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Research article

Succession of tissue microbial community during oat developmental

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ABSTRACT

Investigating oat tissue microflora during its different developmental stages is necessary for understanding its growth and anti-disease mechanism. In this study, 16S rDNA and ITS (Internally Transcribed Spacer) high-throughput sequencing technology were used to explore the microflora diversity of oat tissue. Twenty-seven samples of leaves, stems, and roots from three developmental stages, namely the seedling stage (SS), jointing stage (JS), and maturity stage (MS), underwent sequencing analysis. The analysis showed that 6480 operational taxonomic units (OTUs) were identified in the examined samples, of which 1698 were fungal and 4782 were bacterial. Furthermore, 126 OTUs were shared by fungi, mainly *Ascomycota*, *Basidiomycota*, and *Mucoromycota* at the phylum level, and 39 OTUs were shared by bacteria, mainly *Actinobacteriota* and *Proteobacteria* at the phylum level. The microbial diversity of oat tissue in the three developmental stages showed differences, and the α-diversity of the bacteria and β-diversity of the bacteria and fungi in the roots were higher than those of the stems and leaves. Among the bacteria species, *Thiiopseudomonas, Rikenellaceae RC9 gut group*, and *Brevibacterium* were predominant in the leaves, MND1 was predominant in the roots, and *Lactobacillus* was predominant in the stems. Moreover, *Brevibacterium* maintained a stable state at all growth stages. In the fungal species, *Phomatospora* was dominant in the leaves, *Kondoa* was dominant in the roots, and *Pyrenophora* was dominant in the stems. All species with a high abundance were related to the growth process

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of oats and antagonistic bacteria. Furthermore, connection modules were denser in bacterial than in fungal populations. The samples were treated with superoxide dismutase and peroxidase. There were 42 strains associated with SOD (Superoxide dismutase), 60 strains associated with POD (Peroxidase), and 38 strains in total, which much higher than fungi. The network analysis showed that bacteria might have more dense connection modules than fungi, The number of bacterial connections to enzymes were much higher than that of fungi. Furthermore, these results provide a basis for further mechanistic research.

1. Introduction

Endophytes are defined as organisms, often fungi or bacteria, that are isolated from surface-sterilized plants and do not cause any obvious harm to their host plants. An epiphyte is a microorganism that can be separated from the surface of a leaf, stem, or root without interfering with plant growth [1]. Together, endophytes and epiphytes constitute a unique ecological community. As a plant grows, the relationship between bacteria and fungi becomes increasingly complex, forming a symbiotic phenomenon. These bacteria and fungi are associated with a diverse group of plants, and their associations are critical for nutrient acquisition and distribution, helping to regulate plant growth [2]. Although the current research on microorganisms related to plant growth has paid more attention to the flora in the roots of plants, endophytes are more effective at promoting plant growth in the flora than in the root. Endophytic bacteria play an important role that directly or indirectly promotes plant growth. Furthermore, a previous study confirmed that root flora was more critical than endophytic bacteria because roots promote plant growth by obtaining nitrogen, phosphorus, and iron and regulating hormone levels [3]. The bacterial micro-ecosystem in the roots is widely regarded as one of the primary sources of endophytic bacterial colonization [4]. Endophytic bacterial diversity is a subset of the root sphere and/or root-associated bacterial populations [5]. However, endophytes are generally in constant contact with plant cells, making it easier for them to play a direct beneficial role [6]. Epiphytes also promote and antagonize plant growth [1]. As a type of grain, oats are not only one of the primary sources of food but also an important feed for livestock worldwide and are cultivated in Europe and North America [7]. The main planting areas in China are mountainous areas, plateaus, and high northern cold and cool areas. The Wulanqab area of Inner Mongolia is one of the main producing areas of oats in China, and its planting areas and output rank first in the prefectural-level cities in China. It is an important local grain crop. As the efficacy of oats has been continuously explored [8], research on the cultivation of oats has gradually attracted the attention of agricultural scholars. However, endophytic bacteria and the attached bacteria that promote oat growth have rarely been studied, and the species, abundance, and distribution of these bacteria still need to be clarified. Therefore, in this study, high-throughput sequencing technology was employed to investigate the changes in microbial communities in various plant tissues during the growth of oats and the relationship between these changes and growth indicators.

2. Materials and methods

2.1. Sampling locations, selection, and collection of samples

Because Bai yan No. 2 oat has better resistance to disease, cold and drought than other oat varieties, it was selected as the sample strain in this study. Based on the growth and developmental features of oats (Bai Yan No. 2), we divided the oats into stages of emergence (SS), jointing stage (JS), and mature stage (MS). From June to October 2021, 20 healthy oat strains of 'Baiyan var. 2' from the academician base of the Academy of Agriculture and Animal Husbandry Sciences of Ulanqab City, Inner Mongolia Autonomous Region (40°59′26.16″N, 113°07′26.44″E), Wuchuan County (41°05′47.40″N, 111°27′4.25″E), and the experimental base of the Academy of Agriculture and Animal Husbandry Sciences of Inner Mongolia (40°48′27.79″N, 111°37′22.76″E) were selected. In each region, 20 healthy oat plants were selected and mixed randomly according to three different growth periods in different locations. Roots, stems, and leaves (300 g) in these three stages were sampled individually (the sampling method was shown in Fig. S1). The sample was washed with tap water until clean, wiped with anhydrous ethanol, and then washed with sterile PBS. Then, 150 g of each sample was placed in liquid nitrogen and transferred to our laboratory for use. Additionally, 150-g samples were used for the indicator test.

