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COMMUNITY STRUCTURE OF ENDOPHYTIC FUNGI IN ROOTS AND LEAVES OF *Fagopyrum mill* AND *Avena sativa* IN A CHINESE NORTHERN COLD REGION

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Abstract

In order to explore the endophytic fungi of *Fagopyrum Mill* and *Avena sativa*, Illumina Miseq high-throughput sequencing was used to analyze the community structure and diversity of endophytic fungi in leaves and roots of buckwheat and oat at the mature stage. The results of community structure showed that there were 205 operational taxonomic units (OTUs) in buckwheat roots and 181 OTUs in buckwheat leaves based on 97% sequence similarity level. There were 152 OTUs and 127 OTUs in the root and the leaf of oat, respectively. At the phylum level, Ascomycota and Basidiomycota were the dominant endophytic fungi in buckwheat roots and leaves, while Ascomycota was the dominant endophytic fungus in oat roots and leaves. Alpha diversity analysis showed that the Ace index, Chao index and Shannon index of buckwheat roots were higher than that of buckwheat leaves, and the three indices of oat roots were also higher than that of oat leaves, indicating that the richness and diversity of endophytic fungi community in roots were higher than that in leaves. Biomarkers were found by significant difference analysis in buckwheat and oat. The endophytic functional groups of buckwheat and oat were mainly distributed in Pathotroph and Saprotroph. The results of this study laid a foundation for fully exploiting the dominant endophytic fungal resources of buckwheat and oat and further developing microbial fertilizers.

Keywords: Ascomycota. Basidiomycota. Diversity. Illumina high-throughput sequencing. ITS.

1. Introduction

Buckwheat (*Fagopyrum Mill*), an annual herb, belongs to the Polygonaceae family, *Fagopyrum* genus. It originated in China, and is widely cultivated in the temperate regions of Europe, North America and Asia, particularly Ukraine, Japan, Bhutan, Korea, Nepal, Russia, northwest and southwest China (Ohnishi 1998; Zhang et al. 2017). Buckwheat is suitable for growing in a cool and humid climate. The crop is resistant to environmental changes and ecological pressures, does not require intensive cultivation and has lower production costs. Seeds have high health care value, edible value, nutritional value, and feeding value. The lysine content of buckwheat is much higher than that of rice and corn; buckwheat contains favorable dietary fibers, which can promote intestinal peristalsis and excretion (Bonafaccia et al. 2003; Lee et al. 2010). It can be processed into noodles or other food and medicine products. With the gradual discovery of the pharmaceutical potential of buckwheat, a large number of studies have mainly focused on the bioactive components of buckwheat grains (Krkošková and Mrazova 2005; Eguchi et al. 2009; Ji et al.

2019). Very little research has been performed on the endophytic fungi of *Fagopyrum Mill* (Likar et al. 2008; Zhao et al. 2014).

Oat (*Avena sativa* L.) is an annual grain and feed crop of the Poaceae family, *Avena* genus. It is the sixth most produced grain in the world (Mert 2020). It is an important characteristic crop in China and is widely distributed in northeast, north, and southwest China. Oat has the characteristics of strong adaptability, high nutritional value, and high grass yield (Singh et al. 2013). It is abundant in protein, and contains a number of important minerals, *β*-glucan, lipids, and various other phytoconstituents. Because of the high nutritional and nutraceutical value of oat, research of all aspects of oats has gradually deepened (Stewart and McDougall 2014; Rasane et al. 2015; Leišová-Svobodová et al. 2019; Chen et al. 2021; Kim et al. 2021), and the research on endophytic microorganisms in oats also was paid more and more attentions (Dai et al. 2020; Sun et al. 2020).

The interaction between microbes and plants has attracted wide attention. Especially in the 1980s of last century, plant growth promoting rhizosphere bacteria were proven to promote plant growth (Kloepper and Beauchamp 1992). The isolation and functional researches of plant related microorganisms and the development of new microbial products have become another research hotspot in the field of microorganisms. It also includes growth promoting endophytes that live in plants. These endophytes enter plants through stomata or injured tissues, which do not obviously cause host disease symptoms or change the morphology and structure of plants, and play an important role in promoting plant growth, inducing disease resistance and inhibiting certain pathogens (Hallmann et al. 1997; Brady et al. 2002). Endophytes are the normal microecosystem of plants. They play an important role in different organs and different plant niches. Therefore, beneficial endophytes can be used as microbial pesticides and microbial fertilizers with biological control potential. Endophytes are present in nearly all plants (Petrini 1991). Nowadays, the study of endophytes in different plants mainly focuses on endophytic bacteria, and the isolation and research of endophytic fungi are relatively few, especially in buckwheat and oats. Likar et al. (2008) amplified a putative dark septate endophyte fungus in the root tissue of buckwheat, which can colonize these two economically important plant species, namely common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). Zhao et al. (2014) studied the effects of endophytic fungi on rutin production of buckwheat, and found that the yield of rutin increased by 3.1-3.2 times after treatment with endophytic fungus Fat9. Dai et al. (2020) studied endophytic bacteria and fungi in oat seeds, but did not study the endophytic microorganism in the root and the leaf of oat. Sun et al. (2020) studied the fungal endophyte community in oat stem. With the extensive application of high-throughput sequencing technology in the field of microbial ecology, research on the diversity of plant endophytic fungi, and the isolation and functional study of endophytic fungi will be accelerated.

