ANALYSIS OF MICROBIAL COMMUNITY STRUCTURE FROM RHIZOSPHERIC SOIL OF RAPESEED IN ANKANG, SHAANXI, CHINA

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properties

Abstract

To explore the characteristics and the relationship between rapeseed rhizosphere microorganisms, enzyme activities and soil physicochemical properties of rapeseed rhizosphere soil samples was studied. For this purpose the rapeseed rhizosphere soil along with yellow cinnamon soil, paddy soil, yellow brown soil and yellow brown loam soil samples were collected from different soil types in Ankang, Shaanxi. The results indicated that in case of bacteria the Chao1 index of paddy soil was significantly lower than that of the other three soil types, indicating a relatively low abundance of bacterial flora. There was no significant difference in the richness and diversity of fungi in different soil types. At the class level, the relative abundance of bacteria was highest in Alphaproteobacteria, and the relative abundance of fungi was the highest in Sordariomycetes. Heat map analysis showed that there were similarities and differences in the microbial community structure of four different types of soil.

Introduction

Considering important raw material for high-quality feed, oil and other processed products, rapeseed is an important source of domestic edible vegetable oil (He *et al.* 2022). It is well known that soil type affects the bacterial and fungal community structure of plant rhizosphere microorganisms, and even if the two sites share the same microbiome, their relative abundance is different (Rainey 2010). Soil quality and ecosystem changes reflected by soil microbial communities are important indicators for determining soil properties and ecological functions (Yu *et al.* 2008). Therefore, the characteristics of soil microorganisms can be explored from the perspective of microbial community structure by analyzing and explaining the heterogeneity of different types of soils (Du *et al.* 2019).

The rhizosphere is a place where plants, soil and microorganisms exchange materials, and rhizosphere microorganisms are the most active biotic components in rhizosphere activities and an important index of soil fertility. They participate in the processes of energy flow and nutrient cycling in soil, and it is of great significance to study the community structure of rhizosphere microorganisms for improving ecological environment (Xu *et al.* 2014, Alam *et al.* 2024). Plant rhizosphere soil is directly affected by plant roots and their exudates, and is an important area for the interaction between plants and rhizosphere soil microorganisms. Soil microorganisms are extremely sensitive to the perception of the outside world, and soil enzyme activity and soil physicochemical properties have certain effects on the composition of microbial community structure (Kasel *et al.* 2008, Luo *et al.* 2016). In recent years, microbial sequencing technology has become a major technique for studying soil microorganisms due to its high accuracy, good sensitivity and large sample size for single analysis (Xia and Jia 2014).

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At present, the research on rhizosphere microorganisms mainly focuses on the community structure, diversity characteristics and rhizosphere microbial quantity of rhizosphere microorganisms, etc. As for whether rhizosphere effects exist in plants in different soil types, joint determination of the same plant planted in different soil types is rarely conducted to confirm (William *et al*. 2014). In this study, rapeseed rhizosphere soil in different soil planting areas of Ankang, Shaanxi was taken as the research object to determine the soil physicochemical properties, enzyme activities and microbial community structure, and explore the characteristics and differences of soil microbial composition in rapeseed planting areas of Ankang, in order to reveal the relationship between soil community and soil nutrients, and provide theoretical reference for the efficient utilization of soil in selenium-rich areas.

Materials and Methods

The rapeseed rhizosphere soil samples were collected from different soil types in Ankang, Shaanxi in May 2023, including yellow cinnamon soil, paddy soil, yellow brown soil and yellow brown loam soil. The rapeseed rhizosphere soil samples were collected from diagonal or plum shaped multiple points to form one sample. After collection, the samples were divided into 3 parts, 1 part was stored in the refrigerator at -80℃ for the sequencing of soil microbial community diversity, 1 part was stored in the refrigerator at 4℃ for the determination of soil enzyme activity, and 1 part of the soil was air-dried for the determination of soil physicochemical properties.

The 0.5 g rhizosphere soil sample was weighed and the total soil DNA was extracted using the Fast DNA TMSPIN Kit for Soil (MP, USA). The DNA sample concentration was detected, diluted to 10 ng·µL-1, and stored in the refrigerator at -80℃ for use. Primers 27F and 1492R were used to amplify the full length of bacteria by PCR, and ITS1F and ITS4R were used as primers to amplify the full length of fungi. PCR amplification primers are shown in Table 1.