2.2. DNA extraction and sequencing

The samples in liquid nitrogen were thawed, and the genomic DNA of plant tissue samples consisting of roots, stems, and leaves was extracted according to the kit instructions (Kangwei, Jiangsu, China). 16S rDNA (V3–V4) and ITS (ITS3_KYO2F and ITS4-2409R) sequencing of all samples were performed at Shanghai Biozeron Biotechnology Co., Ltd. PCR amplification conditions were as follows: 94 °C for 5 min; 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min for 28 cycles and a final extension at 72 °C for 10 min. The amplicons were recovered from 2 % agarose gel and purified with an AxyPrep DNA gel extraction kit (AxyPrep, Hangzhou, China). The PCR products were quantified by assays using the QuantiFluor™-ST Blue fluorescence quantification system (Promega, Wisconsin, USA). An Illumina PE250 library was constructed, and sequencing was conducted on a HiSeq 2500 platform (PE250, SpectraLab Scientific Inc., Toronto, Canada). Furthermore, three tests on the oat tissue were performed according to the kit instructions using an indole-3-acetic acid (IAA) kit (Coibo Bio, Shanghai, China), superoxide dismutase (SOD) kit (Coibo Bio, Shanghai, China), peroxidase (POD) kit (Coibo Bio, Shanghai, China) and malondialdehyde (MDA) kit (Coibo Bio, Shanghai, China).

Table 1
Trend of α-diversity profiles of fungi and bacteria during oat development.

Factor	Sample group	α-Diversity (Shannon)[a]						β-Diversity[b]											
		Fungi			Bacteria			Fungal Bray–Curtis		Fungal weighted UniFrac		Fungal unweighted UniFrac		Bacterial Bray–Curtis		Bacterial weighted UniFrac		Bacterial unweighted UniFrac	
		Df	F	P	Df	F	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P
Part	JS	2.6	82.807	0.000	2.6	22.230	0.002	0.976	0.004	0.991	0.003	0.391	0.010	0.627	0.013	0.685	0.004	0.576	0.007
	SS	2.6	5.491	0.044	2.6	74.228	0.000	0.875	0.003	0.884	0.007	0.284	0.251	0.535	0.042	0.667	0.048	0.535	0.013
	MS	2.6	1.274	0.346	2.6	140.443	0.000	0.679	0.009	0.602	0.011	0.380	0.031	0.484	0.051	0.329	0.179	0.544	0.013
	All Stages	2.24	13.130	0.000	2.24	99.033	0.000	0.124	0.103	0.122	0.142	0.098	0.101	0.064	0.585	0.078	0.414	0.091	0.198
Stage	Leaf	2.6	0.496	0.632	2.6	1.866	0.234	0.509	0.005	0.543	0.014	0.240	0.644	0.245	0.393	0.377	0.220	0.391	0.044
	Root	2.6	4.449	0.065	2.6	294.324	0.000	0.948	0.003	0.966	0.005	0.424	0.002	0.942	0.006	0.973	0.004	0.421	0.007
	Stem	2.6	2.636	0.151	2.6	1.193	0.366	0.692	0.016	0.753	0.012	0.232	0.622	0.111	0.874	0.162	0.818	0.364	0.065
	All Parts	2.24	0.692	0.510	2.24	0.293	0.749	0.505	0.001	0.554	0.001	0.174	0.001	0.356	0.001	0.353	0.001	0.339	0.001

2.3. Data analysis

The shared operational taxonomic units (OTUs) among various tissues during the growth and development of high-quality oats were identified from a Venn diagram using R (v3.5.0). The α-diversity and β-diversity estimates were calculated with QIIME using weighted UniFrac distances between samples for bacterial 16S rDNA reads and the Bray–Curtis dissimilarity for fungal ITS reads. Principal coordinate analysis (PCoA) was used to evaluate the distribution patterns of the oat microbiome biogroups. One-way analysis of variance (ANOVA) was used to determine how the samples were classified (e.g., developmental stage or different plant parts). Linear discriminant analysis effect size (LEfSe) using linear discriminant analysis (LDA) was used to estimate the influence of the abundance of each component (species) on their difference effect according to the taxonomic composition on the sample division. Redundancy analysis (RDA) and canonical correspondence analysis (CCA) in the R language "vega" package were used for analysis and mapping. Using the Monte Carlo substitution test, we determined whether environmental factors were significantly correlated with the species community.

3. Results

3.1. Quality metrics of high-through sequencing analysis

A total of 1,131,623 effective sequences of completely endophytic bacteria and attached bacteria were obtained (average: 41,911; maximum: 58,395; minimum: 30,273; average length: 253.80). A total of 1,169,417 effective sequences of superior oat endophytes and attached fungi were obtained (average: 43,311; maximum: 57,955; minimum: 30,110; average length: 256.39) and used for OTU generation. All the high-quality sequences assigned to the same OTU had 97 % similarity, and symbiosis resulted in 6480 OTUs, including 1698 fungi and 4782 bacteria. The curve tended to be flat (Figs. S2a and S2b), indicating that the volume of sequencing data and the overall OTU coverage were sufficient for analysis.

Among all the OTUs found throughout the growth and developmental period of high-quality oats, the fungi shared 126 OTUs (Fig. S2c), in particular *Ascomycota, Basidiomycota,* and *Mucoromycota* at the phylum level, *Aspergillaceae, Chaetomiaceae, Cladosporiaceae, Didymellaceae, Filobasidiaceae, Mortierellaceae*, *Nectriaceae, Plectosphaerellaceae*, and *Sordariomycetes_norank* at the family level, and *Aspergillus, Cladosporium, Filobasidium, Fusarium*, *Mortierella*, and *Trichocladium* at the genus level (average relative abundance >1 %). There were 927 unique OTUs observed in the SS. In addition to the above content of the genus level common strains, this includes *Microdochium, Plectosphaerella,* and *Podospora* at the genus level (average relative abundance >0.1 %), 1068 unique OTUs in the JS, mainly *Alternaria, Aspergillus, Botryotrichum, Cephalotrichum, Chaetomium, Dendrostilbella, Gibellulopsis, Filobasidium*, and *Fusarium* (average relative abundance >0.1 %), and 1111 unique OTUs in the MS, mainly *Acremonium, Penicillium,* and *Vishniacozyma* (average relative abundance >0.1 %).

