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# SUSTAINABLE MANAGEMENT OF SURFACE SUBSTRATE LAYER BASED ON SOIL MICROBIAL DIVERSITY DURING WHEAT GROWTH SEASON

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## Abstract

High-throughput sequencing technology was used to systematically analyze the composition, diversity, and dynamic changes of soil microbial communities during the wheat growing season in Lintong, Shaanxi Province. The abundance of bacterial communities significantly increases after the complete planting cycle of wheat, while the fungal community structure was relatively stable. At the phylum level, Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria constitute the dominant bacterial communities. The fungal community was Ascomycota and Zygomycota. The growth process of wheat significantly changes the structural composition of bacterial communities, enhances enzyme activity and biological regulatory functions of bacterial communities, while inhibiting cellular processes and environmental information processing functions. This study revealed the succession pattern of soil microbial communities during the wheat growing season, identified key microbial groups that maintain soil ecosystem functions, and provided an important theoretical basis and practical guidance for the sustainable management of the surface substrate layer in the study area.

## Introduction

Wheat is the most widely distributed and planted grain crop in Lintong, Shaanxi Province. Soil microbial communities serve as biological indicators of soil quality and ecosystem changes, and their composition and functional characteristics are important indicators for evaluating the ecological functions of surface substrates (Yu *et al.* 2008). Although the current cultivable soil microorganisms account for less than 10% of the total species, breakthroughs in molecular biology technologies such as metagenomics and high-throughput sequencing in recent years have greatly expanded the understanding of soil microbial diversity and its ecological functions (Gao and Guo 2022).

Based on the above background, this study selects wheat rhizosphere soil and non-rhizosphere soil in the Lintong area, Shaanxi Province as research objects, and uses modern molecular ecology techniques to systematically analyze the composition characteristics, diversity distribution patterns, and dynamic changes of soil microbial communities. Exploring the coupling relationship between the ecological benefits of surface substrate and surface cover layer by combining the composition, diversity, and dynamic changes of soil microbial communities during the wheat growing season. The research aims to reveal the key microbial groups that maintain soil ecosystem functions, providing scientific basis and theoretical support for the sustainable management of the surface substrate layer in the study area.

## Materials and Methods

Five experimental plots were selected in the main wheat-producing area of Lintong region for soil sample collection. Three samples of wheat rhizosphere soil and non-rhizosphere soil were collected from each plot, for a total of 60 soil samples.

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The sample groups are G1 which represents rhizosphere soil and F1 non-rhizosphere soil during the emergence period. T1 represents rhizosphere and non-rhizosphere soil during the emergence period. G2 represents mature rhizosphere soil and F2 mature non-rhizosphere soil, T2 represents rhizosphere and non-rhizosphere soil during the mature period.

Sterilized wooden shovel was used to dig up the wheat plants and roots, shake off the soil sample attached to the wheat roots, and put it in a sterile sealed bag. Non-rhizosphere soil samples was also collected accordingly. The collected soil sample should be weighed 2-5 g and immediately placed in a car refrigerator to be sent to the laboratory for storage at -80°C. Based on the Illumina sequencing platform, 16S V3-4 (Youssef *et al.* 2009, Caporaso *et al*. 2011) and ITS1 (Caporaso *et al.* 2012, Degnan and Ochman 2012) information collection was conducted on 60 soil samples, which was completed by Shaanxi Airui Biotechnology Co. Ltd.

The Alpha diversity index of the samples, including Shannon, Chao l, ACE indices, and Weighted Unifrac distance in β diversity, were calculated using the mothur 1.30 software. PCA and Tax4Fun feature annotation clustering heatmap analysis were performed using the ggplot2 software package in R language (version 3.3.1). Community composition analysis was conducted using R language (version 3.3.1), vegan software package, and Python 2.7 software to compare the differences and similarities in microbial community structure and species diversity among each group of samples.

