

## MILLET (*BRASSICA NAPUS* L.) ROTATION MODEL PROMOTED THE YIELD OF MILLET BY IMPROVING THE FUNGAL COMMUNITY STRUCTURE IN DRYLANDS IN CHINA

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(Received 13<sup>th</sup> Dec 2021; accepted 25<sup>th</sup> Mar 2022)

**Abstract.** Plant-crop rotation model plays an important regulatory role in effectively avoiding the occurrence of continuous cropping obstacles in drylands. However, it is not clear how fungal communities change in soils with some different rotation models. To solve this question, we determined the soil nutrient contents, and compared the composition of fungi from soils (0-20 cm) using fungal ITS pyrosequencing techniques. We also tested different crop rotation regimes, including *Triticum aestivum* L. and millet rotation (TaSi), small rye (*Triticale Secalotriticum*) and millet rotation (TsSi), one season of leisure (no any crop) and one season of millet (Si), rape (*Brassica napus* L.) and millet rotation (BnSi). The results showed that BnSi significantly improved soil available potassium contents, the activity of soil catalase, and yields of *Setaria italica*, while reduced soil Alkali-hydrolyzed nitrogen contents compared with other three groups. Fungal alpha-diversity index in BnSi was the highest. The relative abundances of Ascomycota in BnSi (61.53%) and TsSi (62.6%) were higher than that in Si (58.65) and TaSi (55.67%), while Basidiomycota exhibited the opposite trend. Available potassium and organic matter were significant factors affecting fungal communities, explaining 61.4% of total variation. Our findings shed new light on the response of the soil fungal communities to long-term green manure regimes.

**Keywords:** *Setaria italica*, green manure, crop rotation model, soil nutrient, ITS pyrosequencing

### Introduction

The negative effects of continuous cropping on crop production are mainly a reflection of the adverse effects on soil. Continuous cropping changes the physical and chemical properties of soil, and further causes nutrient imbalance (Bending et al., 2000; Yao et al., 2006; Zhang et al., 2019), accelerates the decomposition rate of organic carbon, causes rapid nutrient loss (West et al., 2002; Liu et al., 2003), and destroys the structure of soil aggregates (Six et al., 1999; Nayya et al., 2009). Continuous cropping can cause changes in soil microbial biomass and microbial community structure, resulting in imbalance of microbial community structure, the accumulation of pathogenic bacteria as well as serious diseases (Bever et al., 2012; Zhou et al., 2017), and thus seriously affect the yield and quality of millet.

Compared with continuous cropping, crop rotation system could effectively avoid the occurrence of continuous cropping obstacles and increased crop yields (Zhao et al., 2020). Rotation system could improve soil structure and soils organic matter (Govaerts et al., 2008; Detheridge et al., 2016), and Rotation system could also improve soil microbial diversity, change community structure and biological activity (Chaparro et al., 2012; Trivedi et al., 2015), decreased disease levels (Ai et al., 2018). In addition, the researchers found that fungi are more sensitive than bacteria to crop rotation (Guo et al., 2020). In terms of prevention and control of plant diseases, it was found that in the rotation system, the pathogenic micro-generation was significantly less than that in the continuous cropping system, and can reduce the occurrence of some important crop diseases (Jawson et al., 1993; Li et al., 2014; Palojärvi et al., 2020).

Millet (*Setaria italica*) is one of the main grains for people in northern China. It is widely cultivated in the middle and upper reaches of the Yellow River, and with a small amount cultivated in other areas. Grain of *Setaria italica* has high nutritional value, rich in protein, fat and vitamins.

Due to its important position in agricultural production, people use unreasonable cultivation system in order to pursue yield benefits in limited land, such as continuous cropping or unreasonable crop rotation, and so on. However, long-term continuous cropping has negative impact on agricultural production, which decreased significantly the yield or quality of major grain crops, such as wheat, corn and rice, under continuous cropping conditions (Johnson et al., 1992; Ladha et al., 2000; Lithourgidis et al., 2006). In addition, continuous cropping could also result in yield decline on other main crops, such as soybean, Peanut, and so on (Chen et al., 2014).