In terms of bacteria, 39 OTUs were found in the oat plant tissue microbial community during growth and development (Fig. S2d), with primarily *Actinobacteriota* and *Proteobacteria* at the phylum level and *Burkholderiaceae, Oxalobacteraceae, Rhizobiaceae, Sphingomonadaceae, Xanthobacteraceae* and *Xanthomonadaceae* at the family level. The main microorganisms were *Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium*, *Sphingomonas*, and *Stenotrophomonas* at the genus level (average relative abundance >1 %). Additionally, there were 2869 unique OTUs in the SS. The major classes and genera of microorganisms were consistent with the common ones. We found 3576 unique OTUs in the JS, primarily *Comamonadaceae, Gaiellales_uncultured, Rhizobiaceae, Weeksellaceae, Pseudomonadaceae*, and *Nocardioidaceae* at the family level. At the genus level, the dominant strains were

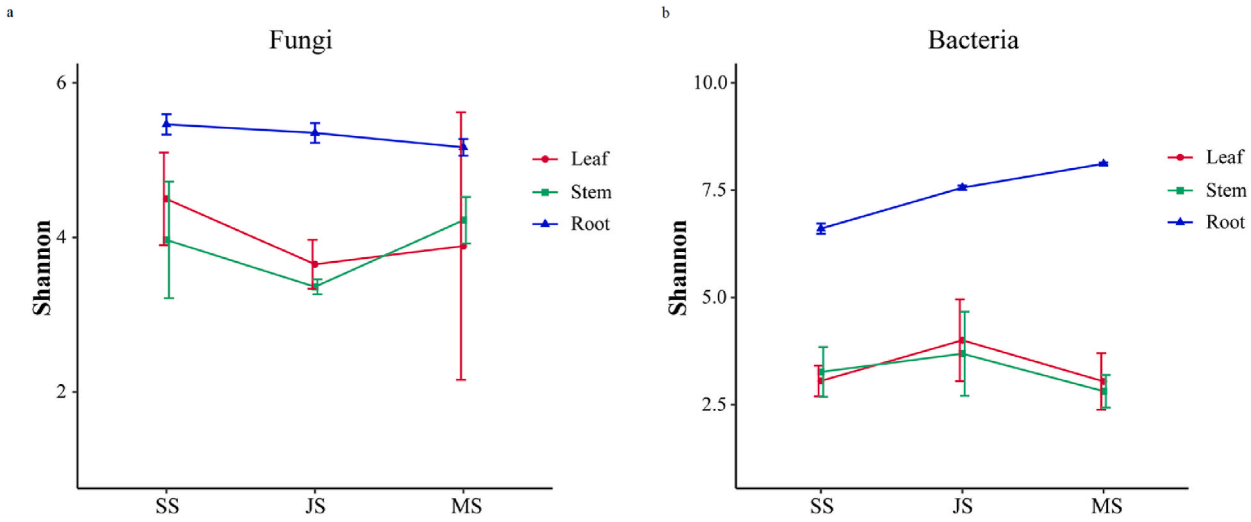


Fig. 1. Changes in fungal (a) and bacterial (b) α-diversity during oat development.

Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium, *Gaiellales_norank*, *Sphingomonas*, and *Pseudomonas* (average relative abundance >0.1 %). There were 2916 distinct OTUs in the MS. *Sphingomonadaceae, Xanthobacteraceae, Rhizobiaceae, Nocardioidaceae*, and *Comamonadaceae* were at the family level. *Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium*, *Bradyrhizobium, Sphingomonas*, and *Stenotrophomonas* were at the genus level (average relative abundance >0.1 %).

3.2. α-Diversity and β-diversity analysis

There were no significant changes in the α-diversity of fungi in the leaf, root, stem, or all parts combined. However, there were significant changes in the SS, JS, and all stages combined but not in the MS (Table 1). As shown in Fig. 1a, the fungal α-diversity of the roots, stems, and leaves had downward trends going from the SS to the JS, but the α-diversity of fungi in the stem and root in the MS did not follow this pattern. This was because the leaf tissue of the MS was declining. The α-diversity of oat bacteria was opposite to that of fungi (Fig. 1b). Table 1 shows no significant changes in the α-diversity of the stems or leaves bacteria of the oats, but the α-diversity of root bacteria increased significantly. Additionally, there were significant changes in the SS, JS, MS, and all stages combined. The bacterial α-diversity of the roots showed an increasing trend in the growth and developmental stage, as shown in Fig. 1b. Overall, the α-diversity of bacteria in the roots was the highest.

We observed significant differences in the Bray–Curtis index of fungal and bacterial β-diversity in the SS, JS, and MS. However, there were no significant differences in all stages combined. Significant differences were found in fungal β-diversity among leaves, roots, stems, and all other parts combined using the Bray–Curtis index (Table 1). In conclusion, the microbial diversity of the oat tissue communities varied with different developmental stages.

In order to eliminate influence of some environmental factors, we also collected plant root soil in the same way, and conducted research on it. The results were as follows: If the three growth stages were taken as groups, there was still no significant difference in the level of bacteria among the groups containing soil samples ($p = 0.38699$). It was indicated that environmental factors (soil) had no significant impact on microbial diversity in the growth and development stage of oat in the level of bacteria (Fig. S3a). If each tissue were taken as groups, there was a significant difference among the groups containing soil samples in the level of bacteria ($p = 0$). It was indicated that there was a great difference between soil factors and the microbial diversity of each tissue of oat (Fig. S3b). The treebar_phylum could find that there were certain differences among soil groups in the level of bacteria, but the similarity was high and there was no significant difference ($p > 0.05$) (Fig. S3c).

3.3. PCoA and LEfSe analysis

PCoA data indicated that the oat tissue had a strong separation of developmental stages, the developmental stage influenced the microbial diversity of the oat tissues, and regardless of whether this was fungal or bacterial community diversity, the roots were usually higher in diversity than the stems and leaves. The LEfSe analysis showed more core bacteria in the MS, at least forty-two bacterial taxa were significantly enriched in the MS (Fig. 2a). The LEfSe results showed more core fungi in the SS and MS than in other stages. Twenty fungal taxa were significantly enriched in the MS (Fig. 2b), and 20 fungal taxa showed significant differences in the SS (Fig. 2c). However, the roots had the highest bacterial microbial communities at all growth stages.