## Results and Discussion

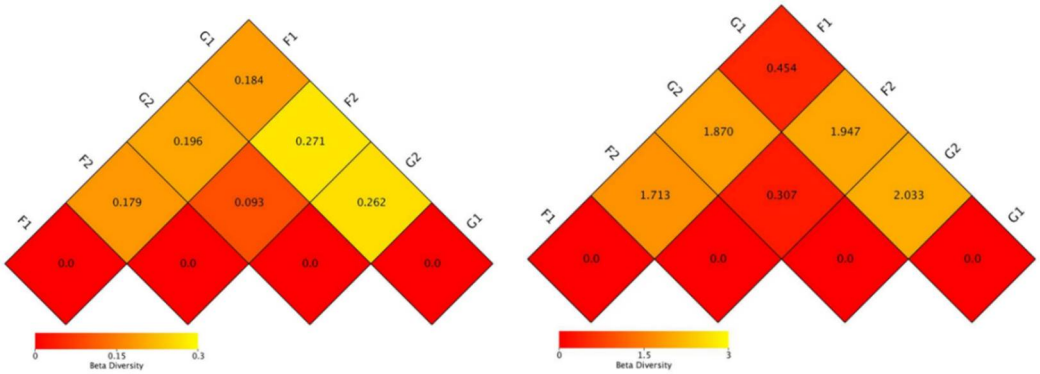
In order to determine and analyze the richness and evenness of soil microorganisms in a specific region or ecosystem, OTUs values of different soil samples were used for calculation. Multiple alpha diversity indices were obtained by analyzing the alpha diversity of different samples at the 97% consistency threshold (Table 1).

The above results indicate that the number of bacteria in all four groups of samples is much higher than that of fungi, which is consistent with the fact that bacteria are the main microorganisms in soil. The number of OTUs of bacteria and fungi in the mature stage is higher than that in the emergence stage, and the Shannon index, Chao1 index, and ACE index of bacteria in the mature stage are significantly higher than those in the emergence stage, indicating that the abundance of microbial communities increases to some extent after a complete wheat planting process.

**Table 1. Bacterial and fungal alpha diversity index.**

| Classification | Group | Observed species | Shannon | Chao1 | ACE | Coverage |
|---|---|---|---|---|---|---|
| Bacteria | G1 | 2046c | 8.863c | 2435.846b | 2489.843b | 0.981a |
| | F1 | 2336b | 9.231b | 2811.195ab | 2861.603b | 0.977ab |
| | G2 | 2635a | 9.552a | 3130.600a | 3248.582a | 0.974b |
| | F2 | 2533ab | 9.457a | 3114.974a | 3185.073a | 0.973b |
| Fungi | G1 | 555a | 5.185a | 602.617a | 610.091a | 0.998a |
| | F1 | 494b | 4.546b | 543.950b | 550.967b | 0.998a |
| | G2 | 584a | 5.212a | 643.512a | 650.767a | 0.998a |
| | F2 | 573a | 5.424a | 624.742a | 633.695a | 0.998a |

*Value Followed by the same letter are not significantly different.

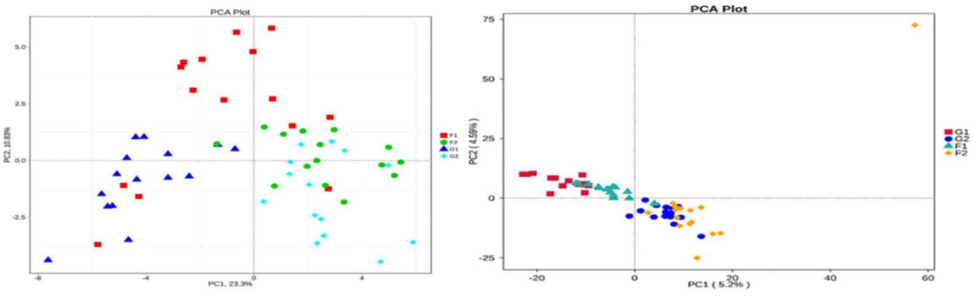



A B

Fig. 1. Weighted unifrac distance of bacterial and fungal community. A: Bacteria, B: Fungi.

As shown in Fig. 1A, for bacterial communities, the differences between G2, F2 and G1, F1 are relatively small compared to F1, F2 and G1, G2, indicating that the impact of planting growth on bacterial communities is greater than the differences between rhizosphere and non rhizosphere soils. The differences between G2, F2 and G1, F1 are significantly greater than those between F1, F2 and G1, G2, indicating that the impact of planting growth on fungal communities is not as significant as the differences between rhizosphere and non rhizosphere soil (Fig.1B). The differences in fungal communities are reflected in the rhizosphere and the rhizosphere soil itself, and the process of planting and growth does not bring about excessive changes in fungal communities.

