon, M. P., Dulaurent, A. M., Guidet, J., Kervroëdan, L., Lamerre, J., Houben, D. (2022): Earthworm communities and microbial metabolic activity and diversity under conventional, feed and biogas cropping systems as affected by tillage practices. – Applied Soil Ecology 169: 104232.
- [6] Dong, L. G., Jiang, Q., Cai, J. J., Zhang, Y. R., Xu, H., Li, S. B. (2011): Analysis of soil microbial functional diversity based on Biolog-ECO technology in alfalfa field with different years of returning to cultivation. – Arid Zone Research 28(4): 630- 637.
- [7] Du, Z. K., Zhang, J., Cheng, C., Shi, B. H., Zhu, L. S., Wang, J., Wang, J. H., Bing, L. (2016): Effects of 3,6-Dibromocarbazole on Soil Health-Based on Soil Enzymes and the Biolog-ECO Test. – Water, Air, & Soil Pollution 233: 1-11.
- [8] Fan, R. Y., Yang, X. Y., Wang, E. H., Zou, L., Chen, X. W. (2014): Vertical distribution characteristics of soil microbial communities in undisturbed black soil. – Chinese Journal of Soil Science 45(2): 389-393.
- [9] Fei, Y. C., Liu, L., Chen, G., Zhang, Y. M., Chen, S. P., Lan, S. R., Wang, D., Cao, G. Q. (2020): Effects of different organic fertilizer treatments on carbon source utilization of soil microbial communities of Camellia oleifera plantation in purple soil area. – Soil and Fertilizer Sciences in China 5: 101-108.
- [10] Field, C. B., Barros, V. R., Dokken, D. J., Mastrandrea, M. D., Chatterjee, M., Estrada, Y. O., Girma, B., Levy, A. N., Mastrandrea, P. R. (2014): Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press(ed.), Cambridge, UK.
- [11] Gorka, S., Darcy, S., Horak, J., Imai, B., Mohrlak, M., Salas, E., Richter, A., Schmidt, H., Wanek, W., Kaiser, C., Canarini, A. (2023): Beyond PLFA: Concurrent extraction of neutral and glycolipid fatty acids provides new insights into soil microbial communities. – Soil Biology and Biochemistry 187: 109205.
- [12] Guo, X., Zhou, Y. (2012): Effects of Land Use Patterns on the Bacterial Community Structure and Diversity of Wetland Soils in the Sanjiang Plain. Journal of Soil Science and Plant Nutrition 21(2): 2-12.
- [13] Guo, W., Zhou, Y. P., Chen, M. Q., Li, D. D., Wang, Q. X., Zhou, T. T., Zhao, B. Z. (2024):

Effects of Combined Application of Straw and Organic-inorganic Fertilizers on Key Microorganisms and Wheat Yield in Fluvo-aquic Soil. – Acta Pedologica Sinica.