There are few studies on the endophytic fungi of buckwheat and oats at present. In this study, the fungal communities in the roots and leaves of mature buckwheat and oat were studied by Illumina high-throughput sequencing technology, and the distributions and diversities of fungi in leaves and roots of buckwheat and oat at maturity were determined. This study provides theoretical support for further isolation and research on endophytic fungi in buckwheat and oat, and the internal mechanisms of their community structure differences.

2. Material and Methods

Field experiment and sampling

The experimental field is located in the Daqing experimental field in Wudalianchi, Heilongjiang Province, China. The longitude and latitude are 126.041851° E and 48.727148° N, respectively. The buckwheat variety is Tianqiao No 2, and the oat variety is Baiyan No 2. Sowing was carried out in May 2019, with a ridge distance of 110 cm, 22 plants per metre and about 350000 seedlings per hectare. Field management practices were normal, and field sampling took place on October 15, 2019.

Sample pretreatment

Three mature buckwheat and oat plants with good growth status were selected. The whole plant was brought back to the laboratory in as insulated box with ice and samples were processed immediately. Pretreatment of buckwheat and oat roots was performed according to the method described by Xiao et al (2017), the experimental process was as follows: (1) The root was placed into a centrifuge tube containing sterile phosphate buffer and vortexed on a vortex oscillator for 10-20 seconds. (2) took out the root and put it into a new centrifuge tube containing sterile phosphate buffer solution, and vortexed for another 10-20 seconds. (3) repeated the previous step until the phosphate buffer in the centrifuge tube turned clear; (4) took out the roots, and washed them in a sterile phosphoric acid buffer solution with ultrasonic oscillation for 5 minutes (oscillation for 30 s, paused for 30 s), then rinsed with phosphoric acid buffer solution to remove microorganisms on the root surfaces; (5) absorbed the water on the surface of the dry root with sterilized filter paper, then stored at – 80 ℃. Oat leaves were rinsed with phosphate buffer to remove microorganisms on the surface; then the leaf surfaces were dried with sterilized filter paper, and then quickly stored in a - 80 ℃ refrigerator for DNA extraction. The root samples of buckwheat were labeled QMG1, QMG2 and QMG3, and the leaves were labeled QMY1, QMY2 and QMY3. The root samples of oats were labeled YMR1, YMR2 and YMR3, and the leaf samples of oats were labeled YMY1, YMY2 and YMY3.

Construction of ITS library and high-through sequencing

Samples of 1 g roots and 1 g leaves of buckwheat and oat were ground with liquid nitrogen. The fungal total DNA of oats and buckwheat tissues was extracted with a fungal total DNA kit (Tian Gen Biochemical Technology Co., Ltd., Beijing) according to the kit instructions. DNA was measured by agarose gel electrophoresis, and the concentration of DNA was determined, then was used for PCR amplification of the ITS region of fungi. The PCR amplification primers used were ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'). The amplification system contains 20 μL, 4 μL 5 × Fastpfu Buffer, 2 μL 2.5 mM dNTP, 0.8 μL primer ITS1F, 0.8 μL primer ITS2, 0.4 μL Fastpfu Polymerase，0.2 μL BSA, 10 ng template DNA. The PCR amplification parameters were as follows: predenaturation at 94 ℃ for 5 min; denaturation at 94 ℃ for 30 s, annealing at 52 ℃ for 30 s, and extension at 72 ℃ for 40 s, 30 cycles; Finally, it was extended at 72 ℃ for 10 min. The amplified products of PCR were detected by 1.8% agarose gel electrophoresis and purified. The high-throughput sequencing library was constructed, and the qualified library was sequenced on Hiseq 2500 platform (Illumina; CA, USA) with two terminal sequencing (150 bp X2) by Beijing Biomarker Technologies Company.

Data processing and information analysis

Reads of each sample were assembled by overlap using FLASH v1.2.7 software to obtain raw tags (Magoc and Salzberg 2011); Raw tags were filtered to obtain good quality clean tags with Trimmomatic v0.33 software (Bolger et al. 2014); UCHIME v4.2 software was used to identify and remove chimera sequences to obtain the final effective tags (Edgar et al. 2011). Then, the sequences were clustered and divided into OTU, and species were classified based on the sequence similarity 97%. Based on the UNITE fungal database, taxonomic analysis of OTU at various taxonomic levels was carried out. The community structure of each sample at the taxonomic levels of phyla and class, and a phylogenetic tree at the taxonomic level of genus was obtained. By alpha diversity analysis, the ACE, Chao1, Shannon and Simpson indices of each sample were calculated. Based on the distance matrix, a sample hierarchical clustering (UPGMA) tree using the corresponding distance was obtained; a statistical analysis of the group differences LefSe (Line Discriminant Analysis (LDA) Effect Size), biomarkers with significant differences were found among different groups (Segata et al. 2011). The ecological function of FUNGuild endophytic fungi was predicted by funguild (functional Guide) software (Nguyen et al. 2016).

3. Results

OTU of root, leaf of buckwheat and oat

A total of 6 samples of root and leaf tissues from mature buckwheat were sequenced by Illumina HiSeq. ITS gene sequencing produced 480,451 paired reads, 456,029 clean tags were obtained after assembly and filtering. An average of 76,005 clean sequences were obtained for each sample. For oat, ITS gene sequencing of 6 samples of root and leaf tissues produced 478,996 pairs of reads, and 454,132 clean tags were obtained after assembly and filtering. An average of 75,689 clean sequences were obtained for each sample. All indices in the sequencing quality evaluation were in accordance with the standard. The dilution curves of root and leaf samples of buckwheat and oat showed sharp increases first and then tended to be smooth with the increase of sequencing quantity, which indicated that each sample species will not increase significantly with the increase of sequencing quantities (Figure 1). Moreover, the coverages of the libraries were more than 99.9%, indicating that the sequencing results were reasonable.

