

Effects of crop rotation on sugar beet growth through improving soil physicochemical properties and microbiome

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ABSTRACT

Soil microorganisms are critical to the sustainable development of agroecosystems. Continuous cropping obstacles refer to crop yield reduction in continuous cropping. Crop diversity can be increased through crop rotation, improving soil-plant interactions to alleviate the continuous cropping obstacles. In our study, rubber dandelion (*Taraxacum kok-saghyz* Rodin, TKS) and sugar beet (*Beta vulgaris* L.) were used for rotation. A two-year field experiment was conducted using a randomized uniform grid design to investigate the effects on soil microbial community structure and physicochemical properties, and supplemented by a pot experiment. We hypothesized that sugar beet and rubber dandelion could change soil microbiome, improve soil physicochemical properties, and ultimately promote the growth of sugar beet. The results showed that compared with continuous cropping, sugar beet and rubber dandelion rotation can enhance the soil microbiome and increase the abundances of *Actinobacteria* and *Streptomyces*. The available nitrogen, potassium and soil organic matter contents were higher in rotation soil than in continuous cropping soil. In addition, urease activity in the soil after rotation with rubber dandelion was significantly increased. In general, the rotation of sugar beet and rubber dandelion could increase the biomass of sugar beet and alleviate the continuous cropping obstacle.

1. Introduction

The biodiversity of soil is essential to microbial communities, soil fertility, and sustainable agriculture. Loss of biodiversity leads to ecosystem degradation, leading to a decline in the functionality of agricultural ecosystems and limiting the prosperity of agriculture (Cardinale et al., 2012; Liu et al., 2023; Banerjee et al., 2019). Continuous cropping is commonly used in modern agriculture due to economic gain and land limitation. However, continuous cropping over a long period leads to an imbalance in soil microbial community structure, an increase in pathogens, and slow crop growth, which are commonly referred to as 'continuous cropping obstacles' (Cook, 2006; Wang et al., 2022). Continuous cropping obstacles can seriously reduce soil fertility, affect crop growth, and lead to lower yield (Sun et al., 2023). Crop rotation is

of increasing interest to growers as a safe and efficient crop cultivation system. It allows crops to obtain soil resources that complement each other in space and time, improves soil fertility, makes agricultural resources more efficient, and enriches soil biodiversity (Jing et al., 2022). Therefore, studying effective crop rotation systems and understanding the underlying mechanisms for sustainable agriculture is essential.

Sugar beet (*Beta vulgaris* L.) is a bioenergy and fodder crop. It is widely cultivated in the major temperate climates of the earth. Sugar beet serves as raw material for many industrial products, and is of economic and strategic importance (Usmani et al., 2022). Long-term planting of sugar beet can lead to increased leaf disease, significant yield loss, and decreased rhizosphere bacterial diversity (Du et al., 2022). Huang et al. also observed that continuous cropping led to slow growth of sugar beet seedlings, and weakened plant defense against

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pests and diseases, ultimately, causing significant reduction in crop yield (Huang et al., 2021, 2019). Continuous cropping obstacles of sugar beet are urgent to resolve.

The microbiota of the rhizosphere is an indispensable element that influences crop production (Ali and Xie, 2020). The interaction between soil microorganisms and plants helps to establish a stable soil environment, which improves soil quality and promotes plant growth (Bai et al., 2022; Hellequin et al., 2021). Crop rotation can effectively alleviate continuous cropping obstacles. It can improve soil microbiome, increase the beneficial microorganisms, and improve soil quality, thus promoting and protecting plant growth (Zhao et al., 2020; Jiao and Yuan, 2019). Yuan et al. indicated that crop rotation between corn and soybeans significantly increased corn yield (Yuan et al., 2022). Xie et al. found that in agricultural practice, fertilization did not affect the remodeling of soil microbiome as much as crop rotation (Xie et al., 2022). Consequently, it is significant to understand the variation in soil microbiome under the rotation and continuous cropping systems for agricultural production.

Plant specialized metabolites selectively recruit specific microorganisms to form microbial communities suitable for plant growth, ultimately creating dynamic and complex interactions at the rhizosphere level (Luo et al., 2022; Yuan et al., 2018; Zhou et al., 2023). Zhou et al. studied a tomato-potato onion intercropping and found that potato onion root secretions contain a class of flavonoids called taxifolin, which can help tomato plants to attract beneficial soil microorganisms such as *Bacillus*, change the composition of rhizosphere microbiota, and reduce plant diseases in tomato (Zhou et al., 2023). Rubber dandelion (*Taraxacum kok-saghyz* Rodin, TKS) is an important resource of Chinese herbal medicine, which has a wide range of antimicrobial effects. Li et al. found that intercropping dandelion with capsicum could prevent phytophthora disease of capsicum (Li et al., 2023). Astafieva et al. isolated four antimicrobial peptides from dandelion flowers, among which ToAMP3 showed better inhibition against *F. oxysporum* and ToAMP4 against *A. niger* and *P. betae* (*P. betae* is a kind of pathogen that causes the bushy root disease of sugar beet) (Astafieva et al., 2012, 2013; Decroës et al., 2022). TKS belongs to the genus *Taraxacum* in the family Asteraceae, it is an important industrial crop for rubber production. Kong et al. analyzed the metabolome of TKS and revealed a large number of specialized metabolites such as terpenoids, coumarins, flavonoids, phytosterols, and phenolic acids (Kong et al., 2021). Flavonoids are an important metabolites produced by the phenylpropanoid pathway. They play an important role in chemical signal transmission between plants and microorganisms, such as the *Rhizobium*-legume symbiosis (Kang et al., 2020). In addition, a flavonoid derivative sakuranetin can help defend the rice blast disease caused by *Magnaporthe oryzae* (Wang et al., 2022). In addition, overexpression of *PalbHLH1* and *PalMYB90* genes in *Populus alba* could significantly increase the content of flavonoids, boost the activities of antioxidant enzymes and enhance pathogen resistance (Bai et al., 2020). Coumarin is another important metabolite in the phenylpropanoid pathway. It was shown to help plants absorb available iron from the soil, and thereby promotes plant growth. In addition, coumarin also affects the soil microbiome by inhibiting pathogenic bacteria *Fusarium oxysporum* and *Verticillium dahliae* in the soil (Stassen et al., 2021). Since TKS has both antimicrobial and rubber-producing properties, we speculate that its metabolites may help to recruit beneficial microorganisms and suppress pathogenic microorganisms, thus improving the soil microbiome and physicochemical properties.

Here we propose two hypotheses: (1) Sugar beet and TKS rotation can change the soil microbiome. (2) The variation in soil microbiome affects the physicochemical properties of sugar beet continuous cropping soil. TKS rotation will promote the growth of sugar beet, thus alleviating the phenomenon of sugar beet's continuous cropping obstacles.