The objectives of this investigation were to clarify the effect of different continuous cropping and crop rotation system on soil physical and chemical properties, microbial community structure and enzyme activities, and further to assess the effect on *Setaria italica* production.

## Material and methods

### *Experimental materials and soil sampling*

The experimental plants millet (*Setaria italica* Henggu 13) was used as the test material, and the experiment was carried out at the Shenzhou Experimental Station of Dry Farming Agricultural Research Institute (37°44'N, 115°42'E, 20 m a.s.l.) of Hebei Academy of Agricultural and Forestry Sciences from 2016 to 2019. The soil type is loam, the average precipitation is 373.1 mm and the average temper is 24.85°C. There were four treatments in a random block design with three replicates: wheat (*Triticum aestivum* L.) and millet rotation (TaSi), small rye (*Triticale secalotriticum*) and millet rotation (TsSi), one season of leisure (no any crop) and one season of millet (Si), rape (*Brassica napus* L.) and millet rotation (BnSi). The management, fertilization and watering of all treatments during millet planting are consistent, and the management measures during wheat, triticale, leisure and rape are consistent. The area of each replicate plot was 12 m×7 m. Soil samples were collected before *Setaria italica* was harvest at the third year (in October of 2019). Five bulk soil (5~10 cm) samples from each treatment were randomly collected and sieved through a 2.0-mm sieve and stored at -80°C for further molecular analysis. The yield of millet in four rotation planning patterns were calculated by per acre.

### ***Analyses of soil physicochemical properties***

For physicochemical characterization, soil samples were air dried at room temperature and sieved through a 2-mm screen. Soil pH value was determined by pH3C pH meter with soil : water=1 : 2.5, and electric conductivity was determined by electrode method. The content of organic matter (OM), alkali-hydrolyzed nitrogen (AN), the effective phosphorus (AP) and available potassium (AK) were determined by external heating method of potassium dichromate, alkaline hydrolysis diffusion method, colorimetric method and flame photometry, respectively.

The activity of soil urease, cellulase and catalase were determined by sodium phenol-sodium hypochlorite colorimetric method, 3, 5-dinitrosalicylic acid colorimetric method and potassium permanganate titration method, respectively.

The concentrations of soil physicochemical properties in soil samples were tested for differences among samples using one-way analysis of variance (ANOVA) by SPSS BASE ver. 21.1 statistical software (SPSS, Chicago, IL, USA).

### ***Soil DNA extraction and Illumina MiSeq sequencing***

Soil genomic DNA was extracted using the MoBio PowerSoil® DNA isolation kit (MoBio Laboratories, Solana Beach, CA, USA), DNA concentration was quantified on a NanoDrop spectrophotometer (Thermo Scientific). Polymerase chain reaction (PCR) was performed using the primers ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA -3) and ITS2 (5'- TGCGTTCTTCATCGATGC-3) (Zhang et al., 2015) for Fungal ITS region. High-throughput sequencing was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at Beijing Allwegene Technology Co., Ltd. (Beijing, China).

The raw data were deposited into the NCBI short reads archive database (accession number : PRJNA733686) The raw sequence reads were initially trimmed using Mothur, and sequences met all the three criteria were kept: (1) the sequence with precise primers and bar-codes; (2) quality score >20; (3) the sequences >230 bp in length. The software package Vsearch was then used to further filter out sequences which were erroneous, chimeric. The remaining high-quality sequences were queried against the GenBank non-redundant nucleotide database (nt) in NCBI using local Blastn. The MEGAN program (Huson et al., 2007) was used to assign BLAST hits to taxa of the NCBI taxonomy. After removing non-fungal sequence reads, the fungal sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level using Uclust. Low-abundance OTUs (fewer than 2 reads, including singletons), which might influence richness and diversity estimates (Dickie, 2010), were excluded from the subsequent analyses. Rarefaction, diversity indices (Shannon and Simpson) of each sample were calculated using the software Mothur. The weighted and unweighted unifrac tests were performed using Mothur to determine the statistical significance of structural similarity among communities across sampling locations. Visualization of beta-diversity information was achieved via ordination plotting with PCA. To compare community characteristics in greater detail, heat maps at the species and genus level were constructed and Venn diagrams were created with R package. The correlations between fungal communities (at the genus level) and physical and chemical properties were determined with redundancy analysis (RDA), by using CANOCO 5.0.

