



The assembly of wheat-associated fungal community differs across growth stages

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Abstract

Plant-associated fungal communities play a vital role in plant adaptations, physiological functions, and productivity. Therefore, it is important to reveal the mechanisms driving the assembly of these communities. Yet it is still not fully understood how community assembly and structure differentiate in plant compartments, growth seasons, and varieties at large geographic distances. In this study, we analyzed bulk soil and plant-associated fungal communities of five wheat varieties across two growth stages in three biogeographic sites with distances of about 324, 534, or 800 km apart between any two locations. Our results indicated that the fungal community varied primarily across the sample types (leaf endosphere, root endosphere, rhizosphere, and bulk soil), followed by growth stage. Compared with the regreening stage, lower α -diversity and more dominance by abundant species in the fungal community were observed in wheat-associated compartments (four sample types except for bulk soil) at the heading stage. Additionally, within each wheat-associated compartment across every growth stage, location had stronger effects on fungal community assembly than the wheat variety. The effects of variety on fungal community assembly were location specific as were the growth-stage patterns of varietal effects on leaf endosphere and rhizosphere fungal communities. We further detected a less diverse but abundant core fungal taxa that could be grouped into three clusters associated mainly with location. This study characterized the interplay effects between plant selection (compartment, growth stage, variety) and environment (location) on wheat-associated mycobiomes by determining drivers of fungal community assembly and core fungal taxa in field conditions.

Key points

- Fungal community assembly was mainly shaped by sample type and growth stage.
- A lower diversity and more abundant core fungal taxa were shown at heading stage.
- Location had stronger effects on fungal community assembly than variety.

Keywords Mycobiome · *Triticum aestivum* L. · Endosphere · Rhizosphere · Growth stage · Location · Core fungal taxa

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Introduction

Mycobiomes are ubiquitous in soil and plants, and the interactions between fungi and plants affect plant growth and health and ultimately ecosystem functions (Berg et al. 2005; Rodriguez et al. 2009). For instance, endophytic fungi form commensal and mutualistic symbiotic relationships with plants (Albornoz et al. 2020; Rodriguez et al. 2009) that can improve plant development by promoting root formation and shoot growth (Sun et al. 2010), enhance resistance to biotic and abiotic stresses (Arnold et al. 2003; Kannadan and Rudgers 2008), and play a significant role in both carbon and nutrient recycling in ecosystems (Sun et al. 2011; Voříšková and Baldrian 2012). However, some endophytic fungi have long latent periods, and some can become pathogenic in

stressful environments (Arnold 2007). Therefore, the mutualistic or antagonistic interaction between endophytic fungi and plants is dependent on genetic and/or environmental conditions (Vincent et al. 2016). Similar to fungal endophytes, rhizosphere fungi also have essential influences on plant fitness and indirectly affect the structure and function of the plant community (Jogaiah et al. 2013; Philippot et al. 2013). Thus, understanding the fungal community assembly, dynamics and the activity of endophyte and rhizosphere fungi could help to develop new strategies for promoting plant growth and health.

Plant mycobiomes may vary between different plant species or varieties, plant compartments, plant health, developmental stages, and geographic locations (Martínez-Diz et al. 2019). The relative contributions of these drivers shaping mycobiomes vary greatly among various studies. Previous studies have revealed that compartment niche is the largest contributor in shaping distinct fungal communities (Cregger et al. 2018; Hamonts et al. 2018). Unique ecological niches could shape plant mycobiomes through interplay with plant species, plant chemical nature (e.g., C/N ratio, cellulose, and lignin contents), edaphic condition, and many other factors. Fungal communities within various plant compartments may have different responses to biotic (e.g., plant variety, growth stage) and abiotic (e.g., location) factors, which could further differentiate fungal community structure.

Studies about soybean rhizospheres (Han et al. 2016) and winter wheat-associated compartments (Grudzinska-Sterno et al. 2016) have shown that fungal communities are shaped more significantly by growth stage than by location, which may be attributed to the versatility of fungi that could interact with plants as pathogens, symbionts, and saprotrophs at different growth stages (Albornoz et al. 2020; Pawłowska et al. 2014; van der Wal et al. 2015). The availability of energy resources for plant-associated microbes differs across plant growth stages. For instance, compared with the vegetative growth stage, plants allocate less sugar to the root during and after the flowering growth stage so that saprotrophic fungi play important roles at this time when decomposing roots are the main ecological niche available (Yu et al. 2012). However, higher fungal diversity (especially in arbuscular mycorrhizal and pathogenic fungi) during the vegetative growth stage may also be explained by sufficient carbon resources (Yu et al. 2012). Additionally, there are positive relationships between saprotroph richness and mean annual precipitation and between ectomycorrhizal fungal richness and host plant species diversity and soil pH. Negative and positive relationships are also observed between pathogen richness and latitude and nitrogen availability, respectively (Wardle and Lindahl 2014). One study suggests that location plays a large role in the fungal community assembly of sorghum rhizospheres (Schlemper et al. 2018). Reportedly, the

growing region rather than the growth stage explains more variation in the fungal community in the sugarcane root and rhizosphere compared to the leaf and stalk because the root and rhizosphere can recruit a larger number of fungi from soil species pools (Hamonts et al. 2018). Therefore, growth stage effects on plant-associated fungal communities may depend on and interact with multiple factors such as plant species, plant compartment niche, and growing field.