- [14] Hawes, M. C., Curlango-Rivera, G., Xiong, Z. G., Kessler, J. O. (2012): Roles of root border cells in plant defense and regulation of rhizosphere microbial populations by extracellular DNA trapping. – Plant and Soil 355(1-2): 1-16.
- [15] Hu, W., Sui, N., Yu, C. R., Yang, C. Q., Liu, R. X., Zhou, Z. G. (2019): Comparative effects of crop residue incorporation and inorganic potassium fertilization on soil C and N characteristics and microbial activities in cotton field. – Journal of Cotton Research 2(4): 11-22.
- [16] Huang, L. F., Song, L. X., Xia, X. J., Mao, W. H., Shi, K., Zhou, Y. H., Yu, J. Q. (2013): Plant-Soil Feedback and Soil Sickness: From Mechanisms to Application in Agriculture. – Journal of Chemical Ecology 39: 232-242.
- [17] Ji, L., Yang, Y. C., Wang, J., Yang, L. (2019): Relationship between soil phenolic acids and the soil microbial community under different land uses. – Acta Ecologica Sinica 39(18): 6710-6720.
- [18] Kim, S. W., Xu, Y. Q., Meidl, P., Bi, M., Zhu, Y. J., Rillig, M. C. (2022): Soil Storage Conditions Alter the Effects of Tire Wear Particles on Microbial Activities in Laboratory Tests. – Environmental science & technology letters 9(12): 1037-1043.
- [19] Li, Q., Yuan, L., Luo, S. Q., Huang, J. G. (2015): Artemisinin and flavonoids in wild Artemisia annuaand surrounding soil and the influence on soil microbes. Acta Prataculturae Sinica 24(11): 29-37.
- [20] Liu, G. F., Bai, Z. J., Cui, G. W., He, W. H., Kongling, Z. L., Ji, G. X., Gong, H., Li, D. D. (2022): Effects of Land Use on the Soil Microbial Community in the Songnen Grassland of Northeast China. – Frontiers in Microbiology 13: 865184.
- [21] Qin, H., Li, C. X., Ren, Q. S. (2017): Effects of different land use patterns on soil bacterial and fungal biodiversity in the hydro-fluctuation zone of the Three Gorges Reservoir region. – Acta Ecologica Sinica 37(10): 3494-3504.
- [22] Qu, T. B., Zhu, Y., Yu, M., Yang, Z. M., Wang, D. L. (2016): Differences in carbon source metabolic fingerprints of soil bacteria in Songnen grassland under different grazing intensities. – Chinese Journal of Grassland 38(12): 68-74.
- [23] Tang, H. M., Xiao, X. P., Li, C., Shi, L. H., Cheng, K. K., Li, W. Y., Li, W., Xu, Y. L., Wang, K. (2021): Microbial carbon source utilization in rice rhizosphere soil with different tillage practice in a double cropping rice field. – Scientific Reports 11(1): 1-9.
- [24] Tian, Y. N., Wang, H. Q. (2011): Application of biolog to study of environmental microbial function diversity. Environmental Science & Technology 34(3): 50-57.
- [25] Verones, F., Pfister, S., Hellweg, S. (2013): Quantifying area changes of internationally important wetlands due to water consumption in LCA. – Environmental Science & Technology 47(17): 9799-9807.
- [26] Xi, J. Y., Hu, H. Y., Qian, Y. (2003): Application of biolog system in the study of microbial community. Acta Microbiologica Sinica 43(1): 138-141.
- [27] Xie, Y., Ma, D. H., Wang, Q. Y., Zhao, B. Z., Zhu, A. N., Zhang, C. Z., Zhang, J. B., Li, X. P. (2023): Spatial and Temporal Variation of Soil Quality in Typical Farmland in Fengqiu County in Recent 30 Years. – Journal of Ecology and Rural Environment 39(3): 1-14.
- [28] Xu, G. W., Duan, H., Wang, Z. Q., Liu, L. J., Yang, J. C. (2009): Effect of Wheat-Residue Application on Physical and Chemical Characters and Enzymatic Activities in Soil. Agricultura Sinica 42(3): 934-942.
- [29] Xue, Z. J., Zhou, Z. C., An, S. S. (2021): Changes in the soil microbial communities of different soil aggregations after vegetation restoration in a semiarid grassland, China. – Soil Ecology Letters 3(1): 6-21.
- [30] Yao, Z. W., Zhang, X. D., Wang, X., Shu, Q., Liu, X. M., Wu, H. L., Gao, S. H. (2022): Functional Diversity of Soil Microorganisms and Influencing Factors in Three Typical Water-Conservation Forests in Danjiangkou Reservoir Area. – Forests 14(1): 67-67.

- [31] Yu, B., Chen, Z. Y., Lu, X. X., Huang, Y. T., Zhou, Y., Zhang, Q., Wang, D., Li, J. Y. (2020): Effects on soil microbial community after exposure to neonicotinoid insecticides thiamethoxam and dinotefuran. – Science of the Total Environment 725: 138328-138340.
- [32] Zhang, J. E., Liu, W. G., Hu, G. (2002): Relationship between soil microbial quantity and soil fertility under different land uses. – Ecology and Environmental Sciences 11(2): 140-143.
- [33] Zhong, W. H., Gu, T., Wang, W., Zhang, B., Lin, X. G. (2010): The effects of mineral fertilizer and organic manure on soil microbial community and diversity. – Plant and Soil 326(1-2): 511-522.
- [34] Zhu, K., Wang, R., Li, G., Xiu, W. M., Wang, J., Li, B., Wang, L. L., Liu, H. F., Zhao, J. N., Yang, D. L. (2018): The response of microbial biomass carbon and metabolic characteristics of albic soil to land use change. Journal of Agro-Environment Science 37(10): 2194-2201.