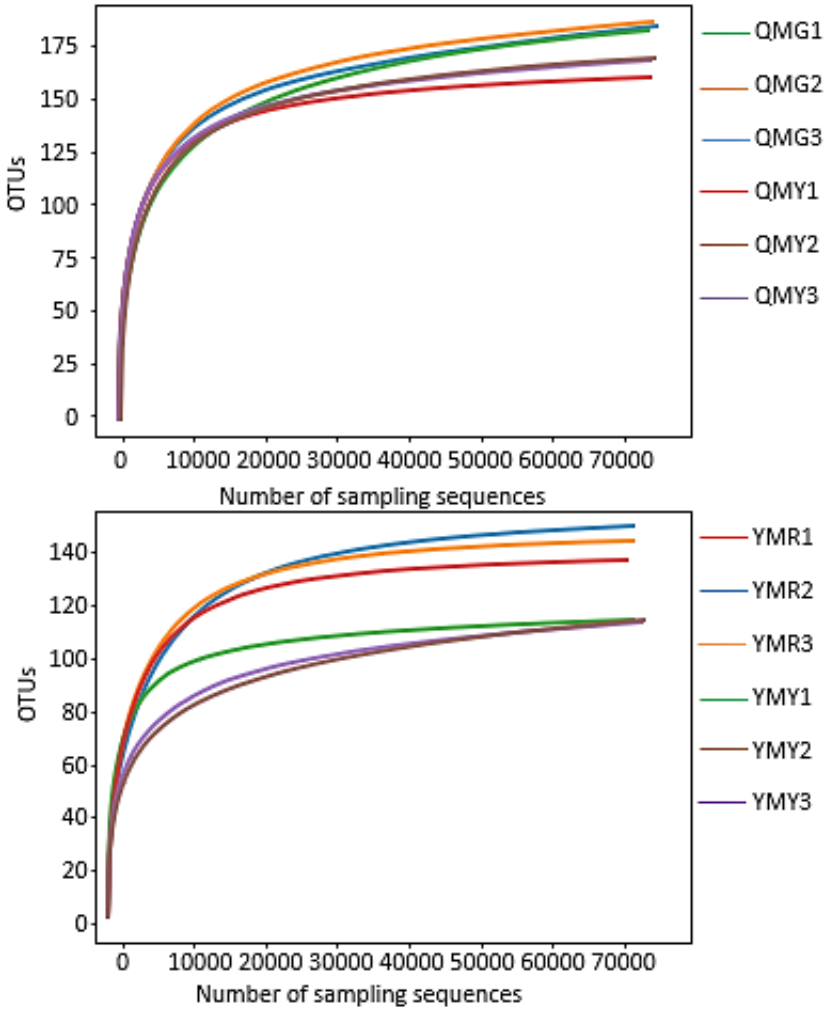

Figure 1. OTU dilution curves of endophytic bacteria in the roots and leaves of buckwheat and oat. Note: QMG, QMY, YMR and YMY represent buckwheat root, buckwheat leaf, oat root and oat leaf samples, respectively.

Non repetitive sequences were clustered into OTUs according to the sequence similarity of 97%, chimeras were removed in the process, and the representative sequences of OTUs were obtained. A total of 228 OTUs were obtained from the 6 samples of buckwheat, and 205 OTUs were detected in buckwheat root samples, and 181 OTUs were detected in leaf samples. The venn diagram showed that 158 OTUs were shared in buckwheat roots and leaves, and 47 and 23 OTUs were unique in buckwheat roots and leaves, respectively. A total of 158 OTUs were obtained from 6 oat samples by resampling and calculation analysis, 152 OTUs were detected in oat root samples and 127 OTUs were detected in leaf samples. The venn

diagram showed that there were 31 and 6 unique OTUs in oat roots and leaves, respectively, and 121 OTUs were shared between oat root and leaves (Figure 2). The results indicated that the roots and leaf of oat and backwheat contained abundant fungal endophytes, and the number of OTUs in the roots of these plants were greater than that in leaves.

Community structure of endophytic fungi in the leaves and roots of buckwheat and oat at maturity stage

The endophytic fungi in the 6 buckwheat samples were mainly distributed in Ascomycota, Basidiomycota and Mortierellomycota. At the phylum level, the dominant endophytic communities in buckwheat roots and leaves were Ascomycota and Basidiomycota, and Ascomycetes are absolutely dominant, 62.43% in roots and 63.40% in leaves, respectively. Meanwhile, Mortierellomycota was also detected in root tissues, accounting for only 0.05% in root tissue, and there was no Mortierellomycota in the leaves. At the class level, the endophytic fungi of 6 samples were distributed in 11 classes. The dominant fungal classes in each sample were Dothideomycetes and Sordariomycetes. Dothideomycetes accounted for 25.52% in root samples and 39.18% in leaf samples; Sordariomycetes accounted for 29.93% in root samples and 15.96% in leaves (Figure 3A).