Additionally, crop variety effects on the fungal community are complex. Significant effects of variety are not detected in wheat rhizosphere fungal communities in eight African and European soils because the large number of fungal taxa is consistently associated with multiple wheat varieties grown in the same field (Simonin et al. 2020). Conversely, wheat variety plays important roles in the leaf fungal community, and the variety effects are larger in older leaves than in younger ones (Sapkota et al. 2017). The sugarcane variety exerts significant effects on fungal assemblages associated with leaf, stalk, root, and rhizosphere soil, and interplay effects between the variety and crop age and between the variety and location are larger than the sole variety effects (Hamonts et al. 2018). Therefore, interactions between plant, growing location, and fungal community should be considered when exploring the mechanisms shaping plant-associated mycobiomes. Thus, a systematic understanding is necessary of how sample type, location, growth stage, and host variety interactively drive the assembly of crop mycobiomes.

The core microbiota represent the microbial populations that are systematically associated with a given host. These plant core populations are considered to be vital for plant health based on evolutionary processes that select microorganisms carrying genes with essential functions for holobiont (e.g., plant plus associated microbiota) fitness (Lemanceau et al. 2017). For example, a small number of core fungal taxa are identified as hub species in wheat rhizospheres based on the cross-domain co-occurrence networks (Schlatter et al. 2020). Thus, defining the core fungal taxa of plant-associated compartments and their responses to biotic and abiotic factors could help us to find key fungal populations for sustaining plant health and improving productivity.

In this study, we investigated fungal community diversity, composition, and core taxa in five varieties of wheat leaf endospheres, root endospheres, rhizospheres, and in bulk soils across two growth stages from three locations based on ITS2 rDNA sequencing. The primary goals aimed to investigate (1) the fungal communities in different compartments of wheat (leaf endosphere, root endosphere, and rhizosphere) and in bulk soils; (2) the interplay effects of major biotic (wheat variety) and abiotic (location) factors on fungal community assembly; and (3) the core mycobiome in wheat.

Materials and methods

Study sites and sampling

Five winter wheat varieties were sown in the same field at each of three locations in China, including Yang Ling, Shan Xi province; Nan Yang, He Nan province; and Su Qian, Jiang Su province, in October 2018 and harvested in early June 2019. These three sites are distributed from west to east along a similar latitude with different annual precipitation and soil properties. Detailed information about the climate, geographical coordination, and soil characteristics of sampling locations were described previously (Zheng et al. 2021). The five winter wheat varieties were Jimai22 (JM22), Xinong511 (XN511), Xiaoyan22 (XY22), Yanzhan4110 (YZ4110), and Zhoumai18 (ZM18). Plant and soil samples were collected in mid-March and late April 2019, representing the regreening and heading stages, respectively. The regreening stage occurs in early spring when the new leaves in 50% of wheat extend 1–2 cm out of the leaf tips. At the heading stage, 50% of young ears of wheat protrude from the tip of the sword leaf in the field.

Three healthy plants were collected separately as triplicate samples for each wheat variety, and nine bulk soil samples were collected at the depth of 0–20 cm several meters from plants at each location and growth stage. To investigate the fungal endophytic community in wheat leaves, we chose different leaves (without the leaf tip fraction) randomly from the same wheat individual and inserted them into a 50-ml sterile Falcon tube. A 40-ml sterile phosphate buffered saline (PBS) solution was added into a sample tube and shaken vigorously for several minutes by hand. Leaves were further cleaned five to six times using a PBS solution in a sterile Petri dish and then collected as a leaf endosphere sample.

Similarly, fresh roots were collected into a 50-ml Falcon tube. We added 40 ml of PBS into the tube, shook vigorously, and removed roots from the tube. The suspension was centrifuged, and the pellet was collected as rhizosphere soil. The root sample was further cleaned similarly to the leaves and was collected as a root endosphere sample. In this way, the root sample may have contained an endosphere plus tightly bound surface microorganisms. In total, we obtained 90 leaf endospheres, 90 root endospheres, 90 rhizospheres (triplicate samples for 5 wheat varieties from 2 growth stages growing at 3 locations), and 54 bulk soil samples (9 soil cores from 2 growth stages growing at 3 locations). All samples were stored at $-20\text{ }^{\circ}\text{C}$ for DNA extraction.

DNA extraction, PCR amplification, and sequencing

Sample genomic DNA (deoxyribonucleic acid) was extracted with a MoBio Powersoil™ DNA isolation kit (San Diego, CA, USA). Primers ITS4 (5'-TCCTCCGCT TATTGATATGC-3') and gITS7 (5'-GTGARTCATCGA RTCTT TG-3') were used for the amplification of a fungal ITS gene tagged with a 12 bp barcode on ITS4 (Ihrmark et al. 2012; Xu et al. 2020).