3.4. Dynamic changes in core microflora in fine oats during development

Our analysis revealed that the core fungal strains of leaves in each developmental stage were as follows: *Phomatospora*, *Ophiobolopsis*, *Candida*, *Panaeolus*, *Botryotinia*, Unclassified, *Camarosporioides*, *Protocrea*, *Humicola*, and *Phaeosphaeria*. *Phaeosphaeria* had a high abundance from the SS and JS to the MS (>33.64 %) but showed a decreasing trend. *Phomatospora* had a higher abundance in the

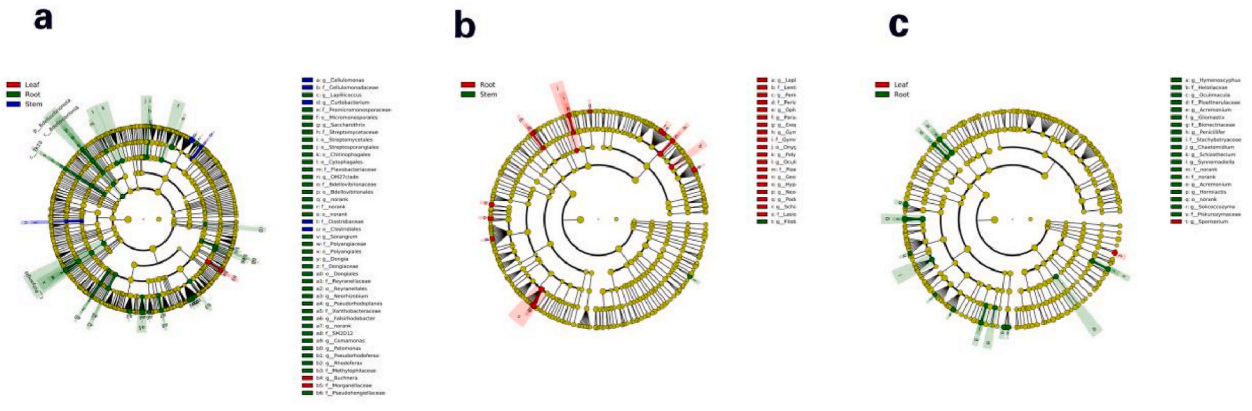


Fig. 2. LEfSe taxonomic cladograms showing significantly different bacterial (a) and fungal (b) taxa associated with oat in the MS and significantly different fungal taxa (c) associated with oat in the SS.

JS (44.39 %) but a lower abundance in the SS and MS (<1.40 %) (Fig. 3a). Second, each developmental stage of the root was analyzed, and its core strain species were *Symmetrospora*, *Gliomastix*, *Penicillifer*, *Talaromyces*, *Naganishia*, *Kurtzmanomyces*, *Humicola*, *Bipolaris Didymella*, and *Kondoa*. *Kondoa* was highly abundant in all stages of root development (>10.10 %) but showed a decreasing trend at each developmental stage. Bipolaris had a higher abundance in the roots of the JS (27.70 %) and a lower abundance in the SS and MS (<5.47 %) (Fig. 3b). Then, we determined the core strain species of the stems in each developmental stage as follows: *Kondoa*, *Lophotrichus*, *Clitocybe*, *Pseudombrophila*, *Plectosphaerella*, *Myxotrichum*, *Pseudeurotium*, *Chaetomium*, *Ustilago*, and *Pyrenophora*. *Pyrenophora* also maintained a high abundance during various developmental periods of the stems (>36.74 %) (Fig. 3c).

First, the core bacterial strains of the leaves in each developmental stage were identified as follows: *Curtobacterium*, *Bacteroides*, *Lysinibacillus*, *Sphingobium*, *UCG-002*, *Comamonas*, *Brevibacterium*, *Rikenellaceae RC9 gut group,* and *Thiopseudomonas*. The abundance of *Thiopseudomonas* was greater than that of the other strains in the SS and MS (>45 %) but lower in the JS (<0.1 %). The leaves of the