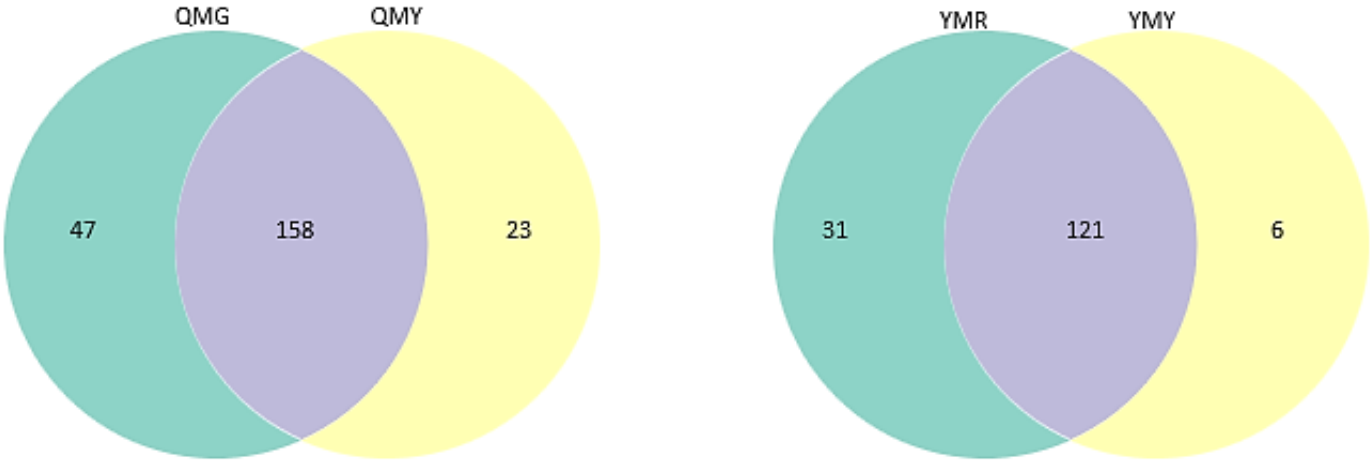

Figure 2. Venn diagram of OTUs in the roots and leaves of buckwheat and oat.

The endophytic fungi in 6 oat samples were mainly distributed in Ascomycota, Basidiomycota, and Chytridiomycota. At the phylum level, the dominant endophytic fungi in oat roots and leaves were Ascomycota, accounting for 53% in root samples and 80.04% in leaf samples. Meanwhile, Basidiomycota accounted for 17.35% in leaf samples, but no Basidiomycota were detected in root samples. At the class level, endophytic fungi of 6 samples were distributed in 11 classes. The dominant fungal classes in root and leaf samples are Dothideomycetes and Sordariomycetes. Dothideomycetes accounted for 22.39% in root samples and 19.22% in leaf samples. Sordariomycetes accounted for 27.83% in root samples and 60.69% in leaf samples (Figure 3B).

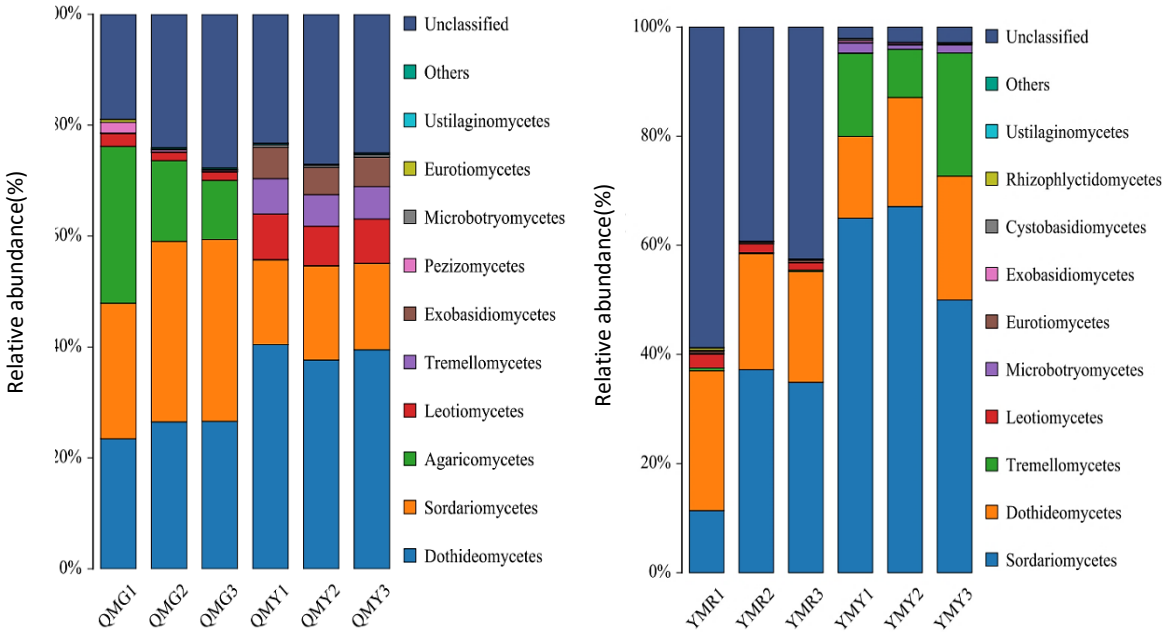

Figure 3. Distributions of endophytic fungi in the roots and leaves of buckwheat and oat at the class level.

The above results showed that Ascomycota and Basidiomycota were significantly dominant in the roots and leaves of buckwheat at the phylum level. Ascomycota was the dominant endophytic fungi in oat roots and leaves which was identical with the previous study in oat seed and stem. Meanwhile, Dothideomycetes and Sordariomycetes were dominant endophytic fungi in the roots and leaves of the buckwheat and oat at the class level, but the proportion were different.