Table 1. Primer design for PCR amplification.

Sequencing region	Primer	Primer sequence
27F_1492R	27F	AGRGTTYGATYMTGGCTCAG
	1492R	RGYTACCTTGTTACGACTT
ITS1F_ITS4R	ITS1F	CTTGGTCATTTAGAGGAAGTAA
	ITS4R	TCCTCCGCTTATTGATATGC

The PCR reaction system was 20 µl (4 µl 5×FastPfu Buffer, 2 µl 2.5 mM dNTPs, 0.8 µl Forward Primer (5 µM), 0.8 µL Reverse Primer (5 µM), 0.4 µL FastPfu Polymerase, 0.2 µL BSA, 10 ng Template DNA, ddH$_2$O supplemented to 20 µl). Bacterial PCR reaction parameters were as follows: predenaturation at 95℃ for 3 min; Denatured at 95℃ for 30 s, annealed at 60℃ for 30 s, extended at 72℃ for 45 s, 27 cycles; Elongation at 72℃ for 10 min; Store at 4℃. The PCR reaction parameters of fungi were as follows: predenaturation at 95℃ for 3 min; Denatured at 95℃ for 30 s, annealed at 55℃ for 30 s, extended at 72℃ for 45 s, 35 cycles. It was extended at 72℃ for 10 min and stored at 4℃. The PCR product was 3µL and detected by 2% agarose gel electrophoresis. The PCR products of bacteria and fungi were sequenced using the third-generation sequencing platform of Pacbio. The sequencing work was completed by Shanghai Meiji Biomedical Technology Co., Ltd.

The ASVs were classified and annotated using the reference sequences provided by NCB database. mothur 1.30 software was used to calculate the Alpha diversity index of samples, including Simpson, Chao l, ACE, Shannon index, etc. (Amato *et al.* 2013). Community composition analysis was conducted using R (version 3.3.1) vegan software package and python 2.7 software to compare the differences and similarities between different samples in microbial community structure and species diversity. The R language (version 3.3.1) vegan software package was used to conduct redundancy analysis (RDA) of soil microbial community and environmental factors to study the effects of soil environmental factors on genetic diversity and dominant groups of microbial communities.

Results and Discussion

The Alpha diversity index is a comprehensive index reflecting the richness and evenness of the microbial community. The larger the values of Chao1 and ACE, the higher the microbial richness of the soil type. Shannon and Simpon are microbial community diversity indices in the soil type. The higher the Shannon value, the higher the community diversity; the higher the Simpon value, also known as dominance index, indicates the lower the community diversity. Relatively speaking, Simpon index is more sensitive to species evenness. Shannon index is more sensitive to species richness (Xu *et al.* 2011). According to Alpha diversity index, in case of bacteria, as can be seen from Chao1 index, the average value of paddy soil is significantly lower than that of yellow cinnamon soil, yellow brown soil and yellow brown loam soil, and the abundance of bacterial flora is relatively low(Table 2). The results of Simpon, ACE and Shannon indexes showed that there was no significant difference between the four different soil types. The average of Simpson index was between 0.003 and 0.008, and the average of ACE index was between 553.285 and 725.082. The average Shannon index ranges from 5.683 to 6.072.

In case of fungi, the analysis results of Simpon, Chao1, ACE and Shannon indexes show that there is no significant difference in the richness and diversity of fungi in four different soil types, and the average Simpson index is between 0.108 and 0.207. The average of Chao1 index ranges from 194.500 to 268.502, the average of ACE index ranges from 197.625 to 272.488 and the average of Shannon index ranges from 2.915 to 3.774.

The results showed that at the class level, Alphaproteobacteria (18.62 ~ 27.42%) had the highest relative abundance in the rhizosphere soil of rapeseed in different soil planting areas of Ankang, followed by Betaproteobacteria (15.73 ~ 20.76%), Gammaproteobacteria (6.47 ~ 20.22%), Actinomycetia (3.71 ~ 5.43%), Chitinophagia (3.71 ~ 4.73%) and Bacilli (2.44 ~ 6.21%) (Fig. 1). Among them, Actinomycetia were dominant in yellow brown soil and yellow brown loam soil. Chitinophagia were more dominant in yellow cinnamon soil and yellow brown loam soil. However, Bacilli were more dominant in paddy soil.