## Results

### *Effects of different planting patterns on soil physico-chemical properties, enzyme activities and yields of Setaria italica*

For analysis whether there was difference of available nutrients among in soils with different green mature plant and *Setaria italica* rotation modes. BnSi significantly improved the contents of soil available potassium, and reduced the contents of soil Alkali-hydrolyzed nitrogen compared to other three groups. And compared with Si, the soil conductivity was decreased (down 42.26%) significantly in BnSi. However, there was no significant difference for pH value, contents of organic matter and available phosphorus among the four planting patterns (Table 1).

**Table 1.** Soil physical and chemical properties

Planting patterns	pH value	Electric Conductivity	Organic Matter (%)	Available Potassium(mg/kg)	Effective Phosphorus (mg/kg)	Alkali-hydrolyzed nitrogen (mg/kg)
BnSi	7.10a	0.08b	2.00abc	130.07a	29.08a	87.15bc
Si	7.44a	0.13a	2.08ab	104.63b	29.95a	91.18ab
TaSi	7.44a	0.11ab	1.98abc	102.04b	31.06a	92.31a
TsSi	7.14a	0.11ab	2.11a	98.78b	30.84a	91.95ab

BnSi is *Brassica napus* L. and *Setaria italica* rotation group; Si is *Setaria italica* continuous group; TsSi is *Triticum secale* and *Setaria italica* rotation group; TaSi is *Triticum aestivum* L. and *Setaria italica* rotation group. Values within the same column followed by the different letters indicate significant differences ( $P < 0.05$ )

BnSi and Si group significantly improved the activity of soil catalase, but there was no significant difference in activity of urease and phosphatases among the four planting patterns (Table 2). Yields of *Setaria italica* in BnSi and TaSi were the highest in the four treatments, and were higher than those in Si and TsSi (Fig. 1).

**Table 2.** Soil enzyme activities

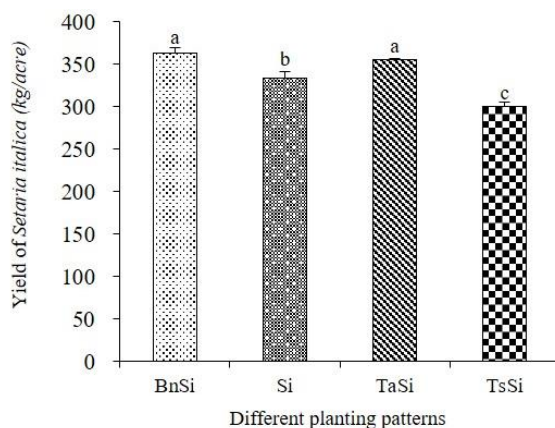
Planting patterns	Urease (mg/(g.24h))	Catalase (0.1mol/L KMnO4)/(h.g)	Phosphatases (mg/g.h)
BnSi	0.34a	0.97a	2.98a
Si	0.32a	0.96a	3.19a
TaSi	0.26a	0.65c	3.20a
TsSi	0.27a	0.72bc	3.14a

BnSi is *Brassica napus* L. and *Setaria italica* rotation group; Si is *Setaria italica* continuous group; TsSi is *Triticum secale* and *Setaria italica* rotation group; TaSi is *Triticum aestivum* L. and *Setaria italica* rotation group. Values within the same column followed by the different letters indicate significant differences ( $P < 0.05$ )