The UPGMA algorithm was used to measure the difference in evolutionary information between buckwheat and oat sample sequences for cluster analysis. Cluster analysis results at the genus level were obtained, as shown in Figure 4. The endophytic fungi of buckwheat were mainly distributed in the following subgroups: *Fusarium*, *Podospora*, *Tilletiopsis*, *Coprinopsis*, *Cyathus*, *Didymella, Alternaria*, *Botrytis*, *Plectosphaerella*, and *Cladosporium*. At the genus level, the third sample of buckwheat root contained more *Coprinopsis*, and the other samples of buckwheat contain more *Cladosporium*. The endophytic fungi in oat were mainly distributed in the following genera: *Alternaria*, *Setophoma*, *Poaceascoma*, *Cladosporium*, *Hannaella*, *Ampelomyces*, *Colletotrichum*, *Saitozyma,* etc. The dominant fungi in oat root were not obvious, but the dominant endophytic fungi in oat leaves was *Colletotrichum*. In addition, the unclassified genus accounted for a large proportions in the roots of buckwheat and oat, and leaves of buckwheat.

Diversity analysis of the endophytic fungal communities in the leaves and roots of buckwheat and oat

The alpha diversities of endophytic fungal communities in buckwheat root and leaf were analyzed. The indexes included Chao1, ACE, Shanon and Simpson. The results showed that the OTU coverage rate reached 99.9%, indicating that the data is reliable (Table 1). The ACE and Chao indices indicated the abundance of species, that is, the number of endophytic fungi in buckwheat roots was higher than that in buckwheat leaves. For the same species abundance, the greater the uniformity of each species in the community, the greater the diversity of the community. According to the Simpson and Shannon indexes, the species diversity of the community of endophytic fungi in buckwheat roots was slightly higher than that in buckwheat leaves.

The alpha diversity analysis results of oat roots and leaves are shown in Table 1. The OTU coverage rate reached 99.9%. The ACE and Chao indexes showed that the number of endophytic fungi in oat roots was significantly higher than that in oat leaves. The Shannon and Simpson indexes showed that the species diversity of endophytes in oat roots was slightly higher than that in oat leaves.

ACE index analysis: buckwheat root > buckwheat leaf > oat root > oat leaf; Simpson index analysis: oat root > oat leaf > buckwheat leaf > buckwheat root. Comprehensive analysis showed that the species

abundance and species diversity of endophytic fungi in the leaves and roots of buckwheat were significantly higher than those in oats.

Figure 4. Community structures and UPGMA clustering tree of endophytes in the roots and leaves of buckwheat and oat at the genus level.

Table 1. α-diversity analysis of endophytic fungal communities in the roots and leaves of buckwheat and oat.

Sample	ACE index	Chao index	Simpson index	Shannon index	Coverage（%）
QMG	200	204	0.0760	3.11	99.99
QMY	171	171	0.0791	3.03	99.98
YMR	136	137	0.2526	2.15	99.99
YMY	116	122	0.2696	2.10	99.99

Analysis of endophytic fungul differences between root and leaf samples of buckwheat and oat

LEfSe (LDA Effect Size) was used to identify the significant biomarkers between groups of roots and leaves of buckwheat and oat. In the buckwheat samples, biomarkers with significant differences in the leaves were much more abundant in the roots according to the biomarker criteria (LDA score > 4) (Figure 5A). The biomarkers in buckwheat leaves included 4 classes, 5 orders, 7 families, 7 genera and 3 species, such as Dothideomycetes, Leotimycetes, Tremellomycetes, Pleosporales, Glomerella, Helotiales, Plectophaerellaceae, Plectophaerella, Botrytis, and Alternaria; the biomarkers in buckwheat roots included 2 classes, 3 orders, 4 families, 4 genera and 4 species, such as Sordariomycetes, Agaricomycetes, Sordarials, Hypocreales, Nidurariaceae, Nectriaceae, Fusarium, and Podospora.

Biomarkers with significant differences in oat leaves were also much more than oat roots (Figure 5B). The biomarkers in oat leaves included 4 orders, 2 classes, 6 families, 8 genera, and 4 species, such as Ascomycetes, Tremellales, Glomeraceae, Trimorphomycetaceae, Colletotrichum, and Dissoconium; The biomarkers in oat roots included Sordariales, Pleosporales, Lasiosphaeriaceae, Nectriaceae, Phaeosphaeriaceae, Podosphaera, Poaceascoma, etc. There were 2 orders, 5 families, 4 genera, and 2 species in total. These biomarkers indicated the difference of endophytic fungul community in the leaves and roots of buckwheat and oat.

Prediction of FUNGuild functional groups of endophytic fungi in root and leaf tissues of buckwheat and oats

To further understand and isolate the functional groups of endophytic fungi in the leaves and roots of buckwheat and oat, endophytic species classification and abundance information were obtained based on the ITS sequences, then the functional groups of endophytic fungi in the roots and leaves of plants were predicted by using the FUNGuild tool. According to the nutrition type, endophytic fungi are divided into three main categories: Pathotroph, Symbiotroph, Saprotroph, and were further subdivided into guilds.