The 25 μL polymerase chain reaction (PCR) system contained 20 ng of template DNA, 1 μL of each primer (10 μM), 9.5 μL of sterilized water, and 12.5 μL of Master-Mix containing Taq DNA polymerase, Mg^{2+} , PCR buffer, and dNTPs. The PCR amplification procedure involved an initial denaturation for 5 min at $94\text{ }^{\circ}\text{C}$, followed by 35 cycles of amplification ($94\text{ }^{\circ}\text{C}$ for 30 s, $56\text{ }^{\circ}\text{C}$ for 30 s, and $68\text{ }^{\circ}\text{C}$ for 30 s), with a final extension at $72\text{ }^{\circ}\text{C}$ for 10 min. The purified PCR products from all samples were mixed at equimolar amounts and sequenced using an Illumina HiSeq sequencer (Illumina, San Diego, CA, USA; 2×250 bp paired ends).

Sequence analysis

The QIIME pipeline (<http://bio.cug.edu.cn/qiime/>) was used to process the original sequences. Chimera sequences were checked using the software Vsearch (Rognes et al. 2016). The sequences not assigned as any fungal phyla were discarded. The operational taxonomic unit (OTU) was identified at the threshold of 97% identity. Taxonomy was assigned using the UNITE database (Koljalg et al. 2013). We resampled 1031 sequence reads for each sample, and 6 leaf samples were discarded for having less than 1000 sequences. Finally, we obtained 318 sequence datasets in this study. Bulk soil and wheat-associated core fungal taxa were defined as those OTUs occurring in at least 80% of samples within each sample type at each growth stage with no relative abundance threshold. Alpha diversity metrics were calculated on the rarefied OTU tables using the Python script in the QIIME pipeline (`alpha_diversity.py`). The raw sequence data were available at the National Center for Biotechnology Information's Sequence Read Archive (NCBI-SRA) with the accession number of PRJNA718428 and Microbiome Database (<http://egcloud.cib.cn>) with project number PRJ-AMPLI-39fd39e613afd113e438cd8e7be15d39.

Statistical analyses

Constrained (CPCoA) and unconstrained (PCoA) principal coordinate analyses with weighted UniFrac dissimilarity were performed to explore the fungal community structure (β -diversity). A significant difference in the fungal community structure was calculated using two-way permutational analysis of variance (PERMANOVA) in the vegan package

(<http://cran.r-project.org/web/packages/vegan/index.html>). Variance analysis (ANOVA) and subsequent post-hoc Tukey's tests were conducted to compare the differences in α -diversity across sample types and growth stages. Differential abundance analysis was performed using the negative binomial generalized log-linear model in the edgeR package (<http://www.bioconductor.org/packages/release/bioc/html/edgeR.html>). Low-abundance OTUs with fewer than five sequences in all samples were removed in this analysis. FUNGuild (Nguyen et al. 2016) was used to assign each OTU into an ecological guild to predict which core taxa belong to which fungal functional group. Indicator species analysis was performed in the labdsv package (<https://cran.r-project.org/web/packages/labdsv/index.html>). All of these analyses were visualized in the ggplot2 package (<https://cran.r-project.org/web/packages/ggplot2/index.html>) in R version 4.0.0. Hierarchical cluster analysis (HCA) and heatmap analysis of the core fungal community structure were performed using the R package pheatmap (<https://cran.r-project.org/web/packages/pheatmap/index.html>).

Variance decomposition

The linear regressions based on principal components across all sample types, locations, growth stages, and varieties were used to determine the variance decomposition of the overall fungal community structure (William et al. 2018). The linear regression was run with the following models:

$PC_i \sim \text{Sample type} * \text{growth stage} * \text{location}$ (or $PC_i \sim \text{Sample type} * \text{growth stage} * \text{location} * \text{variety}$ when the datasets were used without bulk soil data), where PC_i is the i th PC (principal coordinate), and "*" indicates this model included the main effects of the sample type, location, growth stage and variety as well as their interplay effects. The sum-of-squares explained by each model component (including residuals) was used to calculate the variance.

Defining index HEEI, DSI, and DI

According to Xiong et al. (2020), the host-environmental index (HEEI, $HEEI = \text{relative contribution of wheat variety/relative contribution of location}$, based on PERMANOVA analysis in Table 1) is defined to calculate the effects of wheat variety and location within each compartment niche across two growth stages. The lower HEEI value (< 1) represents the higher location effects.

The dissimilarity index (DSI) is defined as the sum of the numbers of enriched OTUs and the numbers of depleted OTUs against bulk soil/total OTUs (low-abundance OTUs with less than five sequences in all samples were removed). The depleted index (DI) is defined as the number of depleted OTUs/the number of enriched OTUs against bulk soil. DSI and DI were used to evaluate the effect extent of each

Table 1 Two-way PERMANOVA test of the variations of wheat-associated fungal communities based on weighted UniFrac distance

Variables		Regreening		Heading	
		R^2 (%)	p	R^2 (%)	P
Leaf					
Single factor	Location	21.46	0.001	36.00	0.001
	Variety	18.09	0.001	9.71	0.048
Two factors' interaction	Location*variety	32.28	0.001	12.82	0.292
Root					
Single factor	Location	35.05	0.001	49.34	0.001
	Variety	11.10	0.004	9.69	0.004
Two factors' interaction	Location*variety	26.15	0.001	14.23	0.014
Rhizosphere					
Single factor	Location	37.96	0.001	20.92	0.001
	Variety	9.55	0.004	14.25	0.001
Two factors' interaction	Location*variety	21.29	0.001	33.09	0.001

enrichment process. The higher DSI value represents greater differences between each compartment niche and bulk soil, and the higher DI value represents a greater depleted effect.