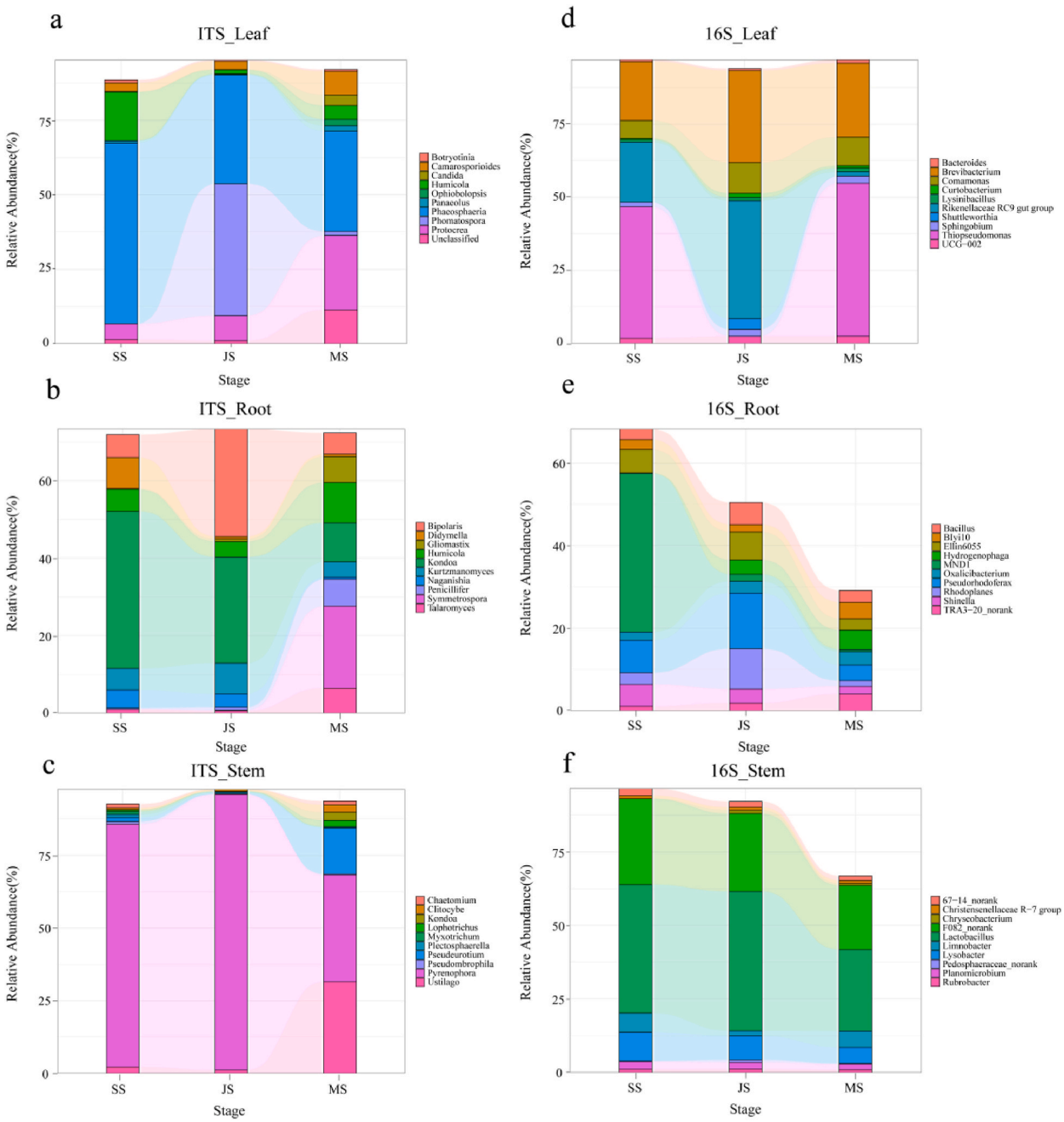
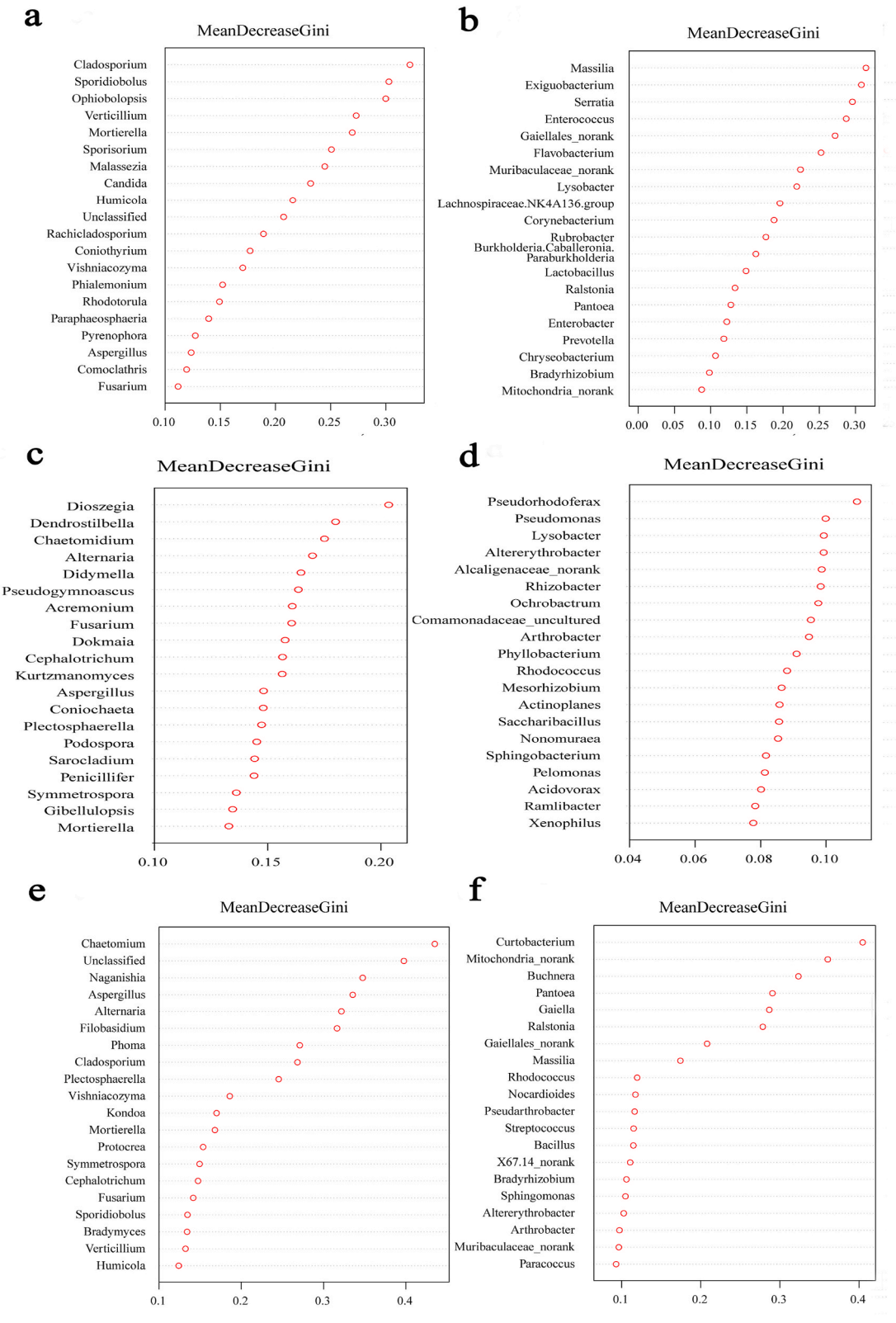


Fig. 3. Relative abundance of core microbiota in oat. SS (a), JS (c), and MS (e) represent the core fungal communities' relative abundancesy, while SS (b), JS(d), and MS(f) represent the core bacterial communities' relative abundances, respectively.