2. Materials and methods

2.1. Experiment field introduction

This experiment was conducted in an experimental field located at the Molecular Biology Laboratory of Heilongjiang University, Harbin, China (E126°62', N45°71'). Sugar beet continuous cropping was carried out in the experimental field from 2009 to 2021 (supplementary Fig. 1), and the sugar beet - TKS rotation zone was set up in 2019 (supplementary Fig. 2).

There were 10 rows in the sugar beet continuous cropping zone and 10 rows in the sugar beet - TKS rotation zone. In the sugar beet continuous cropping zone, sugar beets were transplanted in April and harvested in August every year. In the sugar beet - TKS rotation zone, sugar beets are first planted (transplanted in April and harvested in August), then in the following year, the TKS is planted in April, harvested in August, and the crop is rotated in this way. No fertilizer was added to the black (Mollisol) soil, and the plants were allowed to grow naturally. Cultivation of sugar beet and TKS was at a density of nine plants per m². Groundwater is used for irrigation and weeds were periodically removed. After harvesting the plant materials, the residual roots and debris in the experimental field were removed, and the soil was prepared for the next season.

2.2. Soil and plant sampling

The sugar beet continuous cropping zone and the sugar beet - TKS rotation zone in the experimental field were evenly divided into 20 grids, respectively, each of which was 1 square meter. For sampling, 0–15 cm topsoil samples were collected from the rotation zone and continuous cropping zone, respectively. A random uniform grid design was adopted, and three grids were randomly selected from the 20 grids for sampling, and then pooled together to form a soil sample. The sampling method was repeated three times (Zou et al., 2023). The soil was sieved through a 2 mm sieve. A portion of the freshly sampled soil was air dried (< 30 °C) and used for soil physicochemical property analysis. The rest was used for subsequent soil microorganism assays.

To explore the effect of TKS rotation on the growth of sugar beet seedlings, we carried out a potted experiment side-by-side with field experiment. The site of the potted experiment was right next to the experimental field. The sugar beet pot experiment was conducted using sugar beet continuous cropping soil and sugar beet-TKS rotation soil, respectively. The soil was removed from the experimental field and sieved, then sugar beet was planted, and weeded manually during the experiments. In the experiment, we named the pots as CS, representing the sugar beet continuous cropping group, and RS, representing the sugar beet - TKS rotational crop group. Sampling was carried out at the seedling stage (30 day) and leaf cluster stage (60 day), with three replicates at each stage, and three sugar beets were randomly selected as one replicate. In addition, we also collected soil and air-dried (< 30 °C) from the potting experiments using the same method as aforementioned for the determination of soil physicochemical properties and microorganisms.

2.3. Soil physicochemical analysis

Soil pH was determined using a pH meter, and conductivity was measured by an electrode method (Zhou et al., 2017). A Kjeldahl method was used to determine the soil total nitrogen (TN). Available nitrogen (AN) was determined by an alkaline hydrolysis diffusion method (Zhang et al., 2019). Available phosphorus (AP) and available potassium (AK) were determined by molybdenum-antimony resistance colorimetry (Peng et al., 2021) and flame photometer (Peng et al., 2016), respectively. Soil organic matter (SOM) was determined by heating and titrating with potassium dichromate and sulfuric acid (Kalembasa and Jenkinson, 1973).

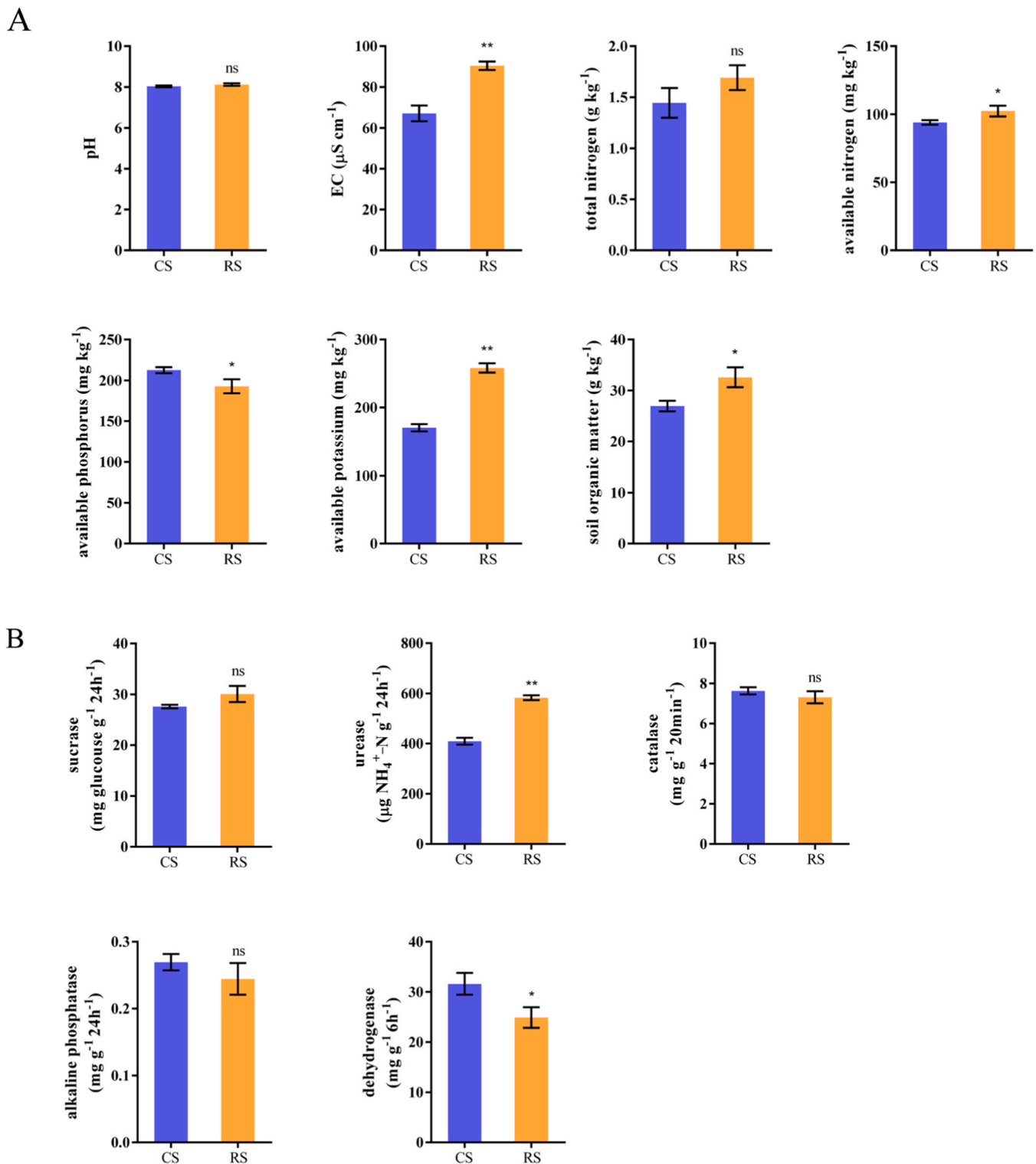


Fig. 1. Physicochemical properties and enzyme activities of the continuous cropping soil (CS) and rotation soil (RS). (A) Physicochemical properties of the soil in the experimental field (B) Activities of soil enzymes. CS is the continuous crop soil group and RS is the rotational soil group. Significance is based on the student's *t*-test, *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, ns indicate not significant.