Results

The assembly of wheat-associated mycobiome was mainly shaped by sample type and growth stage

Unconstrained PCoA analysis based on the weighted UniFrac distance indicated that fungal communities clustered based mainly on sample types in the first axis. Rhizosphere and bulk soil samples clustered more closely, and fungal communities in leaf and root endosphere samples were more similar (Fig. 1). Within each sample type (especially in root endosphere and rhizosphere samples, but not including bulk soil samples), samples from the regreening and heading stages showed distinct mycobiome clusters (Fig. 1). The results of variance decomposition based on each principal coordinate of the PCoAs also indicated that the PC1 of weighted UniFrac analysis was mainly attributed to sample type, and the variance in PC2 was mainly attributed to growth stage (Supplemental Fig. S1; based on all sample datasets including bulk soil samples). Even without bulk soil samples, the variances in PC1 and PC2 could also be attributed to growth stage and sample type (Supplemental Fig. S2; the datasets without bulk soil samples).

The α -diversity indices, including Shannon index, evenness and Faith phylogenetic diversity, and the number of observed OTUs, were all highly dependent on the sample

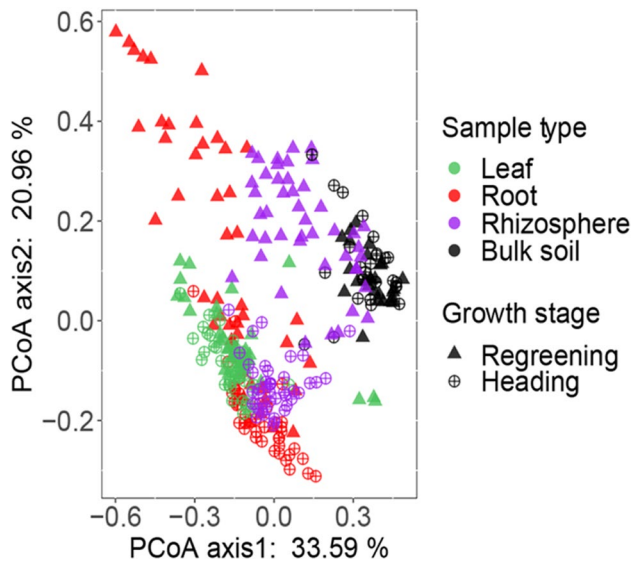


Fig. 1 PCoA plot showing the variations of fungal communities for all samples based on the weighted UniFrac distance

types (Tukey's HSD test: $P < 0.05$), with the highest fungal diversity found in bulk soil, followed by rhizosphere, and consistently lower fungal diversity found in the leaf and root endosphere samples (Supplemental Fig. S3). Within each sample type, the α -diversity indices (Shannon index) in leaf endosphere, root endosphere, and rhizosphere samples were significantly lower in the heading stage than in the regreening stage (Supplemental Fig. S4). However, the α -diversity indices in bulk soil samples were not significantly different between the two stages (Supplemental Fig. S4).

Using the OTU counts in the bulk soil as a comparison, more OTUs were depleted in leaf and root endosphere samples compared with the rhizosphere, resulting in higher DI values in leaf and root endosphere samples and a lower DI value in rhizosphere samples (Fig. 2). The DSI values gradually increased from epiphyte to endophyte, with higher DSI values found in all three wheat-associated compartments at the heading stage compared with the regreening stage (Fig. 2). Additionally, those significantly enriched OTUs, mainly including families *Pleosporaceae*, *Olpidiaceae*, and *Eocronartiaceae*, were overlapped in three plant compartments at the regreening stage (total 7 OTUs). At the heading stage, a total of 14 OTUs, belonging to *Pleosporaceae*, *Mycosphaerellaceae*, and *Didymellaceae*, were significantly enriched in all three wheat-associated compartments. There were 55 and 109 OTUs depleted in all three wheat-associated compartments at the regreening and heading stages, respectively. Among them, families *Chaetomiaceae* (9 OTUs in the regreening stage and 24 OTUs in the heading stage), *Lasiosphaeriaceae* (6 OTUs in the regreening stage and 14 OTUs in the heading stage), and *Pseudeurotiaceae* (5 OTUs

in the regreening stage and 6 OTUs in the heading stage) were all depleted at the regreening and heading stages.