The diversity and richness of the four different types of soil bacterial communities are represented by color gradients and similarity in the heat map (Fig. 2). A blue relative abundance color indicates a low relative abundance, and a red relative abundance color indicates a high relative abundance. There were similarities and differences in the bacterial community structure of four different soil types. The abundance of Cyanobacteria in yellow cinnamon soil was lower than that in paddy soil, yellow brown soil and yellow brown loam soil. The abundance of Fibrobacteres in paddy soil was significantly lower than that in yellow cinnamon soil, yellow brown soil and yellow brown loam soil. The abundance of Deinococcus-Thermus and Tenericutes in yellow brown soil was significantly higher than that in yellow cinnamon soil, paddy soil and yellow brown loam soil. The abundance of Elusimicrobia and Candidatus_Melainabacteria in yellow

Table 2. Alpha diversity analysis of bacteria and fungi in soil samples.

Microbiome	Soil Type	Statistical Item	Simpson	Chao 1	ACE	Shannon
Bacteria	Yellow Cinnamon Soil	Amplitude of variation	0.003~0.014	326.58~961.55	329.04~1018.85	5.25~6.23
		Average value	0.008a	622.498a	645.598a	5.683a
		Standard deviation	0.004	222.172	236.933	0.390
		Coefficient of variation (%)	53.748	35.690	36.700	6.870
	Paddy Soil	Amplitude of variation	0.003~0.006	301.00~720.44	301.00~750.27	5.33~5.99
		Average value	0.005a	538.415b	553.285a	5.763a
		Standard deviation	0.001	158.349	168.780	0.257
		Coefficient of variation (%)	27.348	29.410	30.505	4.457
	Yellow Brown Soil	Amplitude of variation	0.003~0.005	438.50~882.16	444.69~888.66	5.81~6.17
		Average value	0.004a	700.744a	725.082a	5.986a
		Standard deviation	0.001	157.602	163.153	0.130
		Coefficient of variation (%)	22.222	22.491	22.501	2.167
	Yellow Brown Loam Soil	Amplitude of variation	0.002~0.005	464.28~907.34	465.42~935.92	5.65~6.38
		Average value	0.003a	676.648a	697.356a	6.072a
		Standard deviation	0.001	179.120	189.329	0.304
		Coefficient of variation (%)	42.164	26.472	27.150	5.001
Fungi	Yellow Cinnamon Soil	Amplitude of variation	0.039~0.415	93.91~267.53	94.79~269.71	1.66~4.35
		Average value	0.117a	210.397a	212.535a	3.512a
		Standard deviation	0.134	62.414	62.895	0.868
		Coefficient of variation (%)	115.109	29.665	29.593	24.707
	Paddy Soil	Amplitude of variation	0.059~0.487	151.91~259.20	153.04~267.14	1.64~3.76
		Average value	0.207a	194.500a	197.625a	2.915a
		Standard deviation	0.165a	39.960	42.798	0.795
		Coefficient of variation (%)	79.617	20.545	21.656	27.277
	Yellow Brown Soil	Amplitude of variation	0.035~0.625	122.24~342.67	123.02~350.97	1.19~4.42
		Average value	0.187a	245.284a	249.860a	3.360a
		Standard deviation	0.224	80.907	83.432	1.136
		Coefficient of variation (%)	119.979	32.985	33.391	33.804
	Yellow Brown Loam Soil	Amplitude of variation	0.018~0.337	224.00~330.03	224.00~331.59	2.35~4.55
		Average value	0.108a	268.502a	272.488a	3.774a
		Standard deviation	0.118	36.839	38.144	0.817
		Coefficient of variation (%)	109.632	13.720	13.998	21.637

Notes: Different letters indicate significant differences ($P < 0.05$).

brown loam soil was significantly lower than that in yellow cinnamon soil, paddy soil and yellow brown soil. The abundance of Ignavibacteriae in the yellow brown loam soil was higher than that in the yellow cinnamon soil, paddy soil and yellow brown soil.
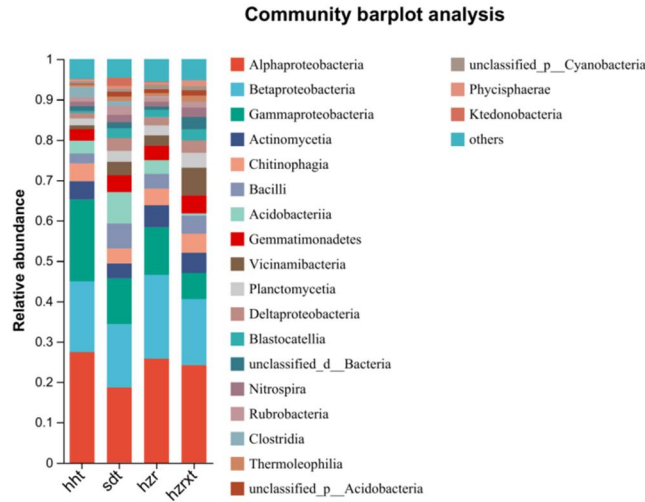