2.4. Analysis of soil enzyme activities

The activity of soil sucrase was measured using a 3, 5-dinitrosalicylic acid colorimetric method (Zhou et al., 2018). Soil urease was measured by using a method of sodium phenol-sodium hypochlorite colorimetry at 578 nm on a multimode plate reader (PerkinElmer Ensignht, USA) (Liu

et al., 2021). Soil catalase was determined by potassium permanganate titration to purplish-red as the endpoint (Sinha, 1972). Soil alkaline phosphatase activity was assayed using a colorimetric method of disodium phenylphosphate with absorbance at 570 nm (Li et al., 2022). The activity of soil dehydrogenase was measured by using the reduction of 2, 3,5-Triphenyltetrazolium chloride (TTC) colorimetry, and its optical

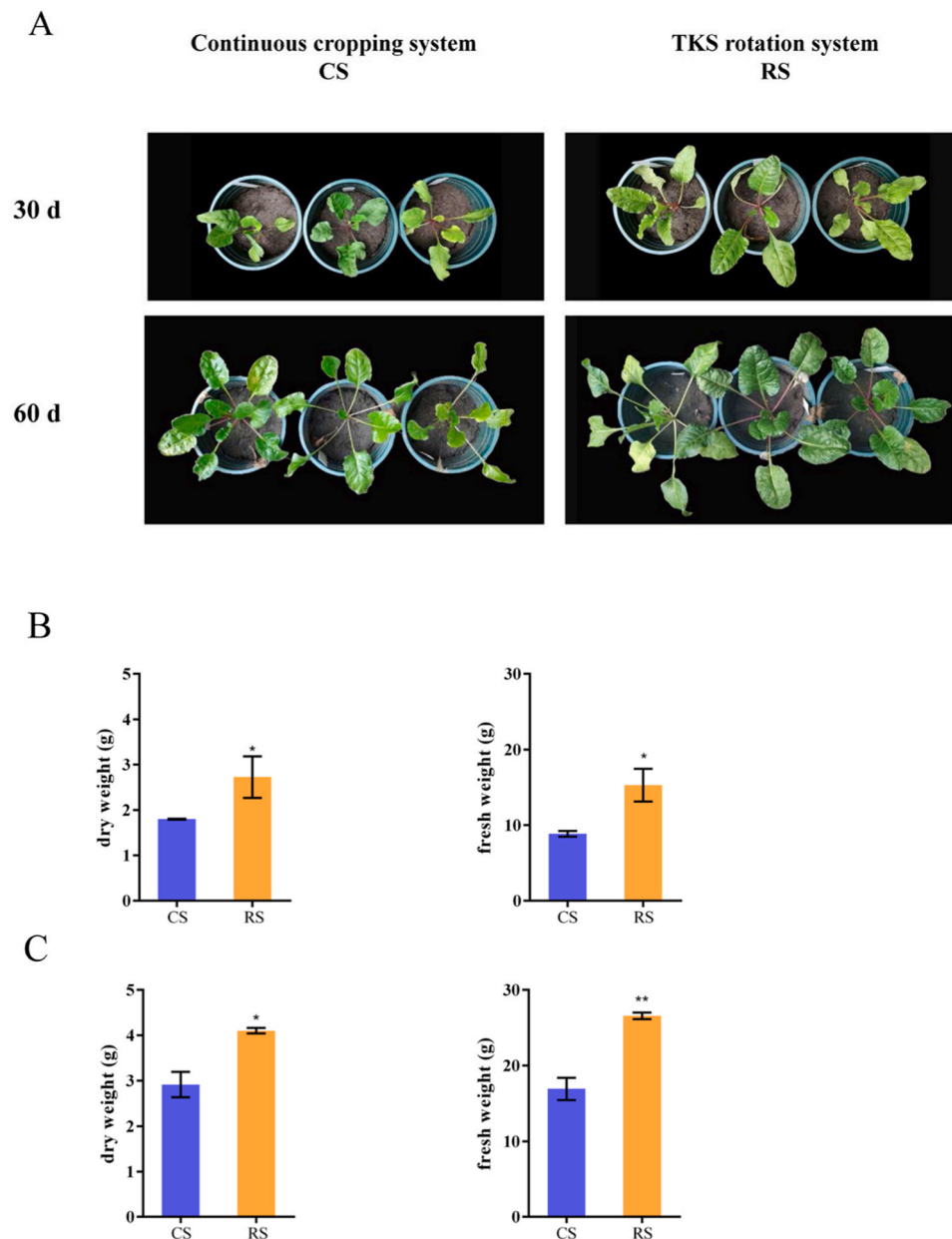


Fig. 2. Morphological phenotypes and growth parameters of potted sugar beet under continuous cropping (CS) and rotational crop (RS) soil conditions. (A) Performance phenotypes of potted sugar beet after 30 and 60 days under CS and RS conditions. (B) Growth parameters of the potted sugar beet (including dry weight and fresh weight) at 30 day. (C) Same as in (B), except at 60 day. Significance is based on the student's *t*-test, *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, ns indicate not significant.

density was measured by a multimode plate reader (PerkinElmer Ensight, USA) at the wavelength of 485 nm (Zhou et al., 2012).

2.5. DNA extraction, Illumina Miseq sequencing, and data processing

DNA extraction, concentration determination, and Illumina MiSeq sequencing of the soil samples were performed by Shanghai Sangong Biotechnology Co., LTD., China. The MiSeq technology was used for sequencing the total DNA of the microbiome of the whole soil sample to analyze the structure of the soil microbial community. The raw sequencing data was assessed by Fast QC and filtered by Trimmomatic in order to obtain reliable data (Bolger et al., 2014). The data is then assembled using IDBA_UD to obtain Clean Reads for each sample (Peng et al., 2012). Prodigal is used for ORF prediction, and genes of no less than 100 bp in length are selected for translation into amino acid

sequences. A non-redundant gene set was obtained through removing redundancy by CD-HIT software (Li and Godzik, 2006). The Clean Reads of each sample were compared to the sequences of non-redundant gene sets using Bowtie2. (Langmead and Salzberg, 2012). Gene abundance information in each sample was obtained using SAMtools (Li et al., 2009). Set filter criteria were: E-value < $1e^{-5}$, Score > 60. The homology of amino acid sequences was compared with the Nr database by Diamond to obtain annotated information for species classification (Buchfink et al., 2015).