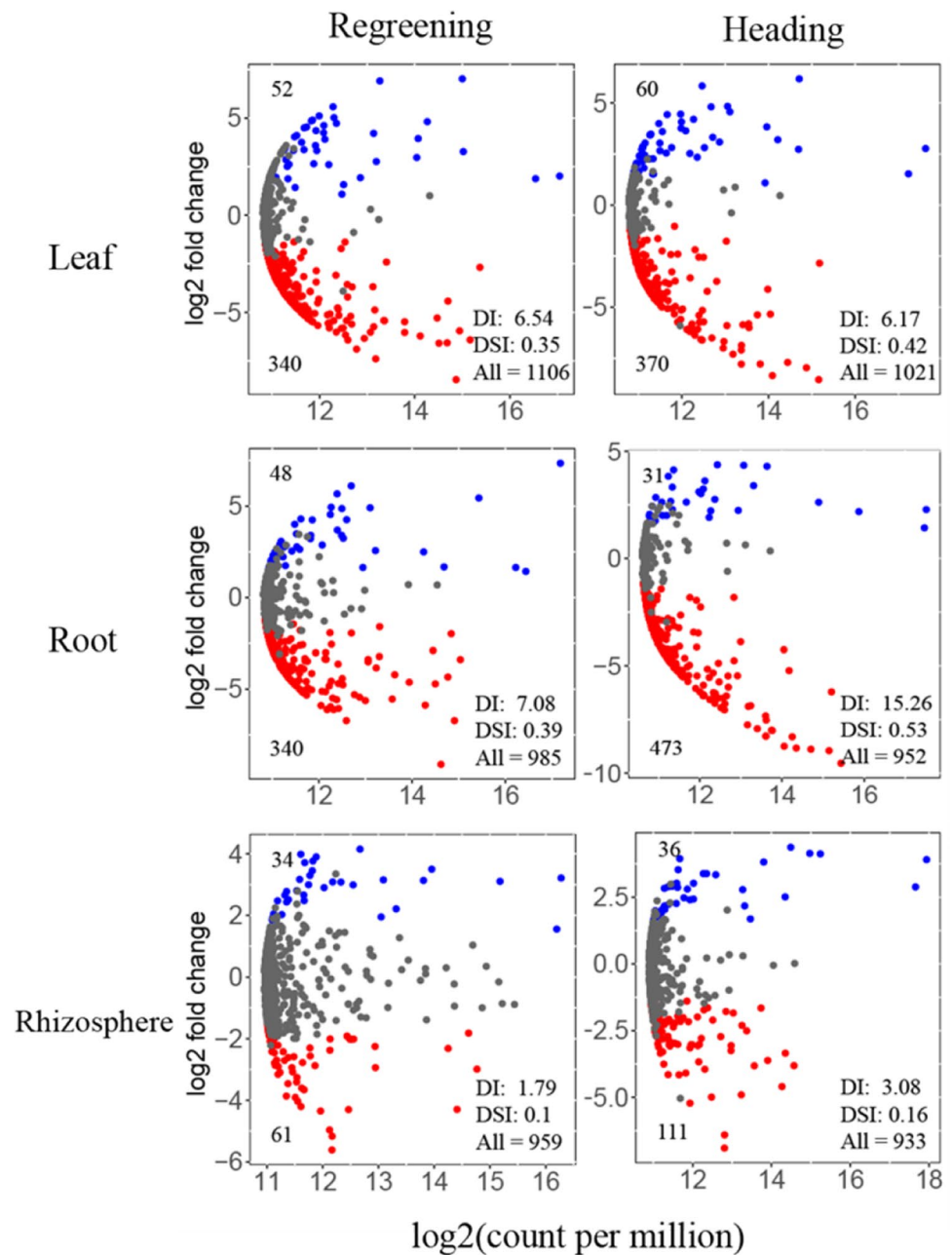
The effects of location and variety on wheat-associated mycobiomes showed distinct growth stage patterns

Within each wheat-associated compartment niche at each growth season, the results of PCoA and PERMANOVA based on the weighted UniFrac distance showed that variation in the fungal community was mainly explained by location (Supplemental Fig. S5, Table 1). In the leaf and root endosphere samples, the significant effects of location contributed a larger source of variation in mycobiomes at the heading stage and in the rhizosphere samples at the regreening stage (Supplemental Fig. S5, Table 1). PERMANOVA results also indicated that the variety had significant effects on mycobiomes in wheat-associated compartments across each growth season. Therefore, the relative host effects (variety effects) and environmental effects (location effects) in wheat-associated compartment niches varied across growth stages. In the regreening stage, the HEEI value gradually increased from the rhizosphere to the root and leaf endospheres. However, in contrast to leaf and root endosphere samples with lower HEEI values at the heading stage, the HEEI value of rhizosphere samples increased sharply and became highest at the heading stage (Supplemental Table S1).

Varietal effects on fungal community were location specific

The PERMANOVA results showed that the interactions between variety and location had significant effects on fungal communities in the leaf endosphere, root endosphere, and rhizosphere. In addition, the interactive effects between variety and location were larger than the sole varietal effect (Table 1). We therefore conducted constrained PCoA analysis based on weighted UniFrac distance for each location to focus on true varietal effects. When we combined the three location datasets together, significant effects of variety contributed a larger source of variation in leaf and root endosphere mycobiomes at the regreening stage, and stronger effects of variety on rhizosphere samples were shown at the heading stage (Table 1). However, these stage patterns of varietal effects on leaf endosphere and rhizosphere fungal communities were location specific. For leaf endosphere and rhizosphere samples, only in the location Yang Ling, the varietal effects on mycobiomes at the regreening and heading stages showed similar patterns to those considering all three location datasets together (Fig. 3).