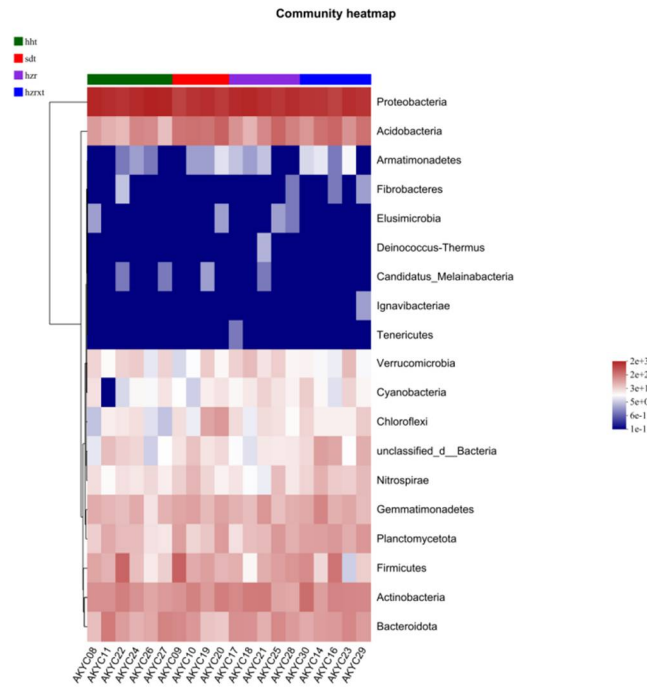Fig. 1. Bacterial composition at the class level of rapeseed rhizosphere soils.

Fig. 2. Heat map analysis of rapeseed rhizosphere bacterial community in different soil planting areas.

At the class level, Sordariomycetes (15.55 ~ 32.84%) had the highest relative abundance of fungi in the rapeseed rhizosphere soil in different planting areas of Ankang, followed by Mortierellomycetes (7.42 ~ 23.10%), Tremellomycetes (6.40 ~ 20.69%), Dothideomycetes (2.59% ~ 16.08%), Eurotiomycetes (2.50 ~ 16.63%) and Agaricomycetes (2.82 ~ 13.89%) (Fig. 3). Sordariomycetes were dominant in paddy soil and yellow brown loam soil respectively. Mortierellomycetes were dominant in yellow cinnamon soil and yellow brown soil respectively. Tremellomycetes were dominant in paddy soil and yellow brown loam soil. Agaricomycetes were more dominant in paddy soil.
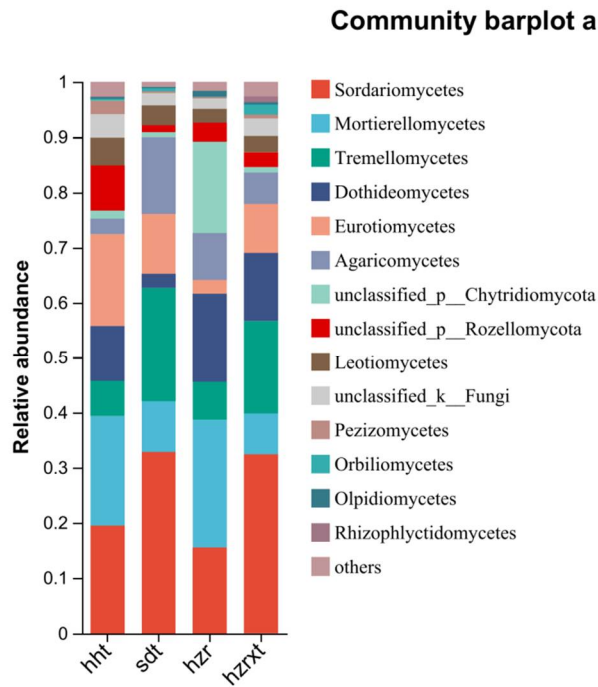

Fig. 3. Fungal composition at the class level of rapeseed rhizosphere soils.