(caption on next page)

Fig. 4. Classifications of random forest models of the leaf, root and steam during various developmental stages of the core taxa. Shown are the important features (top 20) based on the mean decrease Gini (MDG) of random forest models of the core taxa of fungi (a) and bacteria (b) of leaf; Shown are the important features (top 20) based on the mean decrease Gini (MDG) of random forest models of the core taxa of fungi (c) and bacteria (d) of root; Shown are the important features (top 20) based on the mean decrease Gini (MDG) of random forest models of the core taxa of fungi (e) and bacteria (f) of stem.

Rikenellaceae RC9 gut group had a higher abundance (>20 %) in the SS and JS but a lower abundance in the MS (1.43 %). Some bacteria maintained a high abundance at each developmental stage, such as *Brevibacterium* (>20 %) (Fig. 3d). The core strain species of the roots were determined in each developmental stage to be as follows: *Hydrogenophaga*, *TRA3-20_norank*, *Oxalicibacterium*, *BIyi10*, *Bacillus*, *Rhodoplanes*, *Shinella*, *Ellin6055*, *Pseudorhodoferax*, and *MND1*. *MND1* was more abundant than other strains in the SS (38.44 %), with little abundance in the JS and MS (<1.72 %), and there was little abundance of other core bacterial strains (<13.44 %) (Fig. 3e). Then, we determined the core strain species of the stems in each developmental stage to be as follows: *Pedosphaeraceae_norank*, *Christensenellaceae R-7 group*, *Rubrobacter*, *67-14_norank*, *Planomicrobium*, *Limnobacter*, *Lysobacter*, and *Lactobacillus*. *Lactobacillus* showed a high abundance (>27.73 %) in each developmental period but with a decreasing trend with the developmental period. *F082_norank* also maintained a somewhat high abundance (>21.77 %) during each developmental period (Fig. 3f).

To confirm the stability of core taxa in the growth process, we used the random forest-supervised learning model to classify the samples and determine which taxa could explain the strongest variations during the growth process. The results showed that bacterial and fungal core taxa all had better discrimination and were correctly identified in tissue samples of each stage, and discrimination of all stages reached 100 %. The model Gini index showed that all core flora had important characteristics at all developmental stages. In all stages of leaf development, *Cladosporium* and *Massilia* had the greatest variation in fungal (Fig. 4a) and bacterial communities (Fig. 4b). Among all the developmental stages of the roots, *Dioszegia* and *Pseudorhodoferax* had the greatest variation in fungal (Fig. 4c) and bacterial communities (Fig. 4d). Among all the developmental stages of the stems, *Chactomium* and *Curtobacterium* had the greatest variation in fungal (Fig. 4e) and bacterial communities (Fig. 4f). All strains were associated with the oat growth process.

3.5. Effects of various indexes on microorganisms in fine oats during development

According to the CCA and RDA results, during the growth and developmental period of high-quality oats, there were significant differences in the changes in fungal (Fig. 5a) and bacterial (Fig. 5b)communities in each plant tissue, with the MDA, SOD, and POD showing a negative correlation (Table 2). Network analysis revealed 42 strains related to SOD, 60 strains related to POD, 38 strains related to both, and 1 strain related to MDA and IAA in the bacteria. In the fungi, there were 16 strains related to SOD, 15 strains related to POD, 7 strains related to both, and 1 strain related to MDA and IAA. Thus, fungi (Fig. 5c) may have sparser connecting modules than

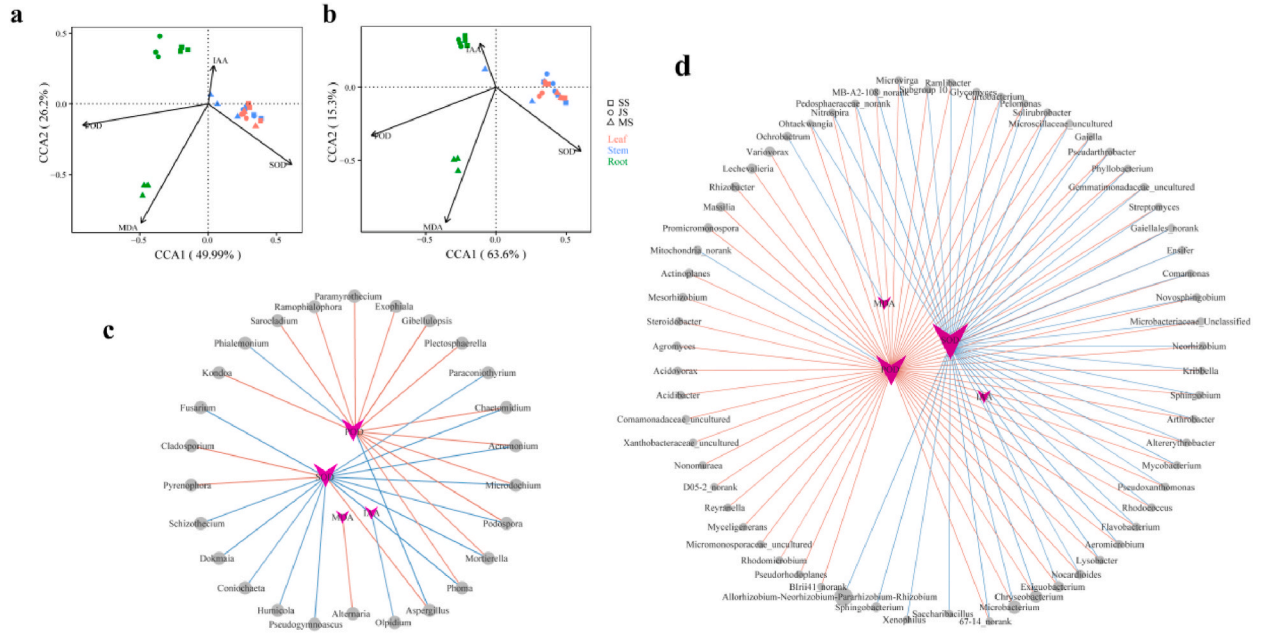


Fig. 5. Correlation maps between microorganisms in oat tissues and IAA, POD, SOD, and MDA during the growth and development of oats. (a) Canonical correspondence analysis reflects the relationship between IAA, POD, SOD, and MDA and fungi in oat tissue during the growth of oats. (b) The relationship between IAA, POD, SOD, and MDA and bacteria in oat tissue during the growth of oats. (c) The co-occurrence relationship between IAA, POD, SOD, and MDA and fungi in oat tissue during their growth. (d) The co-occurrence relationship between IAA, POD, SOD, and MDA and bacteria in oat tissue during the growth of oats.