Fig. 2 The volcano plot illustrating the patterns of enriched and depleted OTUs in each wheat-associated compartment compared with bulk soil. Each point represents an individual OTU (Low-abundance OTUs with less than five sequences in all samples were removed). Each blue point represents an enriched OTU, and a red point represents a depleted OTU. The position along the y-axis represents the fold changes in the relative abundance compared with bulk soil, and the x-axis represents the average abundances of OTUs (as counts per million reads, CPM)



The core mycobiome of field-grown wheat

We identified a core set of fungal taxa for each sample type at each growth season consisting of a small number of relatively abundant fungal OTUs (Fig. 4). The core mycobiome comprised only 0.8–1.7% of all fungal OTU numbers observed in different sample types at the regreening or heading stage (Fig. 4). However, these core OTUs accounted for an average of 53.3–86.3% of the relative abundance of all fungi (Fig. 4). A majority of these core fungal OTUs were classified at the species level, and 12.91–46.54% remained unclassified.

Compared with the regreening stage, the core taxa in wheat-associated compartments (especially in root endosphere and rhizosphere samples) were lower in diversity but higher in abundance at the heading stage (Fig. 4). The species *M. tasiana* and *A. alternata* accounted for high proportions in the core mycobiomes of the leaf endosphere, root endosphere, and rhizosphere at the heading stage (Fig. 4). However, these two fungi species had lower proportions in bulk soils, and the obviously increasing trend at the heading stage in other sample types was not observed in the bulk soil samples (Fig. 4). According to the FUNGuild prediction, all core taxa belonging

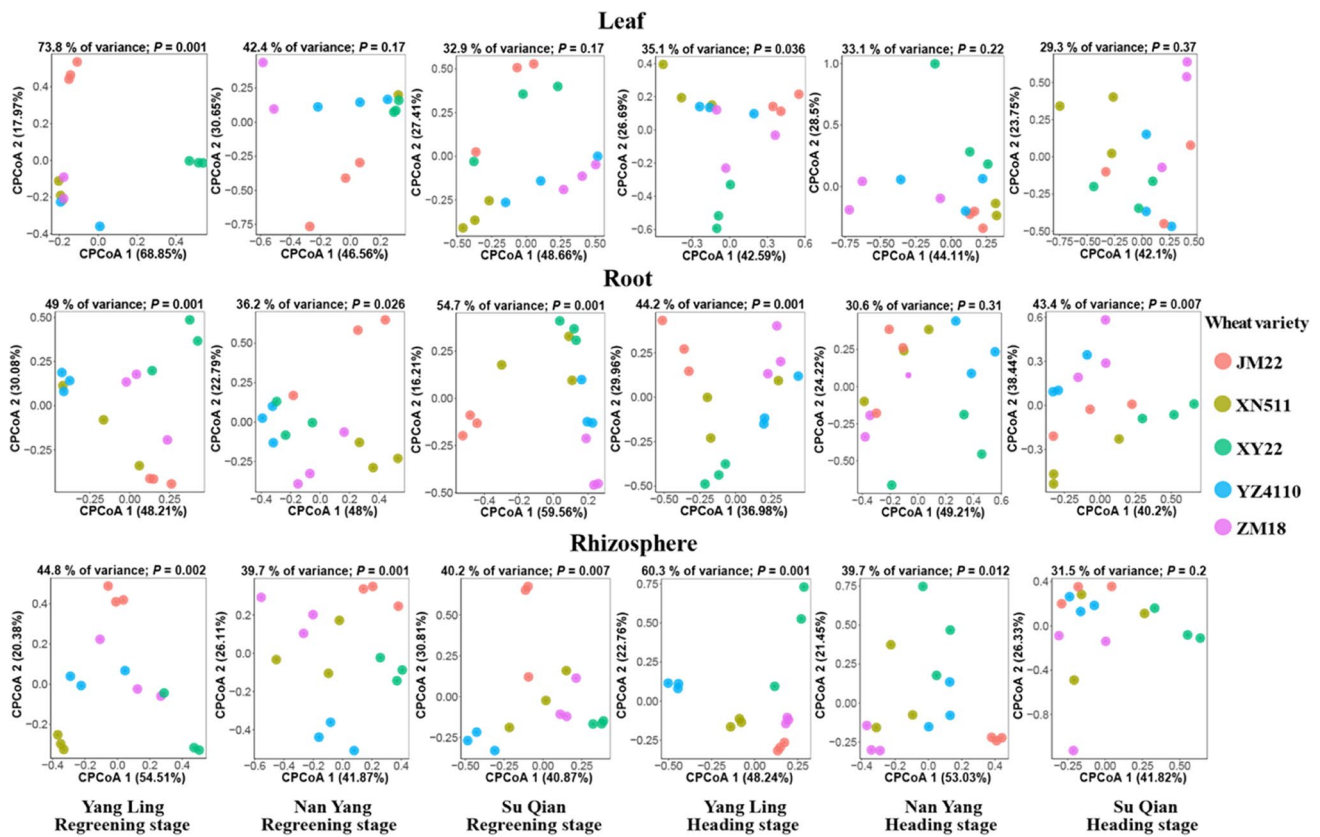


Fig. 3 Constrained PCoA plots showing the variations of fungal communities in three wheat-associated compartments based on the weighted UniFrac distance among five wheat varieties from three

locations across two growth stages. Symbols represent microbiomes and are colored by variety. Total variance attributed to the constrained factor and the significance of the factor are shown in each plot

to *M. tassiana* were pathotrophs, and *A. alternata* could be considered a pathotroph, saprotroph, or symbiotroph.