The relative abundance of fungal communities was further identified by heat map analysis. According to the similarity comparison, it was found that there were similarities and differences in the fungal community structure of four different soil types (Fig. 4). The color of relative abundance of the community changed from blue to red, indicating that the relative abundance of the community changed from low to high (You *et al*. 2023). The abundance of Aphelidiomycota, Kickxellomycota and Basidiobolomycota in yellow cinnamon soil and yellow brown soil was higher than that in paddy soil and yellow brown loam soil. The abundance of Glomeromycota in yellow cinnamon soil and paddy soil is higher than that in yellow brown soil and yellow brown loam soil. The abundance of Monoblepharomycota in paddy soil was significantly lower than that in yellow cinnamon soil, yellow brown soil and yellow brown loam soil. The abundance of Mucoromycota in yellow brown soil was significantly higher than that in yellow brown soil, paddy soil and yellow brown loam soil. The abundance of Zoopagomycota in yellow brown loam soil was significantly lower than that in yellow cinnamon soil, paddy soil and yellow brown soil.
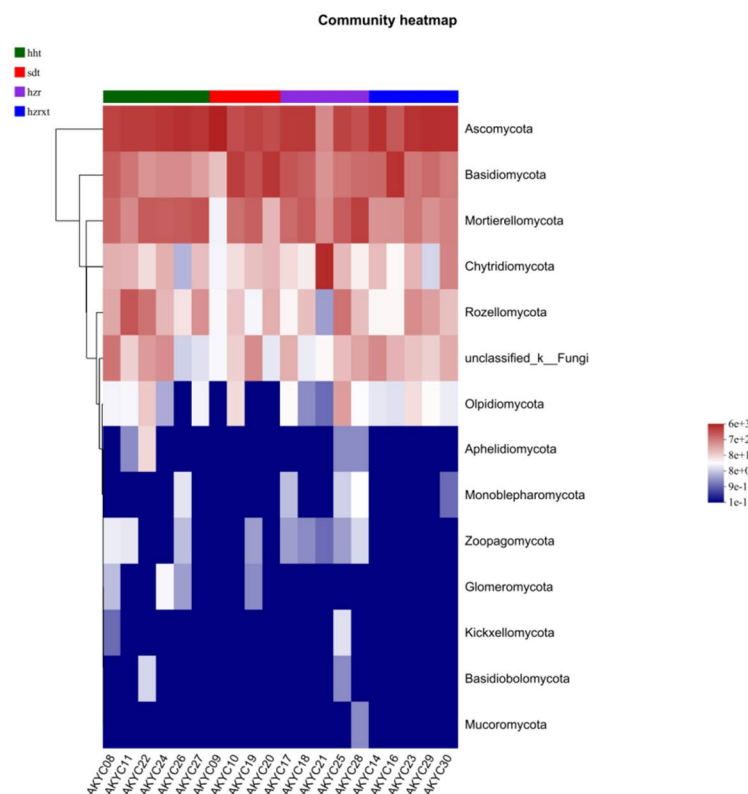
Fig. 4. Heat map analysis of rapeseed rhizosphere fungal community in different soil planting areas.

In order to reflect the influence of soil environmental factors on the composition of bacterial community, Redundancy analysis (RDA) was used to analyze soil physicochemical properties (pH, organic matter (OM), alkali-hydrolyzed nitrogen (N), available phosphorus (P), available potassium (K)), soil enzyme activities (dehydrogenase, invertase, urease and phosphatase) and bacterial community composition at phylum classification level of different soil types, so as to more intuitively and clearly reflect the influence of soil environmental factors on the genetic diversity and dominant groups of soil bacterial community. The longer the rays in Fig. 5, the higher the degree of influence of the factor. The Angle between the two rays is an acute Angle, indicating a positive correlation between the two factors. If the included Angle is obtuse, it indicates that the two are negatively correlated (Wang *et al*. 2021). According to the RDA analysis results of bacterial community composition, soil physicochemical properties and enzyme activities of different soil types, phosphatase activity has the longest ray among soil environmental factors, followed by pH and invertase activity. Therefore, in this study, phosphatase activity, pH and invertase activity have the highest influence on bacterial microbial community structure. At the same time, phosphatase activity was positively correlated with dehydrogenase activity and alkali-hydrolyzed nitrogen, and the correlation was good. pH was positively correlated with organic matter, available potassium and available phosphorus. Invertase activity had a good correlation with available phosphorus and urease. According to the distribution of the four soil