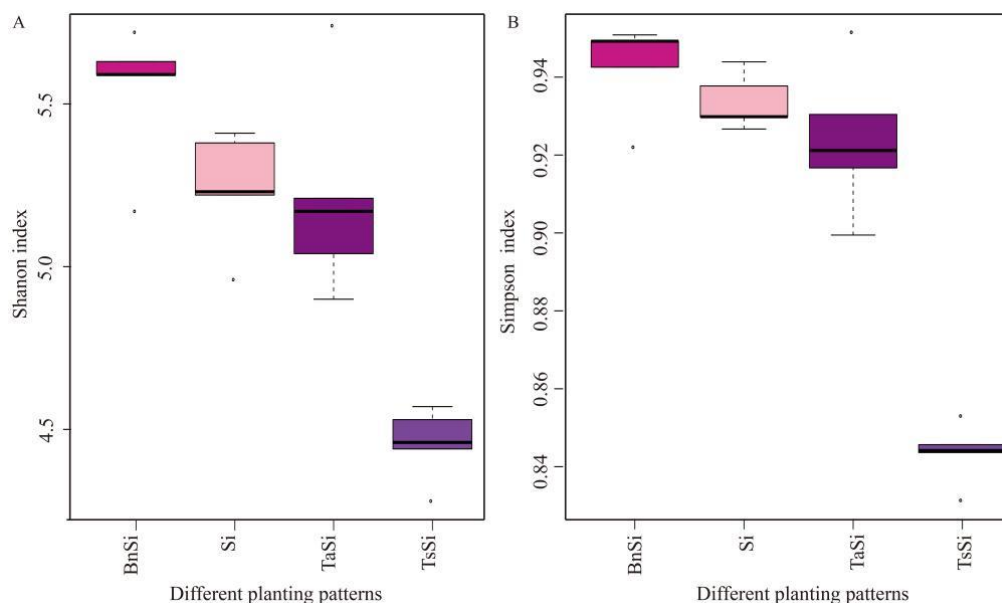
### *Planting patterns changed fungal diversity*

A total of 2,024,148 high quality fungal ITS sequences were obtained from all 20 soil samples. and these were classified into 1,416 OTUs. Good's coverage values were  $> 0.99$  for all samples, indicated that the current numbers of sequences in this study was

sufficient to reveal the diversity of fungal community. Shannon and Simpson indexes were all significantly affected by different planting patterns, Shannon and Simpson indexes in BnSi were highest, followed by Si group, Tasi group and TsSi group (Fig. 2).

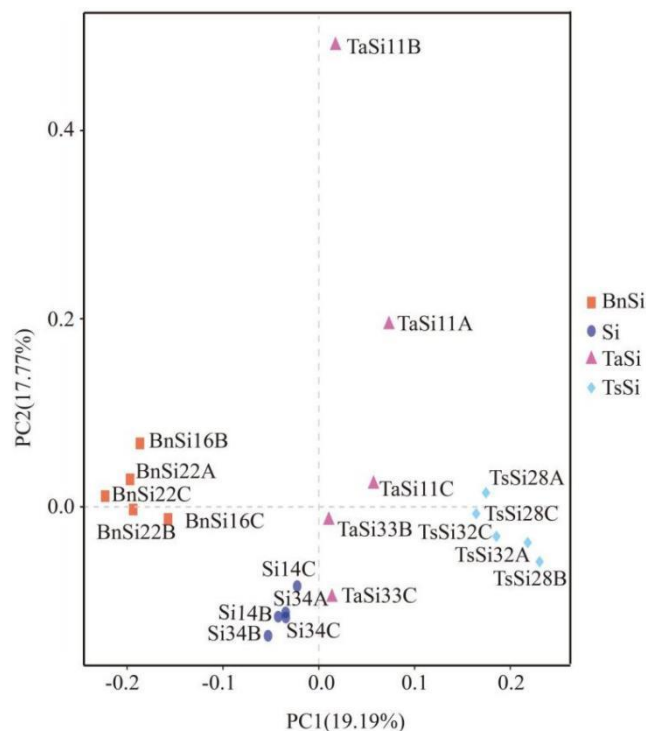


**Figure 1.** Yields of *Setaria italica* in different planting patterns. BnSi is *Brassica napus* L. and *Setaria italica* rotation group; Si is *Setaria italica* continuous group; TsSi is *Triticum secale* and *Setaria italica* rotation group; TaSi is *Triticum aestivum* L. and *Setaria italica* rotation group. Values within the same column followed by the different letters indicate significant differences ( $P < 0.05$ )



**Figure 2.** Alpha diversity indexes in four groups. BnSi is *Brassica napus* L. and *Setaria italica* rotation group; Si is *Setaria italica* continuous group; TsSi is *Triticum secale* and *Setaria italica* rotation group; TaSi is *Triticum aestivum* L. and *Setaria italica* rotation group

The PCA result revealed that fungal communities clustered according to different treatments. Based on the fungal beta-diversity, the four groups of samples were divided into two groups by the first PCA axis, and the left were BnSi and Si, and the right side were TaSi and TsSi (Fig. 3).