2.6. Statistical analysis

The growth status and soil physicochemical properties of sugar beet under rotation and continuous cropping system were compared using *t*-test, with a *p*-value of < 0.05 to indicate significance. Data were

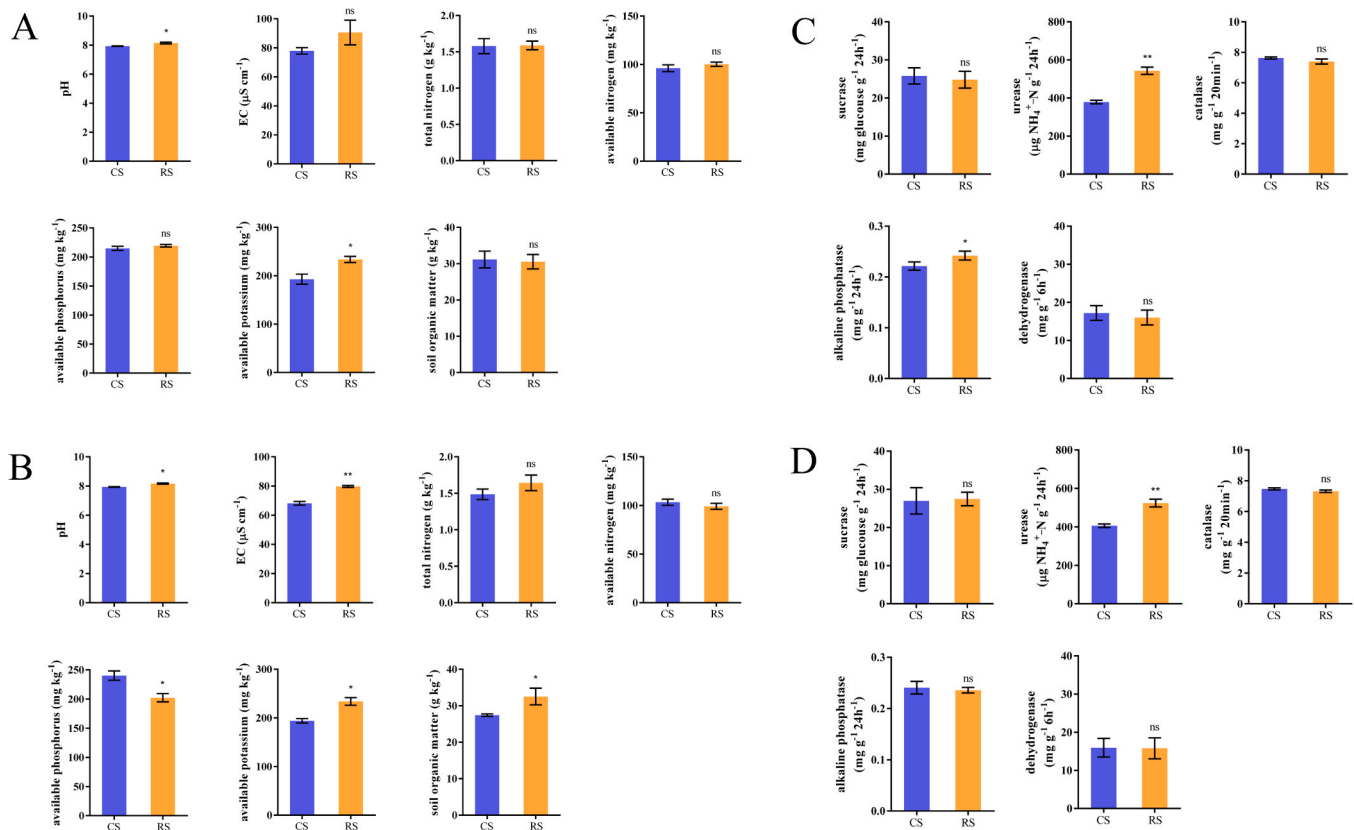


Fig. 3. Physicochemical properties and enzyme activities of continuous crop (CS) and rotational crop (RS) soils. (A) Physicochemical properties at 30 d. (B) Physicochemical properties at 60 d of potted sugar beet. (C) Enzyme activities of CS and RS soils at 30 d. (D) Enzyme activities of CS and RS soils at 60 d of potted sugar beet. Significance is based on the student's *t*-test, *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, ns indicate not significant.

analyzed using SPSS software (version 25.0). Alpha diversity indices and weighted UniFrac distances were calculated using the “vegan” package in “R” (Gong et al., 2021). Microbial community composition was analyzed using principal co-ordinates analysis based on UniFrac distances and used Analysis of similarities (ANOSIM). Canoco 4.5 was used for RDA analysis. Excel, GraphPad Prism 6.01, R language, and Ggplot2 were used to make relevant charts.

3. Result

3.1. The experimental field soil

In the experimental field, the soil physicochemical properties (including soil pH, soil EC value, TN, AN, AP, AK, and SOM) were measured in continuous cropping soil and TKS rotation soil. Compared with continuous cropping, AN, AK, and SOM contents were higher in the rotation soil than in continuous cropping soil (Fig. 1A).

In addition, we measured the activities of soil enzymes, including sucrase, urease, dehydrogenase, alkaline phosphatase, and catalase. Compared with the continuous cropping, urease activity in the soil after rotation with TKS was increased, but the activities of sucrase, dehydrogenase, and alkaline phosphatase were not significantly changed (Fig. 1B).

3.2. The potted plant experiment

3.2.1. Sugar beet morphological indicators

In the potted sugar beet experiment, we observed that sugar beet planted in rotating soils grew better than those planted in continuous soils. The dry weight and fresh weight of sugar beet were measured at 30

d and 60 d after the start of planting. Fig. 2A illustrates the growth of potted sugar beet. Compared with the continuous cropping, the morphological indexes of the TKS rotation were superior to those of the continuous cropping.

At 30 d, the dry weight and fresh weight of potted sugar beet in the rotation system were 1.5 times and 1.7 times, respectively that of the continuous cropping system (Fig. 2B). At 60 d, the dry weight and fresh weight of potted sugar beet in the rotation system were 1.4 times and 1.6 times that of the continuous cropping system, respectively (Fig. 2C). From these morphological and growth indexes, crop rotation promoted the growth of sugar beet very well.