bacteria (Fig. 5d), and the correlation between SOD and POD and bacteria was more significant. For example, in bacteria, *Variovorax* and *Lechevalieria* were related to POD, while *Xenophilus* and *Sphingobacterium* were related to SOD, and *Lysobacter* and *Flavobacterium* were related to both. *Ochrobactrum* was only associated with MDA.

The bacteria most associated with POD were *Agromyces* (cor: 0.9290), *Altererythrobacter* (cor: 0.9324), *Massilia* (cor: 0.9457); The bacteria most associated with SOD were *Microvirga* (cor: −0.9323) and *Gaiellales_norank* (cor: −0.9132), and *Pedosphaeraceae_norank* was the most important strain or main strain during growth.

Schizothecium and *Dokmaia* were associated with SOD, *Gibellulopsi* and *Plectosphaerella* were associated with POD, and *Microdochium* and *Podospora* were associated with both (Fig. 5c). Only *Alternaria* was associated with MDA, and only *Olpidium* was associated with IAA (Fig. 5c). The fungi most associated with POD were *Gibellulopsis* (cor: 0.8871), *Podospora* (cor: 0.8540), *Paramyrothecium* (cor: 0.8365), and *Kondoa* (cor: 0.7946); The fungi most associated with SOD were *Cladosporium* (cor: 0.8365), *Pyrenophora* (cor: 0.7931), *Aspergillus* (cor: 0.7585), *Cladosporium*, and *Aspergillus*, and *Kondoa* was the most significant strain or core strain for growth.

4. Discussion

In this study, the different microbial communities during the developmental stages of oats were studied to investigate whether the microbial community of various oat tissues could promote growth at different developmental stages. Experiments confirmed that the developmental stage had a particular influence on the microbial diversity of oat tissues and on whether fungal or bacterial community diversity was abundant. The α-diversity of bacteria in the roots was usually higher than that of those in the stems or leaves. The root bacterial community was closely related to oat growth, and these microbial communities may promote plant growth. Compared to the JS and SS, the MS had many (up to 40) species of core bacteria. Endophytic bacteria may be closely related to oat maturation, but further research is needed. However, the α-diversity of fungi in the oat MS did not change significantly during growth. Possibly, the α-diversity of fungi had no effect on the growth of oat tissues and had no correlation with maturity.

Many studies have confirmed that *Proteobacteria* dominate the diversity analysis of endobacteria, although members of *Firmicutes* and *Actinomycetes* are some of the most common endophyte groups, and *Ascomycota* is also present [9]. The most commonly found genera of bacterial endophytes were *Pseudomonas, Bacillus, Burkholderia, Stenotrophomonas, Micrococcus, Pantoea,* and *Microbacterium* [10]. According to a previous study, other groups, such as *Planctomycetes, Verrucomicrobia*, and *Acidobacteria,* are not typically endophytes [10]. In this study, the presence of *Actinobacteriota, Ascomycota*, and *Proteobacteria* in oat tissue was consistent with the previous report [9], and some of the other strains had similarities, but there were also many differences. The *Thiopseudomonas* strain, reported to be a probiotic strain isolated from the roots of nodules of *Alnus* trees in New Hampshire, was also found to have a high abundance in the leaves in our study [11]. *Rikenellaceae*, with a high abundance in leaves, are mostly found in the gut but not in plants [12,13]. In addition, *Brevibacterium*, with a high abundance in oat leaves in this study, has been considered by many studies to be a bacterium that promotes plant growth. Other researchers believe that these microorganisms improve plant growth parameters by producing secondary metabolites (including enzymes and antibiotics), which contribute to nutrient absorption, soil fertility, and plant growth and protect plants from pathogens [14,15]. Furthermore, in some studies, *Lactobacillus* has been found in plant roots, while in this study, a high abundance of *Lactobacillus plantarum* was found in the oat stems [16]. A metabolite produced by *Phaeosphaeria* sp., a fungus related to lichens, was found in the leaves. Yi–Jie Zhai et al. found that its metabolites 1 and 8 promoted the growth of rice stem and root elongation in a dose-dependent manner [17]. Many studies have shown that *Kondoa* is a yeast isolated from leaves and soil [18,19]. In this study, *Kondoa* isolates were found in high abundance in the oat roots. Recent studies have shown that although these fungi are pathogenic, they also play an interesting role in agriculture. They act as phosphate solubilizers and produce plant hormones, such as IAA and gibberellinic acid, to accelerate the growth of various plants. Some species play an important role in promoting plant growth under abiotic stresses, such as salt stress, drought stress, heat stress, and heavy metal stress, and act as biological control agents and potential mycoides [20]. *Pyrenophora* is a web spot disease of wheat, which can cause wheat infertility. It rarely occurs on the stems of plants, but a high abundance of *Pyrenophora* was found in the stems in this study [10].

Moreover, the endophytic bacteria population depends on many variables, such as the plant growth stage, plant health status, nutritional status, and soil type and conditions [10,21]. Endophytic bacteria inhabit plants and promote plant growth through three main mechanisms: stimulation, fertilization, and control [22]. Stimulation is the direct production of plant hormones by endophytes that promote plant growth, such as IAA, which promotes the absorption of nutrients by the roots [23]. Santoyo et al. also confirmed this view that endophytes directly or indirectly promote plant growth and development by promoting the synthesis of inert IAA, ethylene, and other hormones in plants [24]. MDA is one of the most important products of membrane lipid peroxidation, and its

Table 2
Correlation maps between oat tissues and IAA, POD, SOD, and MDA during oat growth and development.