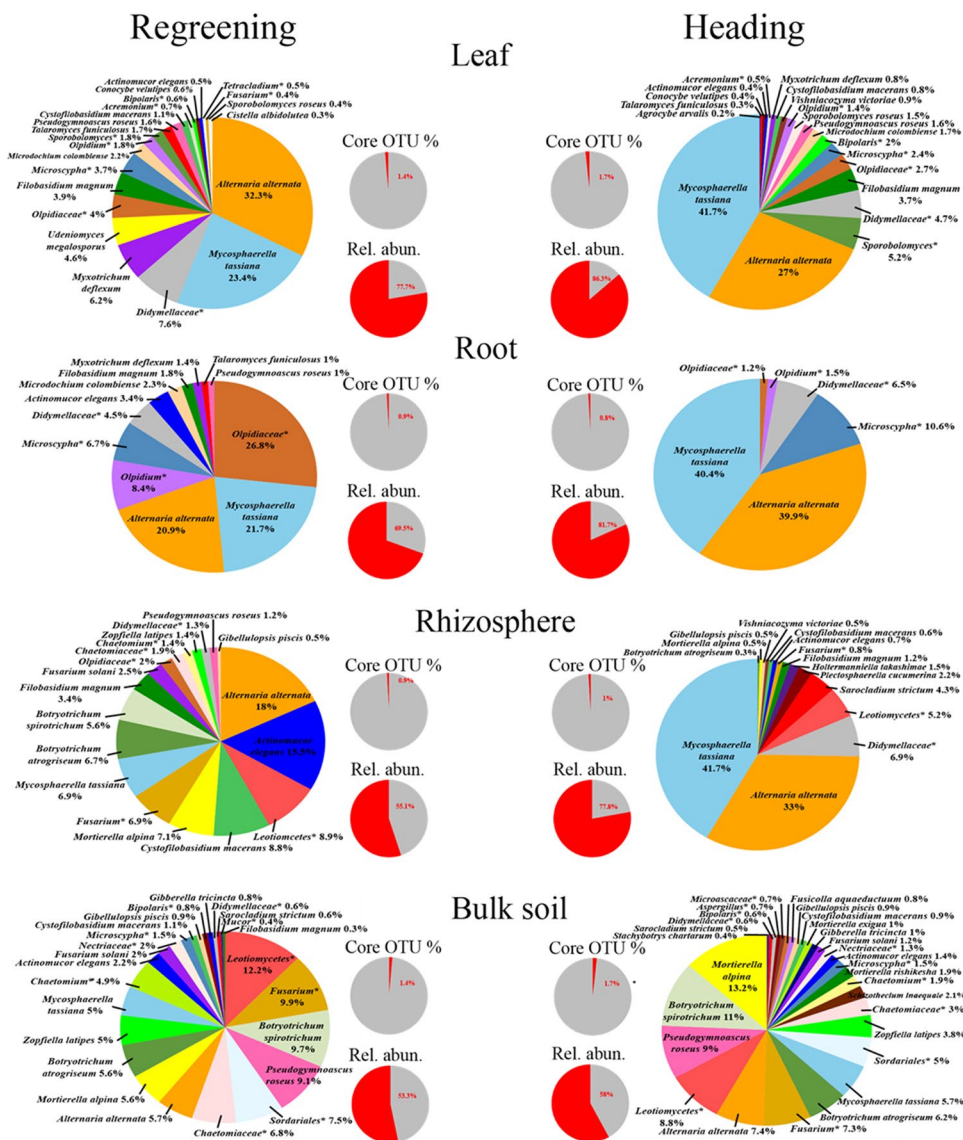
Based on the relative abundance of each core taxon in wheat-associated compartments at the regreening or heading stage, the core taxa of the leaf endosphere and root endosphere at the heading stage and rhizosphere at the regreening stage could be clustered into three groups principally associated with location (Supplemental Fig. S6). A larger number of core fungal taxa varied greatly in relative abundances at different locations (Supplemental Fig. S7). Additionally, we identified several indicator species of core fungal taxa in leaf and root endosphere samples at the heading stage as well as in the rhizosphere sample at the regreening stage. Indeed, the abundances of these fungal indicator species varied greatly in different locations (Fig. 5), and they accounted for an average 32.51–59.23% of the relative abundance of all core fungal taxa.

Discussion

The assembly of wheat mycobiome is primarily determined by compartment niche

Previous studies have shown that plant-associated fungal assemblages are primarily determined by plant compartment (Grudzinska-Sterno et al. 2016; Hamonts et al. 2018). This study also suggested that wheat-associated mycobiome assembly was primarily determined by compartment niche and followed by growth stage based on complete datasets from all locations (Fig. 1 and Supplemental Fig. S1). This determination is likely caused by niche partitioning in various plant compartments, microbial life history differences, and distinct regional species pools that plants can select specific species in different

Fig. 4 The core microbiomes in wheat-associated leaf endosphere, root endosphere, rhizosphere, and bulk soil at the regreening and heading stages, respectively. Core OTU percentage (Core OTU %) representing the core OTU number as a percentage of the total OTU number observed in each compartment at the regreening or heading stage. Relative abundances (Rel. abun.) of fungal core OTUs and their taxonomy are indicated (* = unclassified at the species level)



habitats (e.g., soil microbial species pool for roots and rhizospheres, species in rainfall and aerial dispersal for leaves). Plant compartments leading to different fungal communities indicates that the strength of microbial community differentiation is influenced by biotic (plant property, niche preference of microbes, interactions between wheat and microbes, etc.) and abiotic factors (e.g., nutrient and water availability, light exposure).