3.2.2. Soil physicochemical properties and enzyme activities

Soil physicochemical properties and enzyme activities were measured at 30 and 60 d in the field experiments (Fig. 3). As can be seen in Fig. 3A, soil pH and AK content were significantly higher in sugar beet in the rotation system than in continuous soil at 30 d. At 60 d, the pH value, soil EC value, AK and SOM contents of potted sugar beet in the rotation soil were significantly higher than those in the continuous cropping soil (Fig. 3B). However, the content of AP was lower than continuous cropping soil (Fig. 3B).

As to soil enzyme activities, the activities of urease and alkaline phosphatase in the rotation soil after 30 d of cultivation were significantly higher than in the continuous cultivation. At 60 d, only the soil urease activity in the rotation soil was higher than in the continuous crop soil, while the activities of the other enzymes did not show significant differences (Fig. 3D).

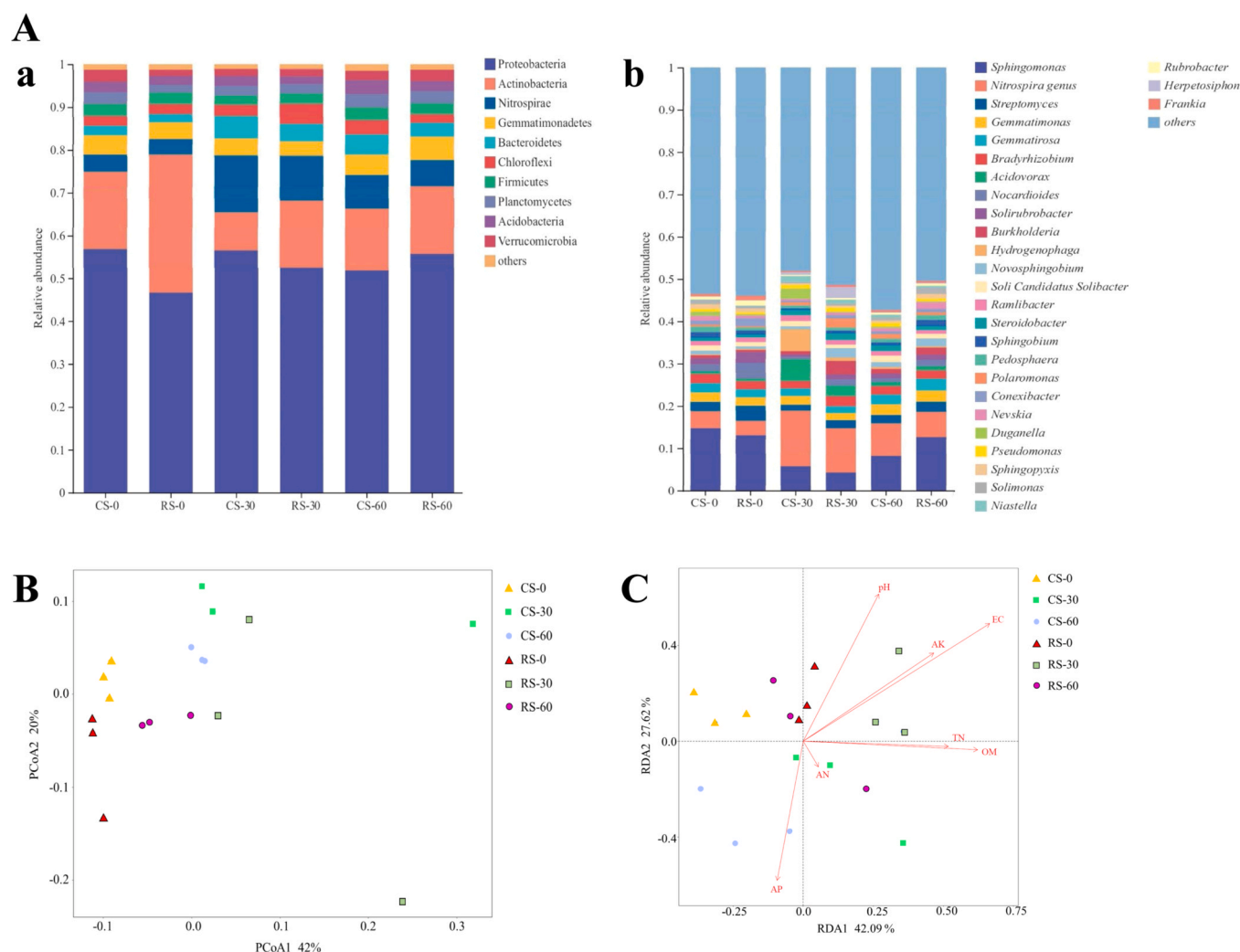


Fig. 4. Soil bacterial abundance, principal coordinate analysis, and redundancy analysis. (A) Relative abundance of bacteria in continuous and rotational soils at the phylum level (a) and genus level (b). (B) Principal Coordinates Analysis (PCoA) plot of bacterial communities based on 18 soil samples, showing the responses of the bacterial composition in continuous crop (CS) and rotational crop (RS). (C) Redundancy analysis (RDA) showing correlations between soil bacterial community and soil physicochemical properties. CS-0 and RS-0 indicate relative abundances of soil bacteria in the experiment field. CS-30 and RS-30 indicate a relative abundance of soil bacteria at 30 d in the pot experiment, and CS-60 and RS-60 indicate a relative abundance of soil bacteria at 60 d in the pot experiment.

3.3. Soil bacterial abundances

Using Diamond for bacterial annotation, a total of 32 Phyla were detected, among which *Proteobacteria*, *Actinobacteria*, *Nitrospirae*, *Gemmatimonadetes*, *Bacteroidetes*, *Chloroflexi*, *Firmicute*, *Planctomycetes*, *Acidobacteria*, *Verrucomicrobia* were the dominant phyla. Their relative abundances account for more than 98% (Fig. 4A).

In the experiment field, the *Actinobacteria* in the soil of the rotation system was significantly higher than that of the continuous cropping system, and the relative abundance of *Proteobacteria* was significantly lower than that of the continuous cropping system (Fig. 4A). Overall, the bacterial microbiome in the experimental field differed significantly.

In the potted sugar beet experiment, the soil microbial community structure was measured 30 d after planting. The result showed that the relative abundances of *Actinobacteria* and *Chloroflexi* in the rotation system were significantly higher than those of the continuous cropping system. At 60 d, the relative abundances of *Proteobacteria* and *Actinobacteria* in rotation soil were greater than those in continuous cropping soil. While *Nitrospirae*, *Gemmatimonadetes*, and *Bacteroidetes* were lower than in the soil of the continuous cropping (Fig. 4A).