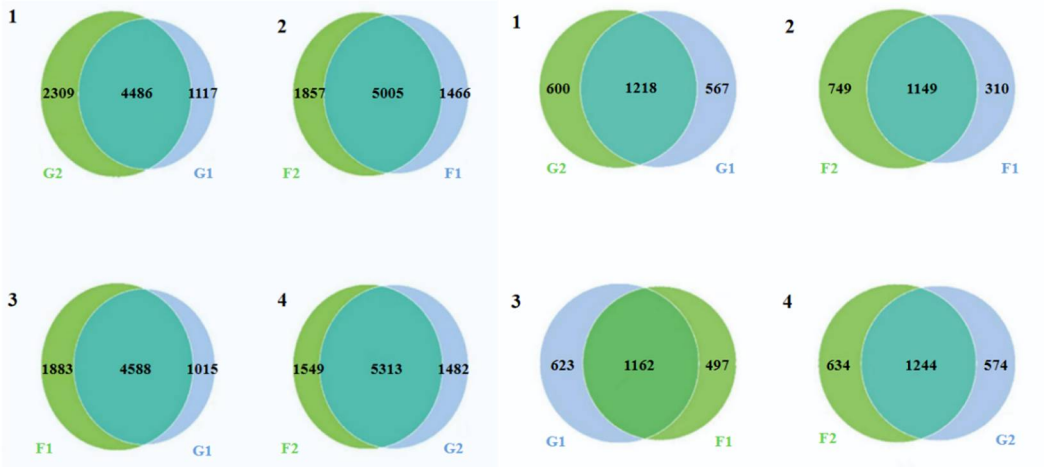
By applying PCA, it is possible to extract the two coordinate axes that best reflect the differences between samples, thereby reflecting the differences in multidimensional data on a two-dimensional coordinate graph and revealing simple patterns in complex data backgrounds. If the community composition of the samples is more similar, their distances in the PCA graph will be closer.




A B

Fig. 2. Principal component analysis of bacterial and fungal communities. A: Bacteria, B: Fungi

As shown in Fig. 2A, there is slight cross overlap among the four groups of samples, but their respective clusters can be delineated and have considerable distance, indicating that each group of the bacterial community has significant independent characteristics. As shown in Fig. 2B, for the fungal community, the distribution of the four groups of samples is relatively concentrated, with small inter-group distances and large overlapping areas of their respective clusters, indicating that the differences between the fungal communities are relatively small.



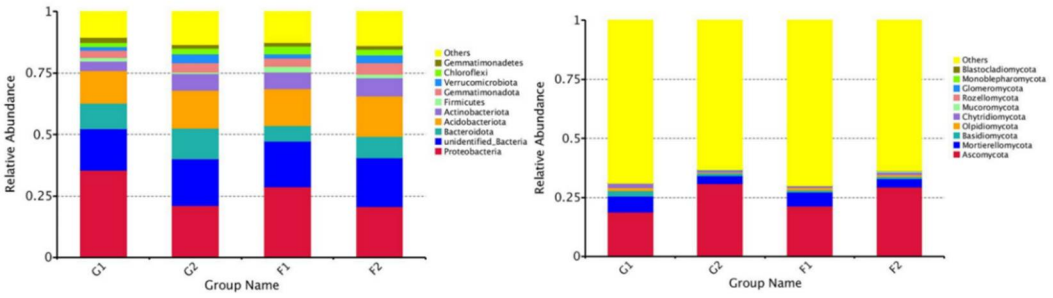

A B

Fig. 3. Bacterial and fungal venn diagram. A: Bacteria, B: Fungi

The bacterial Venn diagram analysis based on OTU comparison showed that there were 4486 common bacterial species in the rhizosphere soil, accounting for 56.70% of the total, 1117 unique bacterial species in the seedling stage, and 2309 unique bacterial species in the mature stage, compared between the samples at the seedling and mature stages (Fig. 3A-1); There are a total of 5005 bacterial species in non-rhizosphere soil, accounting for 60.10% of the total. There are 1466 bacterial species unique to the seedling stage and 1857 bacterial species unique to the mature stage (Fig. 3A-2). The number of bacterial species in non-rhizosphere soil during the mature stage is higher than that during the seedling stage, and the number of bacterial species in rhizosphere soil during the mature stage is significantly higher than that during the seedling stage. After a complete wheat growth cycle, the number of bacterial species in both rhizosphere and non-rhizosphere soils showed an increasing trend. Comparing rhizosphere and non-rhizosphere soil samples, there were 4588 common bacterial species during the seedling stage, accounting for 61.53% of the total. There were 1015 unique bacterial species in the rhizosphere soil and 1883 unique bacterial species in the non-rhizosphere soil (Fig. 3A-3). There are 5313 common bacterial species in the mature stage, accounting for 63.67% of the total. There are 1482 unique bacterial species in the rhizosphere soil and 1549 unique bacterial species in the non-rhizosphere soil (Fig. 3A-4). At different growth stages of wheat, the proportion of common bacterial species in both rhizosphere and non-rhizosphere soils exceeds 60%.