The prediction results of functional groups of endophytic fungi in leaves and roots of buckwheat showed that the endophytic fungi were mainly pathotrophs and saprotrophs, but there were no symbiotrophs. Among the pathotrophs, *Coniothyrium* accounted for 99%. In addition, *Stephylium* accounted for 1%. Among the saprotrophs, *Coniothyrium* accounted for 59.51%, *Podospora* accounted for 34.23%, and there was small amount of *Filobasidium* and *Schizothecium* (Figure 6A). According to the detailed classification results of species function, dung saprotroph was dominant in the root samples, while there was little or no dung saprotroph in the leaf samples. The proportion of plant pathogen-wood saprotroph in the three buckwheat leaves were as high as 98%, while the proportion of QMG1 in roots was 38.36%, QMG2 was 2.73% and QMG3 was 2.25%. Few or no undefined saprotroph were presented in each sample (Figure 6B). The functional groups were different between roots and leaves of buckwheat.

The prediction results of the functional groups of endophytic fungi in the leaves and roots of oat showed that endophytic fungi were mainly pathotroph and saprotroph, but there was no symbiotroph. Among the pathotrophic types, *Coniothyrium* accounted for 100%. *Cercophora* was dominant in the saprophytic type, accounting for 61.84%; *Filobasidium* and *Phialophora* accounted for 22.37% and 15.79%, respectively (Figure 7A); the functional subdivision of functional communities is shown in figure 7B. The proportion of dung saprotroph in oat roots were 0%, 45.03% and 40.78%, respectively; the proportion of plant pathogen-wood saprotroph was 90.16%, 47.02% and 44.69%, respectively; the proportion of undefined saprotroph was 9.84%, 0% and 1.11%, respectively. Wood saprotroph accounted for 0%, 7.95% and 13.41%, respectively. Two types of functional endophytic fungi were detected in oat leaves, namely plant pathogen-wood saprotroph and undefined saprotroph. The proportion of plant pathogen-wood saprotroph were 98.70%, 89.91% and 65.75%, respectively; the proportion of undefined saprotroph was 1.30%, 10.09%, and 34.25%, respectively. Plant pathogen-wood saprotroph were dominant functional group in leaf of buckwheat and oat, which indicated this type of functional group has important ecological functions in leaves.

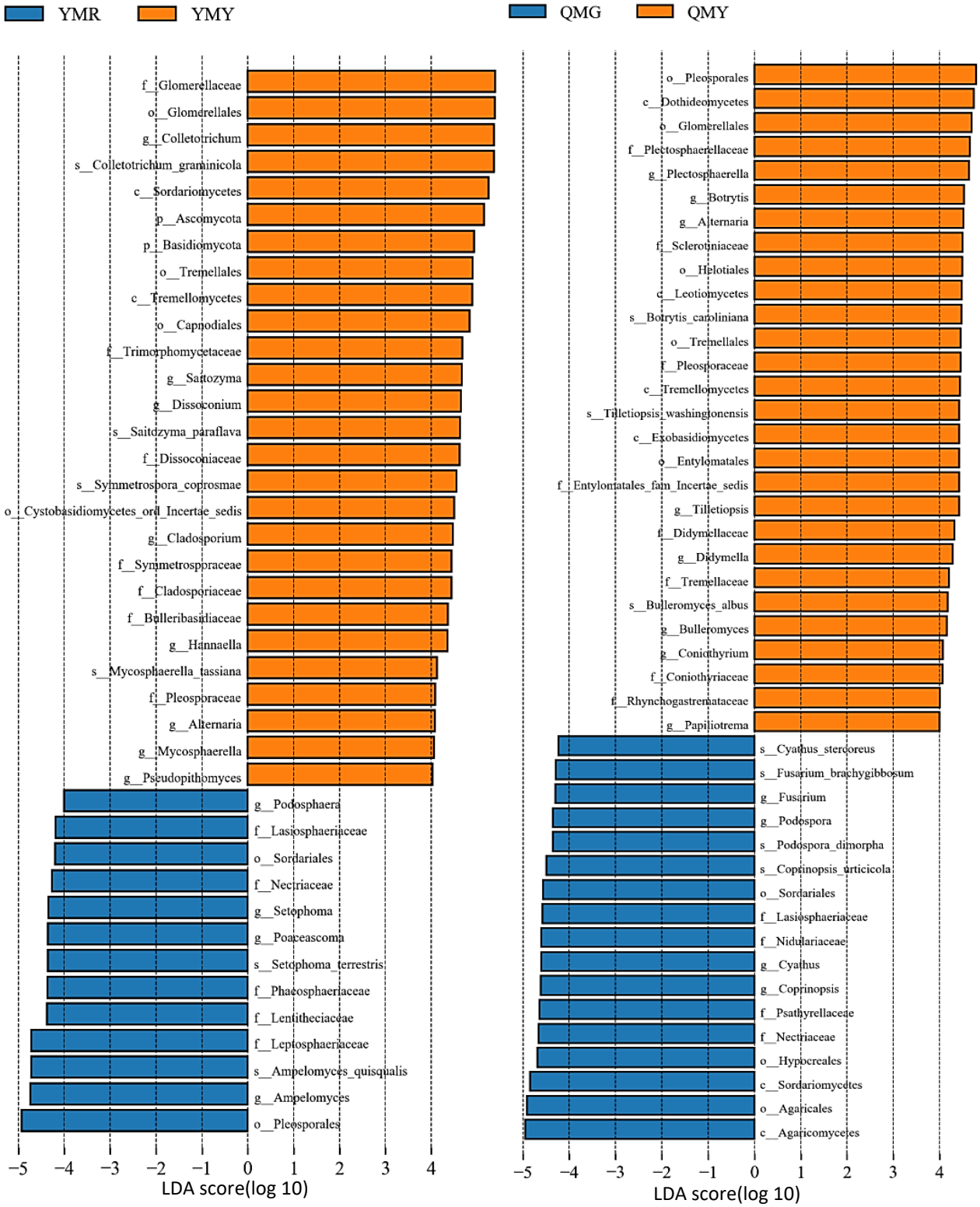

Figure 5. Histogram of LDA value distributions.