At the genus level, the *Streptomyces*, *Conexibacter*, *Solirubrobacter*, and *Nocardia* in the rotation system were higher than in the continuous

cropping system, while the relative abundances of *Sphingomonas* and *Nitrocellulose* in the soil of rotation system were lower, and other bacterial flora were also significantly different (Fig. 4A). After 30 d of pot planting, *Streptomyces*, *Burkholderia*, and *Bradyrhizobia* have higher relative abundances in crop rotation, but *Sphingomonas*, *Nitrospira*, and *Acidovorax* were lower. After 60 d, *Sphingomonas* and *Streptomyces* showed an increase of relative abundances in crop rotation, while *Nitrospira* was decreased. At both the phylum and genus levels, the effect of crop rotation on the structure of bacterial communities was marked.

3.4. Soil bacterial community composition and structure

Assessment of soil microbial structure are reflected by Alpha and Beta diversity indexes. Bacterial Alpha diversity indexes were measured in the soil samples from the experimental field and the pot experiment at 30 d and 60 d (Supplementary table 1). The results showed that soil samples from the experimental field and the 60 d potting experiment showed a higher Shannon index in the potted soil of the rotational system than in the continuous crop system, while the Simpson index was on the contrary (Supplementary table 1). This indicates that crop rotation can increase the bacterial diversity in the soil.

PCoA analyses demonstrated Beta diversity, where samples from the

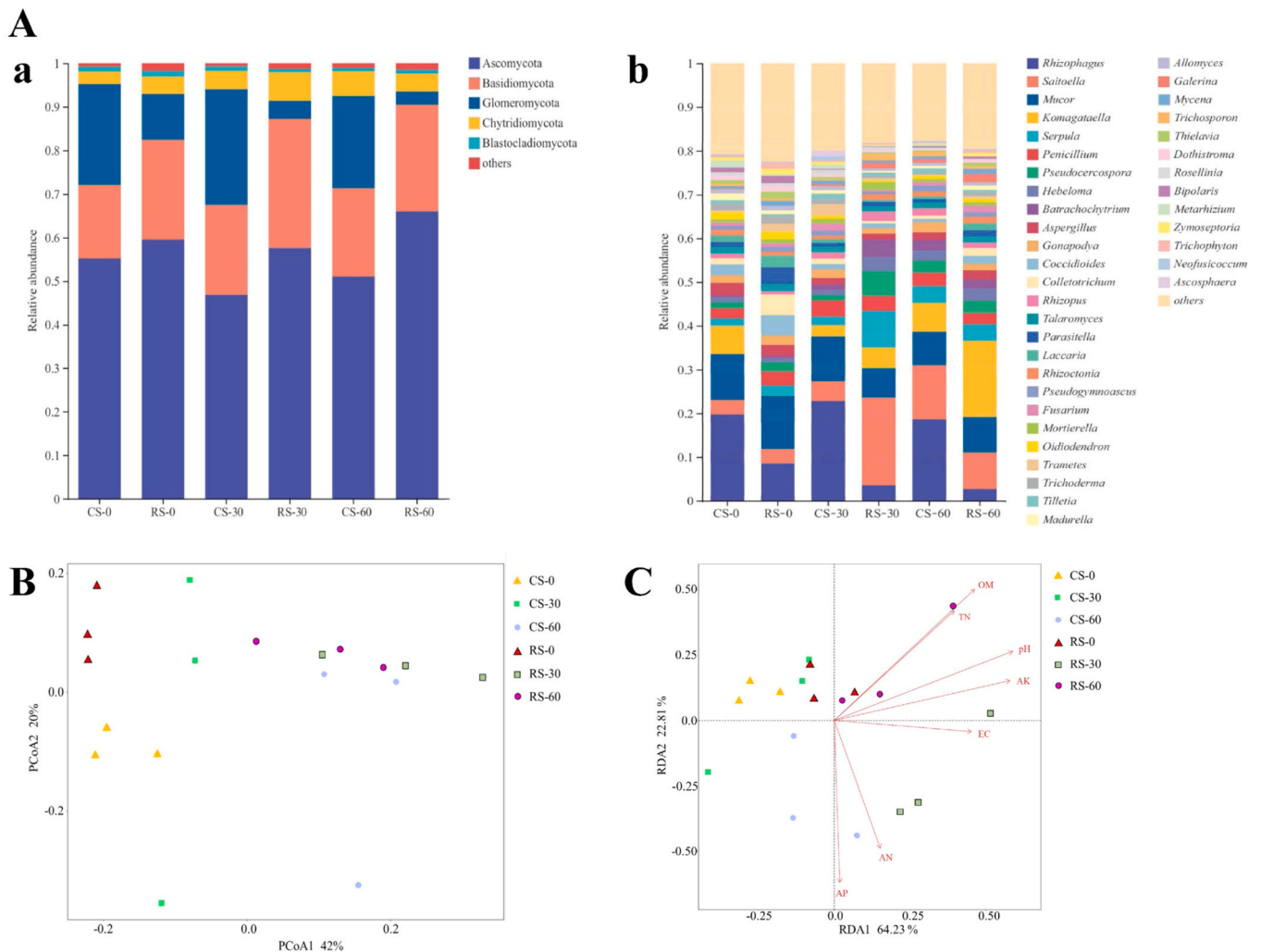


Fig. 5. Soil fungal abundance, principal coordinate analysis, and redundancy analysis. (A) Relative abundance of fungi in continuous and rotational soils at the phylum level (a) and genus level (b). (B) Principal Coordinates Analysis (PCoA) plot of fungi communities based on 18 soil samples, which showed the response of the fungi composition in continuous crop (CS) and rotational crop (RS). (C) Redundancy analysis (RDA), which shows the correlation between soil fungal communities and soil physicochemical properties. CS-0 and RS-0 indicate soil fungi composition in the experiment field. CS-30 and RS-30 indicate soil fungi composition at 30 d in the pot experiment, and CS-60 and RS-60 indicate soil fungi composition at 60 d in the pot experiment.

same treatment clustered together and samples from all the six treatments were distinguishable. The first and second principal coordinates together explained 62% of the variation in community structure ($R=0.4947$, $p=0.001$) (Fig. 4B).

3.5. Relationships between soil bacterial communities and soil physicochemical properties

The correlation between soil bacterial communities and soil physicochemical properties was determined by Pearson correlation analysis (Supplementary table 2). RDA was employed to further determine the relationship between bacterial communities and soil physicochemical properties ($p < 0.05$). RDA1 and RDA2 together explained 69.71% of the compositional changes. As shown in Fig. 4C, we analyzed soil physicochemical factors that may affect soil microbial communities, including soil pH, EC value, TN, AN, AP, AK, and SOM. The RDA showed AN to be a crucial environmental factor that was significantly correlated with the soil bacterial microbiome of the samples.