**Figure 3.** Principal Component Analysis (PCA). PCA of the pyrosequencing reads obtained from soils subjected to different fertilization regimes based on the weighted Fast UniFrac metric. Different colors or shapes represent different samples or groups. The scales of horizontal and vertical axes are relative distance and have no practical significance. PC1 and PC2 respectively represent the suspected influencing factors of the genetic composition deviation of the two groups of samples, which need to be summarized by combining the characteristic information of the samples. The closer the distance between the two points, the smaller the difference of gene abundance composition between the two samples, and the higher the similarity. BnSi is *Brassica napus* L. and *Setaria italica* rotation group; Si is *Setaria italica* continuous group; TsSi is *Triticum secale* and *Setaria italica* rotation group; TaSi is *Triticum aestivum* L. and *Setaria italica* rotation group

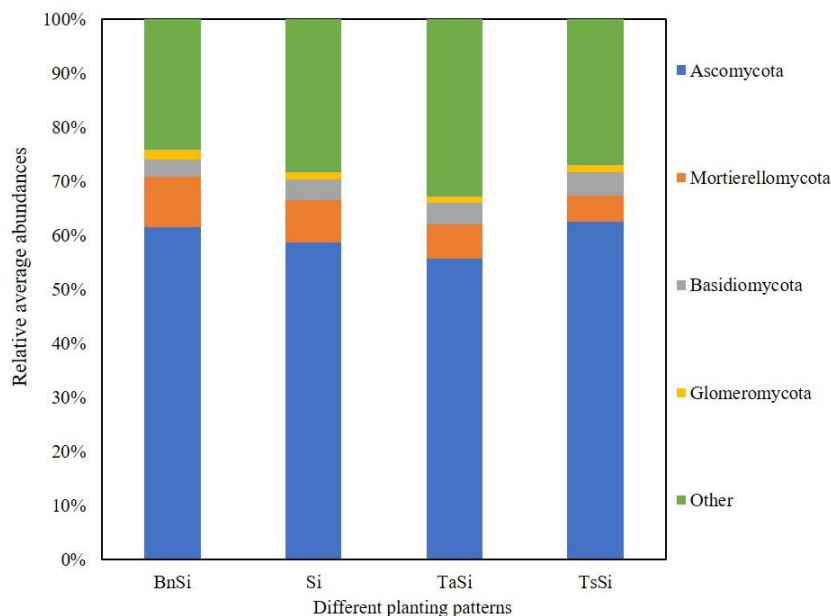
### **Fungal community composition**

The relative abundance of different phyla in the four groups are shown in Fig. 4. The dominant fungi phyla in soils were Ascomycota (55.67%-62.6%), Mortierellomycota (4.73%-9.36%), Basidiomycota (3.15%-4.40%) and Glomeromycota (1.20%-1.90%).

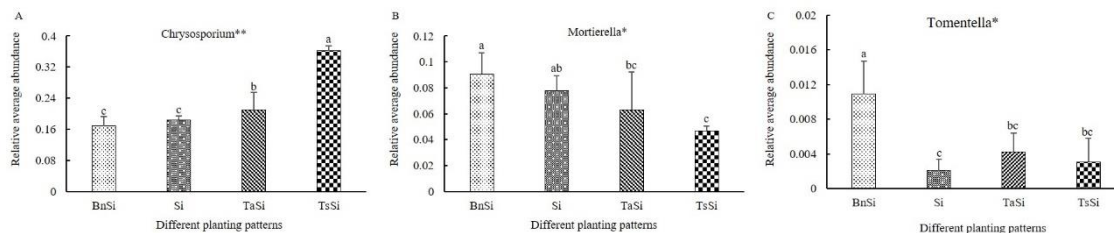
Different planting patterns significantly changed the relative abundance of the main phyla. For phyla Mortierellomycota, their relative abundance was significantly lower from BnSi, Si, Tasi than TsSi. The relative abundance of Ascomycota in BnSi (61.53%) and TsSi (62.6%) were higher than in Si (58.65) and TaSi (55.67%), while Basidiomycota showed the opposite trend.