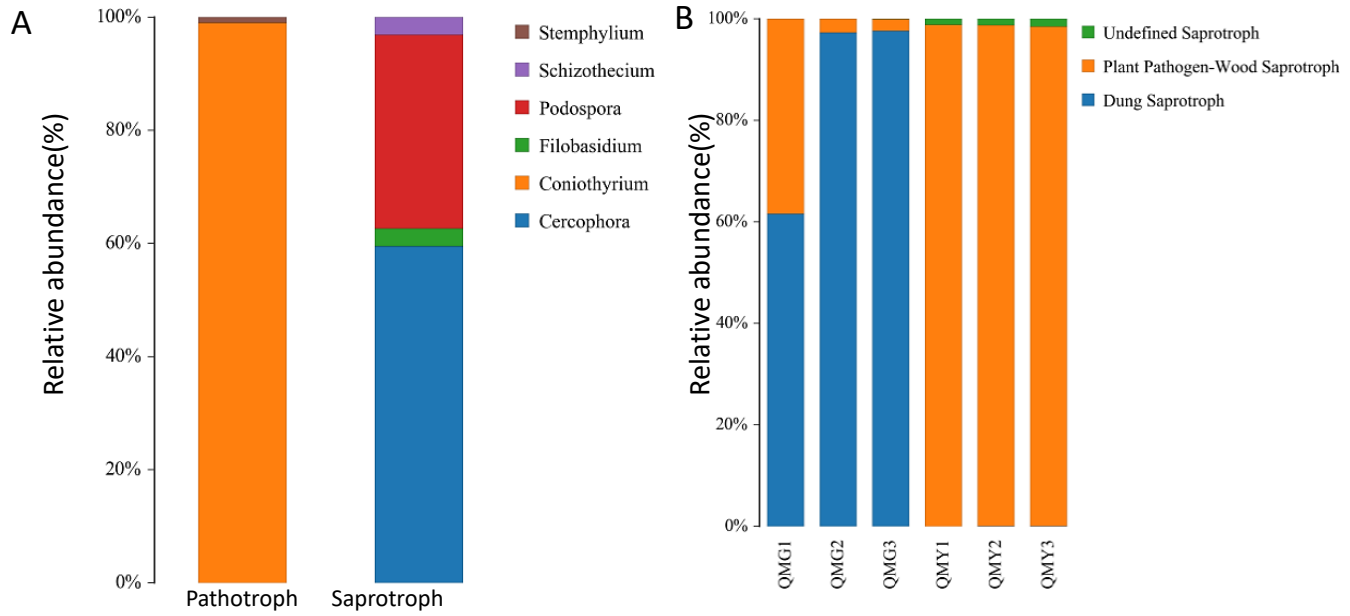

Figure 6. Funguild fungi A - functional classification and B - species histogram of buckwheat roots and leaves.

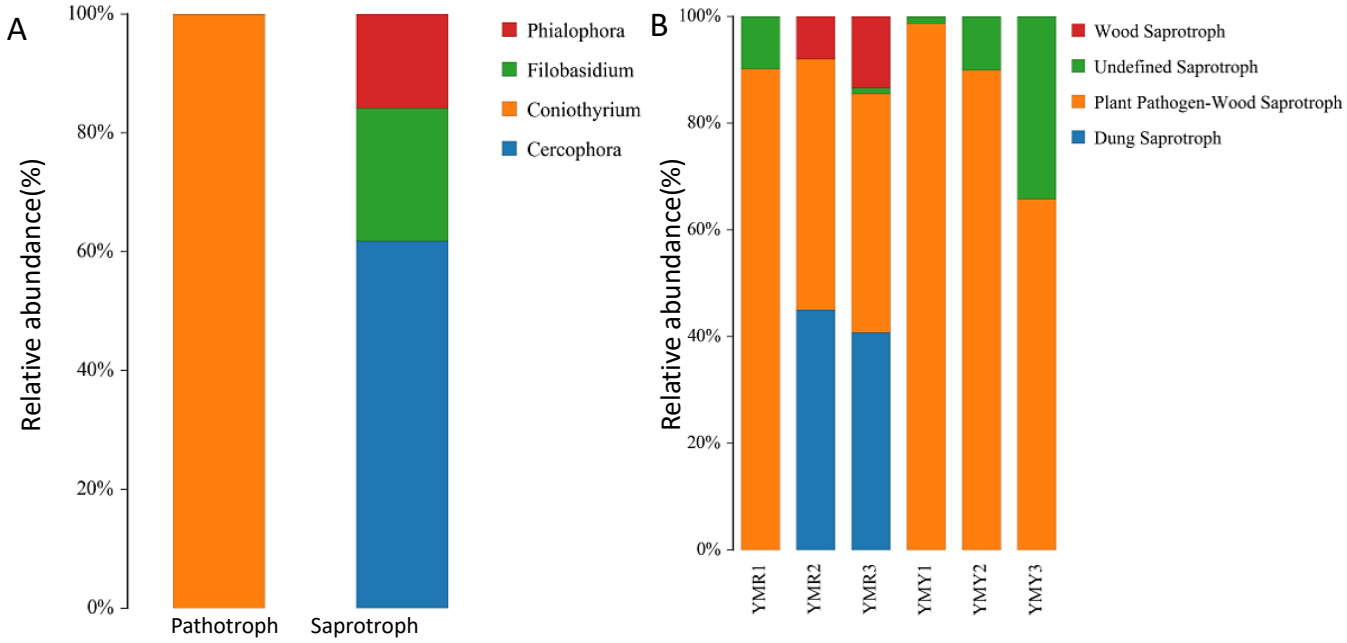

Figure 7. A - Functional classification of fungi and B - species histogram of oat roots and leaves.