3.6. Soil fungal abundances

Annotation results of fungi in the soil revealed 32 Phyla (Fig. 5A).

Among them, the dominant fungi were *Ascomycota*, *Glomeromycota*, *Basidiomycota*, *Chytridiomycota*, and *Blastomycota*, representing over 99% of the overall relative abundance.

In the experiment field, *Ascomycota* and *Basidiomycota* were more abundant in the crop rotation soil. However, the relative abundance of *Glomeromycota* in the rotation soil was lower than that in the cropping soil. The trend in relative abundance was the same in the pot experiment.

The genus-level annotation results revealed that in the experiment field, the relative abundances of *Rhizophagus*, *Saitoella* and *Mucor* were the dominant species in both systems. In the rotation system, the relative abundances of *Penicillium*, *Pseudocercospora*, and *Trametes* were higher (Fig. 5A). After 30 d of pot sugar beet planting, compared to continuous cropping, the relative abundances of *Saitoella* and *Serpula* in the rotation soil system were higher, and *Rhizophagus* and *Mucor* were lower. Higher levels of *Mucor* and *Komagataella* were found in the rotational soil after 60 d of potting. At both levels, the effect of crop rotation on the structure of soil fungi communities was highly significant (Fig. 5A).

3.7. Soil fungal community composition and structure

The composition and structure of the soil fungal community were

evaluated by Alpha and Beta diversity indexes (Supplementary table 3). After the rotation of TKS, the Shannon index in the rotational soil was higher than the continuous cropping system, while the Simpson index was the opposite, indicating that the fungal diversity in the rotational soil was significantly higher than the continuous cropping system. The TKS rotation improved the fungal diversity in the soil.

PCoA analyses demonstrated Beta diversity, where samples from the same treatment clustered together and samples from all six treatments were distinguishable (Fig. 5B). The first and second principal coordinates together explained 62% of the variation in community structure ($R=0.5416$, $p=0.001$).

3.8. Correlation between soil fungal community and soil physicochemical properties

The correlation between soil bacterial communities and soil physicochemical properties was determined by Pearson correlation analysis (Supplementary table 4). RDA was used to further determine the relationship between fungal communities and soil physicochemical properties ($p < 0.05$). RDA1 and RDA2 together explained 87.04% of the compositional changes. We analyzed soil physicochemical factors that may affect soil microbial communities. The results of RDA showed that the soil pH, EC, TN, AP, AK, and SOM were crucial physicochemical factors that significantly correlated with the soil fungal community structures (Fig. 5C).

4. Discussion

4.1. Effect of TKS crop rotation on soil microbial community structure

The soil microorganisms may form complex symbiotic interactions with plants, and they have a critical impact on plant growth and development (Korenblum and Aharoni, 2019; Xu et al., 2018). An increasing body of research suggests that plants alter their rhizosphere composition, recruiting specific soil microorganisms and forming complex mutualistic relationships that ultimately improve plant fitness (Zhalnina et al., 2018). This work here focused on the effect of TKS rotation on soil microbiome. The results showed that TKS rotation reshaped the soil bacterial (Fig. 4) and fungal (Fig. 5) microbiome, and improved rhizosphere environment. Li et al. used rotations of potato with oats and potato with maize to show that the rotations significantly improved the microbiological composition of the soil, ultimately decreasing the abundance of harmful pathogenic bacteria (Li et al., 2023). This is consistent with the results of our study.

The Illumina Miseq sequencing results showed that the relative abundances of the major bacterial (Fig. 4) and fungal (Fig. 5) communities in the soil changed significantly after TKS crop rotation. In terms of bacterial phylum community structure, *Actinobacteria* and *Proteobacteria* were the two main groups in the bacterial community (Fig. 4A). Our results showed that the relative abundance of *Actinobacteria* in the soil was significantly increased from 18% to 32%, and the abundance of *Proteobacteria* was significantly decreased from 57% to 47% after the crop rotation. *Actinobacteria* are beneficial bacteria, which contribute to organic matter decomposition, nitrogen fixation, and phosphate dissolution, thus increasing soil nutrient content (AbdElgawad et al., 2020). In addition, it has been shown that manure application increases soil nutrient content and also affects soil microbial community structure, resulting in an increase in the abundance of *Actinobacteria* and a decrease in the abundance of *Proteobacteria* (Tang et al., 2023). These results are in line with our studies with sugar beet.

Streptomyces are the bacteria that cause the most concern because they are involved in complex substance metabolism (Olanrewaju et al., 2020). Worsley et al. have shown that some soil-dwelling *Streptomyces* produce a variety of anti-pathogenic and anti-parasitic metabolites with growth-promoting effects on *Arabidopsis*, while other *Streptomyces* can help crops resist pathogens (Worsley et al., 2020). *Solirubrobacter* is a

potentially beneficial soil bacterium (He et al., 2021; Franke-Whittle et al., 2015). It was found to be a marker flora and a potential rhizosphere growth-promoting bacterium in the soil of healthy black pepper orchards (Obieze et al., 2023). In our study, the relative abundances of *Streptomyces* and *Solirubrobacter* increased significantly after crop rotation (Fig. 4A), both more than doubled, and the increase of soil beneficial bacteria could better protect crop growth. Also, we found that the abundance of the genus *Trametes* increased nearly 4-fold in the soil after crop rotation (Fig. 5A). The genus *Trametes* contains a large number of lignin-degrading fungi, which have an essential role in the natural carbon cycle and in protecting the environment (Chen et al., 2023). To summarize, TKS crop rotation can significantly improve the structure of the soil microbiome, making the rhizosphere soil environment optimal and protect crop growth. Therefore, our first hypothesis was supported.