types, paddy soil and yellow brown loam soil were distributed in the positive direction of RDA1 axis, while yellow cinnamon soil and yellow brown soil were distributed in the negative direction of RDA1 axis. On the RDA2 axis, paddy soil and yellow brown loam soil were mainly distributed in the positive direction, while most yellow cinnamon soil is distributed in the negative direction, and yellow brown soil is near the axis. The distribution distance between the yellow cinnamon soil and the paddy soil indicates that the bacterial community structure of these two soils is unique, while the distribution of yellow brown soil and yellow brown loam soil is close, indicating that the bacterial community structure of these two soil types is similar. Phosphatase activity has great influence on yellow cinnamon soil and yellow brown soil, while pH has great influence on yellow brown loam soil. In summary, phosphatase activity, pH and invertase activity had significant effects on soil bacterial community diversity, and were the main influencing factors of the bacterial communities of the four soil types (Fig. 5).
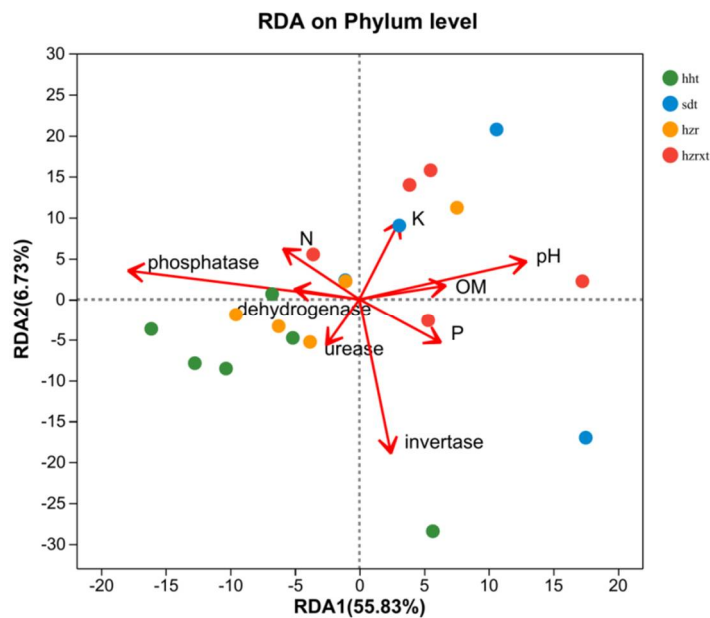

Fig. 5. Redundancy analysis of rhizosphere bacterial communities and environmental factors of rapeseed in different soil planting areas.

According to the RDA analysis results of fungal communities, soil physicochemical properties and enzyme activities of different soil types (Fig. 6), it can be seen that invertase activity has the longest ray among soil environmental factors, followed by available potassium and pH. Therefore, in this study, invertase activity, available potassium and pH have the highest influence on fungal microbial community structure. The invertase activity was positively correlated with urease activity and organic matter. pH was positively correlated with available phosphorus and organic matter, and the correlation is good. According to the distribution of the four soil types, paddy soil is distributed in the positive direction of RDA1 axis, yellow cinnamon soil and yellow brown loam soil were distributed in the negative direction of RDA1 axis, and yellow brown soil is near the axis. On the RDA2 axis, yellow cinnamon soil and yellow brown soil were mainly distributed in the positive direction, while paddy soil and yellow brown loam soil