### **Distribution of the fungal community at the genus level**

At the genus level, we compared the differences in the top ten abundant genera in different groups. Mortierella was typically declined in relative abundance, while Chrysosporium increased. the relative abundance of genera Tomentella in BnSi was highest than other groups (Fig. 5).



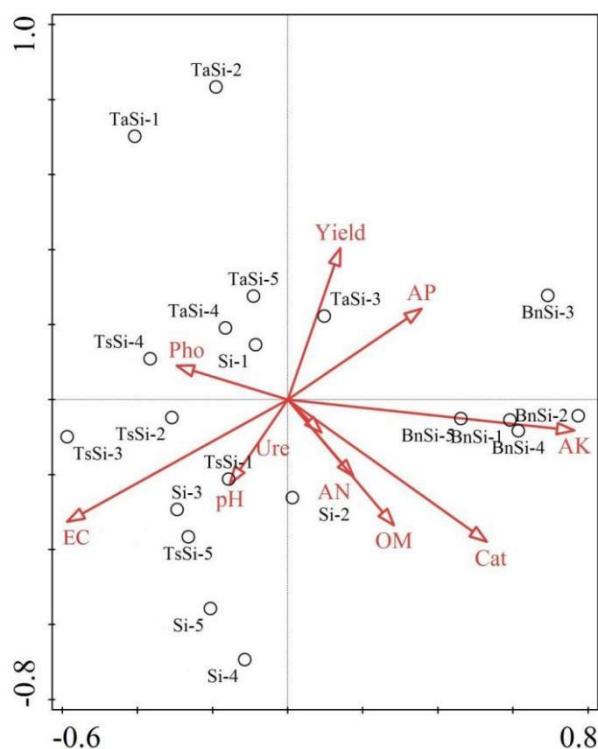
**Figure 4.** Relative average abundances of the four most abundant phyla across different planting patterns. BnSi is *Brassica napus* L. and *Setaria italica* rotation group; Si is *Setaria italica* continuous group; TsSi is *Triticum secale* and *Setaria italica* rotation group; TaSi is *Triticum aestivum* L. and *Setaria italica* rotation group



**Figure 5.** Relative average abundances of the top three abundant genera under different planting patterns. A: The relative average abundance of the *Chrysosporium* genus; B: The relative average abundance of the *Mortierella* genus; C: The relative average abundance of the *Tomentella* genus. Error bars indicate the standard deviation of relative abundance between five replicate samples (\*\* $P < 0.01$ , \* $P < 0.05$ ). BnSi is *Brassica napus* L. and *Setaria italica* rotation group; Si is *Setaria italica* continuous group; TsSi is *Triticum secale* and *Setaria italica* rotation group; TaSi is *Triticum aestivum* L. and *Setaria italica* rotation group

### Redundancy analysis

RDA showed that physicochemical parameters explained 28.49% of changes in fungal communities. The axis1 explained 15.43%, while the axis 2 explained 13.06% of the changes (Fig. 6). AK ( $F = 2.2$ ,  $P = 0.016$ ), OM ( $F = 1.8$ ,  $P = 0.022$ ) (Table 3) were significant factors affecting fungal communities, explaining 61.4% of total variation.



**Figure 6.** Results from redundancy analysis (RDA) of different nitrogen added treatments bacterial and soil chemical characteristic. pH is pH Value; EC is electric conductivity; OM is organic matter; AP is available potassium; available phosphorus; AN is alkali-hydrolyzed nitrogen; Pho is phosphatases; Cat is catalase; Ure is Urease

**Table 3.** Analysis of constrained forward selection results

Name	Explains (%)	Contribution (%)	pseudo-F	P-value
AK	10.7	17.4	2.2	0.016
OM	8.6	14	1.8	0.022
AP	6.4	10.4	1.4	0.168
Alkali hydrolyzed nitrogen	6.1	9.9	1.3	0.142
Conductivity	6.4	10.4	1.4	0.126
Yield	5.3	8.6	1.2	0.26
Catalase	5.4	8.9	1.3	0.218
pH	3.8	6.2	0.9	0.578
Urease	4.1	6.6	0.9	0.504
Phosphatase	4.6	7.4	1.1	0.388

pH is pH Value; OM is organic matter; AK is available potassium; AP is available phosphorus

## Discussion

Our study explored the effects of soil physicochemical property analyses and soil fungal communities under different crop rotation combinations, and further to clear which crop rotation combinations was better.