4.2. Effect of TKS crop rotation on soil physicochemical properties and enzyme activities

Soil microorganisms strongly influence soil physicochemical properties and soil enzyme activities (Zou et al., 2023), which are the most significant factors in determining soil fertility (Neupane et al., 2021; Guo et al., 2022). Our study showed that compared with continuous cropping, the relative abundances of *Rhizobia*, *Pseudomonas*, *Burkholderia*, and *Trichosporon* increased after crop rotation (Fig. 4A). *Rhizobia* can be endophytic with legumes, thereby fixing free atmospheric nitrogen and providing nitrogen nutrients for plant growth (Ju et al., 2019). *Pseudomonas*, *Burkholderia*, and *Trichosporon* convert soil nitrate into dinitrogen, increasing soil nitrogen content for plant utilization (Hartmann and Six, 2023). Our results were consistent with previous studies that the abundances of all these genera in the soil increased (Fig. 4A), which in turn fixed the free nitrogen in the soil and increased the available nitrogen content in the soil (Fig. 1A). For example, the AN content in the experimental field significantly increased by 8.8% after the crop rotation, and then improved soil fertility, and promoted crop development. Hu et al. verified that with the process of vegetation restoration, *Ascomycota* and *Basidiomycota* gradually became dominant bacteria in the soil, and their increases closely correlated with soil SOC (Hu et al., 2022). Here soil organic matter content was significantly increased by 21% after TKS crop rotation (Fig. 1A), and the *Ascomycota* and *Basidiomycota* in crop rotation soil were significantly higher (Fig. 5A). This finding suggests that the increase in SOM was due to the increase in the relative abundance of *Ascomycota* and *Basidiomycota* after crop rotation. *Aspergillus*, *Pseudomonas* and *Penicillium* can increase soil potassium content by releasing organic acids to dissolve potassium from minerals (Soumare et al., 2023). Here we found that these microbes significantly increased (Fig. 4A), and a concomitant increase of 51.8% in the AP content in the experimental field. It is reasonable to expect that the elevated soil potassium content was due to the elevated abundance of these microbes (Fig. 1A). Phosphorus is a vital nutrient in agroecosystems. *Rhizophagus* can assist plants in absorbing phosphorus from the soil (Kobae, 2019). In this study, a significant decrease of 9.3% in soil AP content was found after crop rotation (Fig. 1A), probably due to the decrease in the abundance of *Rhizophagus* bacteria in the soil (Fig. 5A). In general, TKS rotation significantly increased soil AN, AK, and SOM contents by increasing the relative abundances of beneficial bacteria in the soil, which in turn improved soil physicochemical properties (Fig. 1A).

Soil enzymes are derived from various types of plants, animals, and microorganisms in the soil. They participate in the decomposition and synthesis of humus, breakdown of animal and microbial residues, and the synthesis and decomposition of inorganic and organic chemicals in the soil (Sun et al., 2020). Importantly, they act as catalysts for various biochemical reactions during various types of soil transformations and as important indicators of soil health and ecosystem sustainability (Ananbeh et al., 2019; de de Andrade Barbosa et al., 2019). Guo et al. found that urease and dehydrogenase activities could be significantly

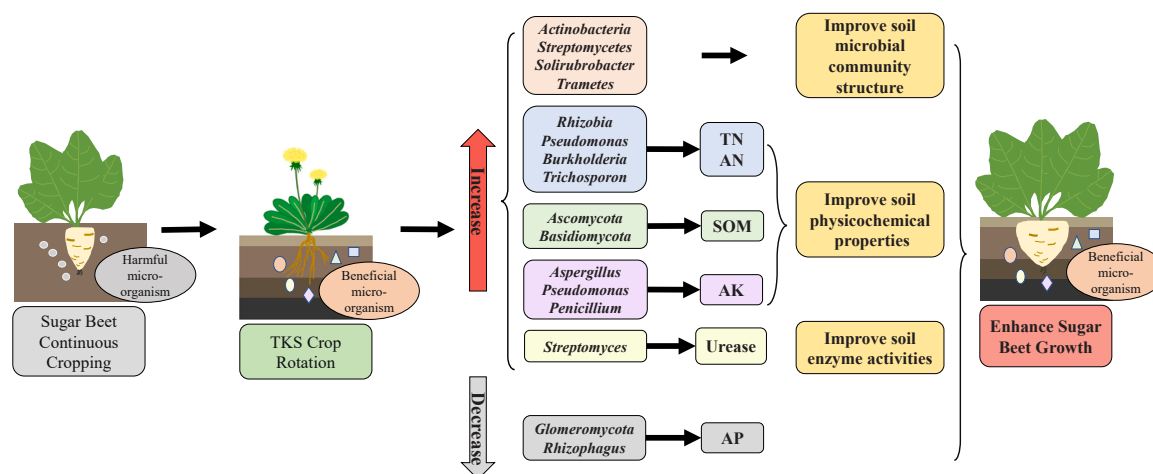


Fig. 6. Conceptual model for the TKS Crop rotation promoted the growth of sugar beet by improving soil microbial community structure and soil physicochemical properties. Total nitrogen, TN; available nitrogen AN; soil organic matter, SOM; available potassium, AK; and available phosphorus, AP.

increased by inoculation with *Streptomyces pactum*, thereby enhancing the phytoremediation of PTE-contaminated soils (Guo et al., 2021). Our results demonstrated that TKS rotation significantly increased urease content by 42.5% in the experimental field (Fig. 1B), which could help promote soil N cycling and improve soil nitrogen content. This is also consistent with the increase of soil available nitrogen content in our experiment. Overall, the results supported our second hypothesis.

4.3. Crop rotation promotes sugar beet growth by improving the soil microbiome and physicochemical properties

Considerable research has been done on soil microbial composition and crop growth. For example, Li et al. confirmed that crop rotation-fallow treatments can improve bacterial composition in rice paddies, thereby significantly improving soil quality (Li et al., 2022). In this study, the PCoA analysis confirmed that the soil microbiome could be enhanced after crop rotation. We observed that the soil bacterial microbiome in 30 d crop rotation was positively correlated with all the soil physicochemical properties (Fig. 4C). There was a significant positive correlation between soil fungal microbiome and soil physicochemical properties at 30 d and 60 d of crop rotation, especially soil TN, AP, AK, and SOM (Fig. 5C).

In this study, TKS rotation significantly increased soil AN, AK, SOM, and soil urease activities in the experimental field (Fig. 1). In the complementary potting experiments, sugar beets grown in crop rotation soil were superior to those grown in continuous soil in terms of morphological and growth indexes (Fig. 2). Therefore, sugar beet and TKS rotation can promote sugar beet growth (Fig. 6).

5. Conclusions

Sugar beet and TKS are two important industrial crops, our results showed that TKS rotations increases sugar beet biomass by creating optimal plant-soil interaction systems. It optimized the structure of soil microbial communities, and enhanced soil quality and fertility, ultimately providing a boost to sugar beet growth. Sugar beet - TKS rotation is expected to help overcome soil continuous cropping obstacles. In the future, we will extend the study to further investigate the allelopathic effects of TKS specialized metabolites on sugar beet, with the ultimate goal to develop more economical and sustainable crop rotation systems.

CRediT authorship contribution statement

Yu Song: Writing – review & editing. **Sixue Chen:** Writing – review & editing. **Haiying Li:** Writing – review & editing. **Chunquan Ma:**

Writing – review & editing. **Chuanqi Guo:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Chao Yang:** Data curation, Formal analysis, Investigation. **Junsheng Fu:** Data curation, Formal analysis, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.indcrop.2024.118331](https://doi.org/10.1016/j.indcrop.2024.118331).

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