4. Discussion

Endophytes play an important role in plant growth, defence, stress tolerance, and yield and so on. The in-depth research in this field is still being conducted and has attracted more and more attention. Research on plant endophytes has not only been carried out on model organisms, but also extended to some important plants with economic value. Buckwheat and oats are coarse grain crops with important health care functions, and there are few studies on endophytic fungi (Mcinroy and Kloepper 1995). High-throughput sequencing technology is widely used in the research on microbial community diversities in plants, animals, food, and environment (Tian et al. 2019; Wu et al. 2021) and has become comprehensive and efficient means to study the microbial community structures at the molecular level. High-throughput sequencing technology can not only provide information of endophytes community structure in plants, but also provide a theoretical basis for isolating of beneficial microorganisms.

In this study, the community structures of endophytic fungi in the roots and leaves of buckwheat and oats were analyzed by high-throughput sequencing. The results showed that there were abundant endophytic fungi in the roots and leaves of buckwheat and oats. They generally shared same fungi, while some fungi were different. Ascomycota and Basidiomycota were dominant in buckwheat; the main endophytic fungi in oats were from Ascomycota. According to previous studies, most plant endophytes are similar at the phylum level. Endophytic bacteria were mainly distributed in Proteus, Firmicutes and Actinomycetes, and the endophytic fungi were mainly distributed in Ascomycetes and Basidiomycetes (Mcinroy and Kloepper 1995). There were 258 OTUs endophytic fungi in oat seeds, and Ascomycota (78%) and Basidiomycota (11%) were dominant (Dai et al. 2020). In oat stems, Ascomycota and Basidiomycota were also the dominant endophytic fungi (Sun et al. 2020). It was basically consistent with the results of this study, which fully demonstrated that plant endophytes have similarities in the larger taxa. At the class level, Dothideomycetes were dominant in oat seeds and stem (Dai et al. 2020; Sun et al. 2020), which is consistent with our study in oat roots and leaves. On the other hand, the differences in species and abundances of endophytic fungi existed in different hosts and different tissues, and biomarkers were identified in every tissue of buckwheat and oat. The endophytic fungal communities in the roots and leaves of buckwheat or oat were quite different at the genus level, which may be related to the degrees of light exposure and nutrient accumulation in different plant tissues. Dai et al. (2020) also found substantial differences in community compositions across host species and locations. More experimental data would further provide a basis for studying effects of host and environment on the endophytic fungul community structures.

Prediction of FUNGuild functional group showed that the pathophytotrophic groups included the genus *Coniothyrium minitans*. *C. minitans* is an important parasitic fungus, which can degrade oxalic acid secreted by the *Sclerotia* and enhance the activity of extracellular lyases, such as chitinase and *β*-1.3-glucanase. Thus, it can destroy the mycelia and sclerotia of *Sclerotia*, promote the absorption of nutrients and then promote the growth of plants (Lou et al. 2016; Sun et al. 2017). It is a very potential biocontrol fungus. This study also found that *Alternaria and Cladosporium* were the dominant genera in the fungal endophyte communities in the roots and leaves of buckwheat and oat. The genera *Alternaria* and *Cladosporium* were also prevalent in oat stems (Dai et al. 2020). Cladosporium can synthesize a variety of compounds, which can endow plants with antibacterial and cytotoxic activities, and have good inhibitory effects on pathogens. In buckwheat, a beneficial endophytic fungi Fat9 was used for hairy root cultures, and it enhanced rutin production in tartary buckwheat. So, there are many beneficial endophytic fungi in various tissues of buckwheat and oat, and further work is required to isolate these fungi and understand their potential roles.

This study provided important guidance for the subsequent isolation of endophytic fungi, and provided a basis for studying the relationship between endophytic fungi and plants. In addition, it contributes to understand the relationship between oat and buckwheat health value and plant nutrition components from view of microorganism and enhance their economic value.

5. Conclusions

In this study, the endophytic fungi in the roots and leaves of buckwheat and oat were analyzed by high-throughput sequencing technology. There were 205 OTUs in buckwheat roots and 181 OTUs in buckwheat leaves based on 97% sequence similarity level. There were 152 OTUs in the roots and 127 OTUs in the leaves of oat. At the phylum level, Ascomycota and Basidiomycota were the dominant endophytic fungi in buckwheat roots and leaves, while Ascomycota was the dominant endophytic fungus in oat roots and leaves. The richness and diversity of the endophytic fungal community in roots were higher than those in leaves. Biomarkers were found by significant difference analysis in buckwheat and oat. The endophytic functional groups of buckwheat and oat mainly consisted of pathotroph and saprotroph.

Authors' Contributions: GAO, Y.: conception and design, acquisition of data and analysis and interpretation of data. LI, Y.: analysis and interpretation of data and drafting the article; HAN, Y.: drafting the article and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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