### ***Effect of crop rotation on soil physicochemical properties***

Soil enzyme activities and soil physical and chemical properties, are greatly related with soil microorganisms (Sun et al., 2013). In our study, we measured the activity of urease, catalase and phosphatases under four crop rotation combinations, the result showed the activity of catalase was higher in BnSi and Si than in Tasi and TsSi. Catalase was important oxidoreductases, which could break down hydrogen peroxide to water and oxygen, and reduced the toxic effects of hydrogen peroxide produced by plant or microorganisms metabolism on organisms and soil. In addition, Catalase was also reported involving in soil antimicrobial defense, and was positive related to plant disease resistance (Zhou et al., 2012; Xu et al., 2013), this was consisted with the previous research that the activities of catalase were significantly increased in the healthy soil (Wang et al., 2017).

In modern intensive agriculture, potassium depletion is a biggest cause of crop yield stagnation and low nutrient efficiency in the soil (Hahane et al., 2020), Soil available potassium is a kind of potassium that can be absorbed easily by plant, increased content of available potassium in BnSi showed *Setaria italica*-oilseed rotation combination was more sustainable agricultural system. Electric conductivity was lowest in BnSi may suggest that *Brassica napus* L. could have positive effects in reducing salinity levels (Gura and Mnkeni, 2019).

### ***Effects of crop rotation combinations on soil fungal diversity***

Compared with continues cropping, crop rotation system could also improve soil microbial diversity, change community structure and biological activity (Chaparro et al., 2012; Detheridge et al., 2016), But the intensity of improvement was different under different crop rotation combinations (Ai et al., 2018). Microbial diversity had positive impacts of soil environment. Rich microbial diversity in soil can lead to a more stable and healthy soil ecological system (Chaer et al., 2009; Pietras et al., 2013; Wang et al., 2017). In our study Soil samples in BnSi (*Setaria italica*- oilseed rotation combination) had highest fungal diversity, followed by Si and Tasi. TsSi significant decreased the soil fungal diversity. These results were consistent with PCA, compare to TaSi and TsSi, BnSi and TsSi significantly changed the fungal diversity and the sample clustering were separated far from the other two groups.

### ***Effect of crop rotation on fungal community composition***

In our study Ascomycota was dominant fungal phylum in all bulk soils, but there was significant difference in Mortierellomycota. Besides, Mortierella is an important genus of Mortierellomycota shown significant changes in different crop rotation, Mortierella has been shown to be a beneficial fungi for crops, and can assist crops and mycorrhizal fungi in phosphorus acquisition (Wang et al., 2020; Etesami et al., 2021), and some members of Mortierella was plant fungal endophyte can enhances biosynthesis and stress tolerance in the host plant (Wani et al., 2017), Tomentella belongs to Ectomycorrhizal fungi, increasing abundance of Tomentella can facilitate nutrient and water supply to the plants.

## Conclusion

In conclusion, the soil phy-chemical characteristics, soil enzyme activities, yields of millet, and the composition of fungal communities in soils were clearly affected by long term plant-millet rotations. The microbial community data presented support the idea that rape (*Brassica napus* L.) and millet rotation have positive effects (i.e. higher alpha-diversity index) on fungal communities, yields of millet, and the activity of soil catalase. Therefore, we preliminarily concluded that millet and rape rotation is a good choice for the stability of soil ecosystem and sustainable development of production under drought conditions.

**Acknowledgments.** This work was Funded by Basic Research Funds of Hebei Academy of Agriculture and Forestry Sciences, [No.2021040202], Key Research and Development Program of Hebei Province, [No. 20326321D], and Construction of Modern Agricultural Industrial Technology System [NO. CARS-06-13.5-B3].

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