	Bacteria		Fungi	
Factor	R2	P	R2	P
MDA	0.945851479	0.001	0.89095367	0.001
SOD	0.491821399	0.003	0.51209499	0.003
POD	0.830676635	0.001	0.806529362	0.001
IAA	0.098557799	0.434	0.069164964	0.461

Note: IAA is indole-3-acetic acid, POD is peroxidase, SOD is superoxide dismutase, and MDA is malondialdehyde.

production can aggravate membrane damage. Therefore, the MDA content is a common index in the study of aging physiology and resistance physiology of plants. MDA can be used to understand the degree of membrane lipid peroxidation, which indirectly measures the degree of membrane system damage and plant stress resistance. MDA can effectively protect wheat seedlings from drought damage [25]. Almost all aerobic bacteria rely on enzyme-promoted and non-enzyme-promoted antioxidants to combat the presence of reactive oxygen species. SOD is one of the most important antioxidant enzymes as part of the enzymatic antioxidant system. The life span of an organism is intrinsically related to the SOD activity and antioxidant capacity [26]. Plants contain a large amount of POD, an enzyme with high activity. It is related to respiration, photosynthesis, and auxin oxidation. Its activity changes continuously during plant growth and development. Generally, its activity in aging tissues is high, and its activity in young tissues is weak, so peroxidase can be used as a physiological index of tissue aging. Many researchers believe that SOD and POD are related to drought resistance and saline–alkali resistance [26,27]. In this study, MAD, SOD, and POD showed significant differences in the diversity of microorganisms, which may be related to the resistance of microorganisms in oat tissue. *Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium* and *Sphingomonas* were the main microorganisms only associated with SOD in the oat plant tissue microbial community during growth and development. Moreover, Phomatospora and Chaetomium were dominant only related to POD in fungi. *Aspergillus* and *Mortierella* were also the strains associated with SOD and POD, which were the main microorganisms in the oat plant tissue microbial community during growth and development. This indicates that some microorganisms in each tissue at each growth stage are related to their growth. Kim et al. confirmed that *Lysobacter* sp. CJ11T was isolated from the root of *Glycine* max L from Goyang City, South Korea. A new exopolysaccharide purified from its fermentation broth also has antioxidant properties [28]. Another study confirmed that *Sphingomonas* is salt tolerant and can increase the SOD value of plants [29]. A similar study found that under no salt or salt stress, the dry weight, fresh weight, plant height, root length, SOD activity, and chlorophyll content of wheat and soybean plants inoculated with *Cladosporium* were higher than those of uninoculated control plants [21]. *Bacillus*, with antibacterial properties, also showed antioxidants in the core of the cherimoya roots. Pedro Ulises Bautista–Rosales et al. used it to control the softening of *Annona muricata* L. in 2022. The activities of SOD and catalase (CAT) increased by 1.35 and 1.78 times, respectively, on the first day after *Bacillus* inoculation. However, on the third day of storage, SOD and POD expressions increased 18.7 and 4.5 times, respectively. *Bacillus* was found to enhance the antioxidants of cherimoya fruit [30]. *Lactobacillus* with antibacterial properties also has antioxidants in the core of the stem [31]. The resistant *Lactobacillus* CD101 antioxidant has also been found to be mainly distributed on the cell surface and extracellular secretions [31]. In this study, we explored the strains related to oat growth via the microbial diversity of oats to promote their growth and inhibite pathogenic bacteria by studying the strains that have certain regional limitations. However, it was undeniable that the present analysis maybe partly truly reflects the diversity microbial feature of oats in different growth stages. further, increase the sample size of each province and region, and conduct separate studies is recommended. At the same time, other crops of the same growing period were collected in other areas as controls in order to draw more objective and correct conclusions. Second, a comparison microbial diversity study of samples from sick and healthy groups is recommended, which will aid the understanding of the microbial diversity of oats. And We also continued to demonstrate that bacterial communities, rather than fungal communities, promote oat tissue growth.

5. Conclusion

In this study, oat tissues mainly comprised *Ascomycota, Basidiomycota,* and *Mucoromycota* at the phylum level of fungi and *Actinobacteriota* and *Proteobacteria* at the phylum level of bacteria. All strains were related to the growth process of oats and antagonistic bacteria. The α-diversity of bacteria and β-diversity of bacteria and fungi of the roots were higher than those of the stems and leaves. The bacteria had denser connection modules than fungi. The effects of MDA, SOD, and POD analysis on bacterial and fungal communities in plant tissues were significantly different, showing a negative correlation, which may be related to the stress resistance of microorganisms in oat tissue.

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Availability of data and materials

All the data generated or analyzed during this study are included in this published article, and the supplementary information files will be freely available to any scientist wishing to use them for non-commercial purposes upon request via e-mail.

CRediT authorship contribution statement

Chao Cheng: Writing – review & editing, Writing – original draft, Resources, Methodology, Conceptualization. **Yahong Zhang:** Writing – review & editing, Supervision, Methodology, Investigation. **Linchong Zhang:** Writing – original draft, Resources, Methodology, Investigation. **Jianjun Guo:** Visualization, Validation, Formal analysis. **Songhe Xu:** Validation, Supervision, Resources, Conceptualization. **Pengfei Gao:** Software, Resources, Methodology, Formal analysis. **Kongxi Fan:** Investigation, Formal analysis, Data curation. **Yiwei He:** Visualization, Supervision, Investigation, Formal analysis. **Yanchun Gong:** Project administration, Formal

analysis, Data curation. **Gang Zhong:** Validation, Supervision, Data curation. **Shaofeng Su:** Resources, Project administration, Investigation, Data curation. **Zhiguo Liu:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

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Appendix A. Supplementary data

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