were distributed in the negative direction. Yellow brown soil and yellow brown loam soil samples were distributed far away, indicating that the fungal community structure of these two soils had their own unique characteristics. Invertase activity had a great influence on yellow cinnamon soil and yellow brown loam soil, while available potassium had a great influence on paddy soil. In summary, invertase activity, available potassium and pH had significant effects on soil fungal community diversity, and were the main influencing factors of fungal communities in the four soil types(Fig. 6).
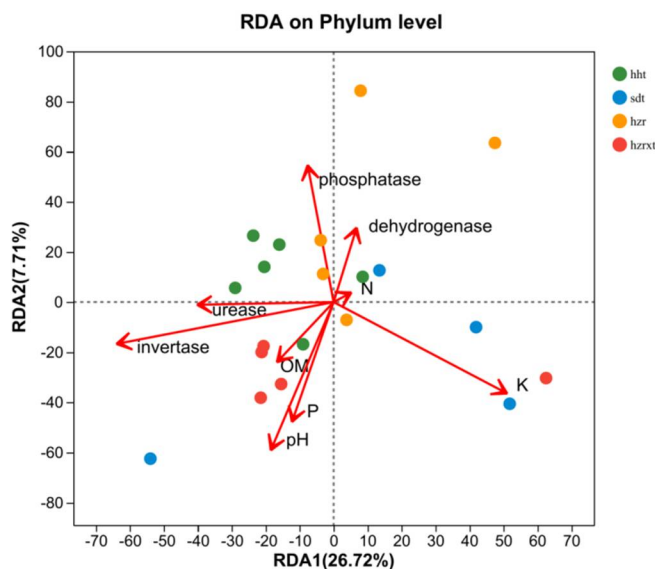

Fig. 6. Redundancy analysis of rhizosphere fungal communities and environmental factors of rapeseed in different soil planting areas.

This study found that soil physicochemical properties, enzyme activities and microbial community structure of rapeseed rhizosphere soil in different planting areas of Ankang were similar, but to some extent there were also differences.

The results of soil physicochemical property analysis showed that the pH of yellow brown loam soil was alkaline, and the pH of yellow cinnamon soil, yellow brown soil and paddy soil was obviously acidic. Soil nutrient is an important index to measure soil comprehensive productivity, and there is a certain relationship between soil available nutrient and soil alkali-hydrolytic nitrogen, available phosphorus and available potassium (Yu and Shi 2015). The results showed that the soil type with low available potassium content was yellow brown soil and the soil with high available phosphorus content was paddy soil. The more alkaline the soil is, the less soil available phosphorus content is. The main reason is that soil pH has a certain impact on the utilization and availability of soil phosphorus. With the increase of pH, the adsorption of soil phosphorus decreases (Devau et al. 2011).

Soil enzymes are active proteins that catalyze the decomposition of soil organic matter and are very sensitive to factors such as vegetation, water and nutrient content (Yang et al. 2015). Dehydrogenase can reflect the amount of active microorganisms in the soil system and their degradation activity to organic matter, and can be used as the degradation performance index of soil microorganisms. Invertase, also called sucrase, plays an important role in increasing soluble

nutrients in the soil. Urease can decompose urea into ammonia, carbon dioxide and water, and play an important role in nitrogen transformation. Phosphatase can be used as an indicator of soil fertility. The results of this enzyme activity analysis were consistent with those of Li *et al.* (2012), and there was a significant positive correlation between urease activity and invertase activity.

Soil microorganisms are one of the key drivers of soil fertility and participate in about 90% of soil reaction processes (Konopka *et al.* 1998). Different types of microorganisms were distributed in different soil types in different regions, and there were obvious differences in microbial diversity and structure in soils with different climatic conditions and different parent materials. In particular, there is a difference in microbial abundance between alkaline yellow brown loam soil and acidic yellow cinnamon soil, yellow brown soil and paddy soil. Redundancy analysis shows that pH has a significant impact on it, possibly because pH affects the growth, metabolism, substrate transformation and flora composition of microbial communities (Chen *et al.* 2019). In this study, it was found that there was no significant difference in fungal diversity among the four different soil types, possibly because the fungal communities in the soil were very likely to be similar in different regions with similar local ecological conditions (Liu *et al.* 2008).

In this study, we systematically studied the relationship between rhizosphere microorganisms and enzyme activities and soil physicochemical properties in rapeseed in Ankang City, Shaanxi Province. The results show that: in case of bacteria the Chao1 index of paddy soil was significantly lower than that of the other three soil types, indicating a relatively low abundance of bacterial flora. There was no significant difference in the richness and diversity of fungi in different soil types. At the class level, the relative abundance of bacteria was highest in *Alphaproteobacteria*, and the relative abundance of fungi was the highest in *Sordariomycetes*. There were similarities and differences in the microbial community structure of four different types of soil. In case of soil phosphatase activity, pH and invertase activity had significant effects on the diversity of soil bacterial microbial community, and soil invertase activity, available potassium and pH were the main influencing factors of fungal microbial community based on the RDA analysis. Other results clarify the relationship between soil communities and soil nutrients, and provide theoretical reference for soil efficient utilization in selenium-rich areas.

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