The number of microbial communities in the fungal Venn diagram is significantly lower than that in the bacterial Venn diagram, indicating that bacteria are the main microorganisms in soil. The analysis of fungal Venn diagram based on OTU comparison showed that there were 1218 common fungal species in the rhizosphere soil, accounting for 51.07% of the total, 567 unique fungal species in the seedling stage, and 600 unique fungal species in the mature stage, compared between the samples at the seedling and mature stages (Fig. 3B-1). There are a total of 1149 fungal species in non-rhizosphere soil, accounting for 52.04% of the total. There are 310 fungal species unique to the seedling stage and 749 fungal species unique to the mature stage (Fig. 3B-2). Comparing rhizosphere soil and non-rhizosphere soil samples, there were 1162 common fungal species at the emergence stage, accounting for 50.92% of the total. There were 623 unique fungal species in the rhizosphere soil and 497 unique fungal species in the non-rhizosphere soil (Fig. 3B-

3). At maturity, there are 1244 common fungal species, accounting for 50.73% of the total. There are 574 unique fungal species in the rhizosphere soil and 634 unique fungal species in the non-rhizosphere soil (Fig. 3B-4). Overall, the proportion of shared fungi in each group of Venn diagrams is lower than the proportion of shared bacteria.




A B

Fig. 4. Relative abundance of bacterial and fungal species at the phylum level. A: Bacteria, B: Fungi

Analysis of Fig. 4A shows that the dominant bacterial communities in the four groups of samples are Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria. The content of these four dominant bacteria in rhizosphere soil and non-rhizosphere soil at different time points is around 55%. Among them, the relative abundance of Proteobacteria during the emergence stage was significantly higher than that during the mature stage, with a relative abundance of 35.52% in the rhizosphere soil and 28.65% in the non-rhizosphere soil during the emergence stage. In the mature stage, the relative abundance of Proteobacteria in rhizosphere soil decreased to 21.09%, while in non-rhizosphere soil it decreased to 20.64%. The relative abundance of Bacteroidetes is significantly higher in rhizosphere soil than in non-rhizosphere soil, with a relative abundance of 10.34% in rhizosphere soil during the seedling stage and 12.34% during the mature stage. The relative abundance in non-rhizosphere soil at two time points was 6.4% and 8.65%, respectively. In the rhizosphere soil during the seedling stage, the relative abundance of Actinobacteria was only 3.69%, but in the rhizosphere soil during the mature stage, its relative abundance was 6.77%. In the non-rhizosphere soil at two time points, its relative abundance was 6.56 and 7.39%, respectively. The relative abundance of Acidobacteria in the four samples showed little difference, with relative abundances of 13.35% and 15.41% in rhizosphere and non-rhizosphere soils during seedling emergence, respectively and 15.08% and 16.54% in rhizosphere and non-rhizosphere soils during maturity, reapectively.

Fig. 4B intuitively presents that the dominant fungal phyla in this area are Ascomycota and Mortierellomycota. The relative abundance of Ascomycota shows an increasing trend over time, while the relative abundance of Mortierellomycota shows a decreasing trend over time. During the emergence period, the relative abundance of Ascomycota was 18.74% in rhizosphere soil and 21.27% in non-rhizosphere soil. The relative abundance of Mortierellomycota was 6.72% in rhizosphere soil and 6.06% in non-rhizosphere soil. In the mature stage, the relative abundance of Ascomycota was 30.85% in rhizosphere soil and 29.32% in non-rhizosphere soil. The relative abundance of Mortierellomycota was 3.31% in rhizosphere soil and 3.73% in non-rhizosphere soil.

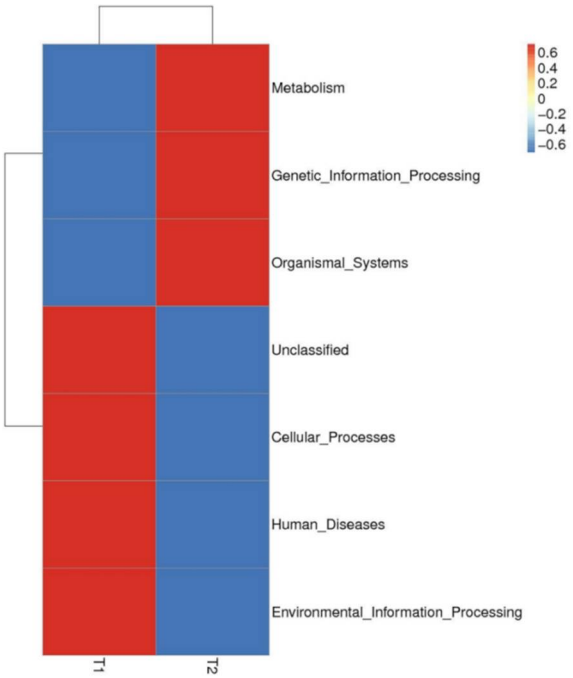



Fig. 5. Cluster heatmap of Tax4Fun functional annotation of wheat rhizosphere soil bacteria.

Using the Tax4Fun method to predict the functional annotation of bacterial communities in wheat rhizosphere soil that are significantly affected by crop growth. Based on the database annotation results, select the top 7 functional information with the highest abundance ranking at each annotation level for each sample or group, and generate a functional annotation clustering heatmap (Fig. 5) to visually view the functions with relatively high abundance and their proportions at different annotation levels for each sample.

This study revealed the dynamic changes in soil microbial communities during wheat cultivation through alpha diversity analysis. The results showed that the number of bacteria in all four samples was significantly higher than that of fungi, which is consistent with previous reports that soil microorganisms are mainly composed of bacteria (Yu 2009). It is worth noting that the number of OTUs of bacteria and fungi in the mature stage is higher than that in the emergence stage, and the Shannon index, Chao1 index, and ACE index of bacteria in the mature stage are significantly higher than those in the emergence stage. These findings indicate that with the growth of wheat, the abundance of soil microbial communities shows a significant increasing trend, which may be closely related to ecological processes such as increased root exudates and soil organic matter accumulation (Van Rensburg *et al.* 2024).

The PCA results confirm the above findings from the perspective of spatial distribution. Although there is slight cross-overlap among the four groups of samples in the bacterial community, they can still form clear independent clusters with large inter-group distances, indicating significant inter-group specificity of the bacterial community. In contrast, the distribution of the four groups of samples in the fungal community is relatively concentrated, with small inter-group distances and large cluster overlap areas, further confirming the relative stability of the fungal community structure. This result is consistent with the research conclusion of Sun *et al*. (2023) that soil fungal communities have strong environmental adaptability.

From an ecological perspective, the stability of soil microbial communities is closely related to the stability of their growth environment (Wang *et al.* 2014). In this study, at the phylum level, Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria formed the dominant bacterial communities. The fungal community is dominated by Ascomycota and Zygomycota, with Ascomycota showing an increasing relative abundance over time and Zygomycota showing a decreasing trend. This dynamic change may reflect the response of soil microbial communities to changes in plant demand, soil environment, and agricultural management measures during the wheat growth cycle (Perez 2015).

The characteristics of soil microorganisms are an important indicator for evaluating the sustainable utilization potential of the surface substrate layer. The impact of the wheat planting and growth process on bacterial community is greater than the difference between rhizosphere soil and non-rhizosphere soil, and the abundance of bacterial community increases to some extent after a complete wheat planting process. The differences in fungal communities are mainly reflected between rhizosphere soil and non-rhizosphere soil, while the impact of planting and growth processes on their community structure is relatively small. Each group of bacterial communities has significant independent characteristics, while the differences between groups of fungal communities are relatively small. At the phylum level, Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria constitute the dominant bacterial communities, while the dominant fungal phyla are Ascomycota and Zygomycota. The growth process of wheat significantly changes the functional characteristics of bacterial communities. Specifically, the enzyme activity and biological regulatory functions of bacterial communities are significantly enhanced, while cellular processes and environmental information processing functions are inhibited. In contrast, the functional characteristics of fungal communities remain relatively stable throughout the entire growth cycle.

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