Compared with leaf and root endospheres, fungal diversity was higher in rhizospheres and in bulk soils (Supplemental Fig. S3). This is consistent with previous studies (Beckers et al. 2017; Cregger et al. 2018). The leaf endosphere fungal community had greater diversity than the root endosphere community (Supplemental Fig. S3). Leaves are subject to harsh conditions, such as ultraviolet radiation exposure, limited nutrient availability, and sharp temperature

differences throughout the diurnal rhythm (Remus-Emsermann and Schlechter 2018). Thus, microbial competitions may be weaker in leaf endospheres than in root endospheres that allow the co-occurrence of more tolerant fungal species.

The effects of plant selection and location on wheat fungal communities are influenced by growth stage

Fungal diversity indices in leaf endosphere, root endosphere, and rhizosphere communities were significantly higher in the regreening stage than in the heading stage (Supplemental Fig. S4). The DI value in the root and rhizosphere was also higher at the heading stage (Fig. 2). With plant growth, carbon relocating to roots and its release through both rhizodeposition and respiration decrease, for instance, in wheat from 33% of pulse labeled ^{14}C at early tillering to

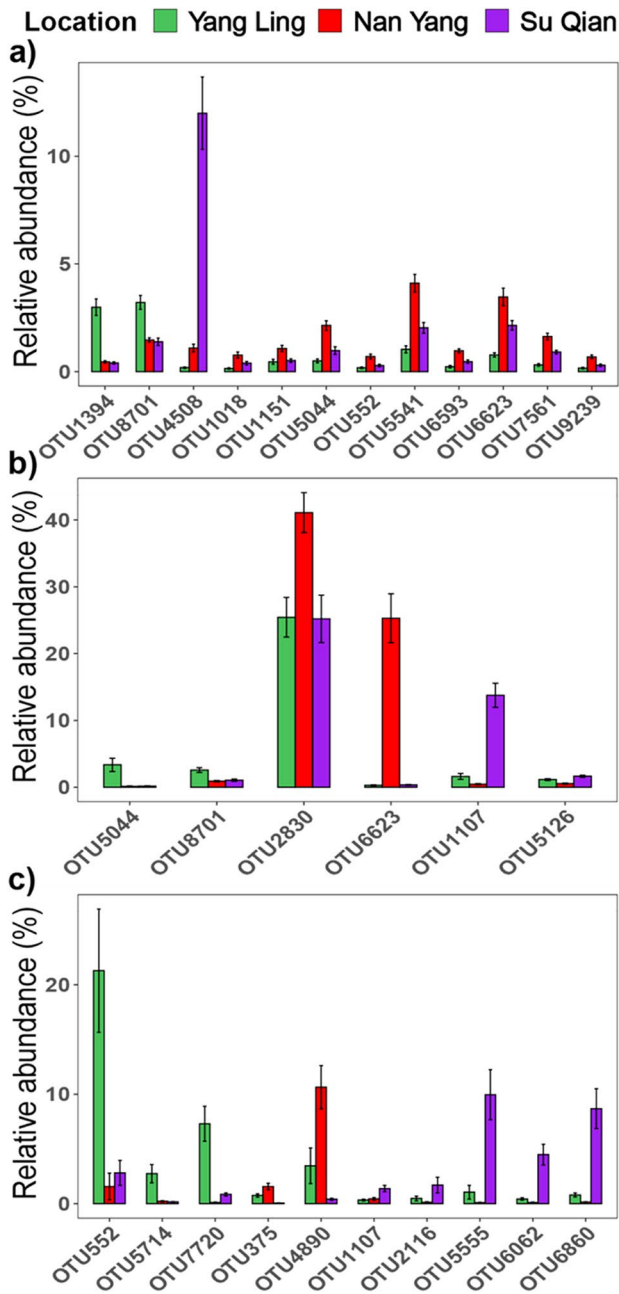


Fig. 5 The average relative abundances of indicator species using the core fungal datasets in leaf (a) and root (b) endospheres at the heading stage and rhizosphere (c) at the regreening stage. Values are means \pm standard errors

9% after anthesis (Gregory and Atwell 1991). Additionally, plants release sugars that could be used by diverse microorganisms at earlier growing stages while also releasing more specific exudates at later growing stages, possibly to attract more specific microbes (Chaparro et al. 2013). Together, these releases indicated much stronger filter effects in wheat-associated compartments at the heading stage, resulting in less diverse fungi to colonize.

For each sample type, both location and variety had significant effects, but location accounted for a larger proportion of variations in the mycobiome of wheat-associated compartments at both the regreening and heading stages. These results are consistent with previous work showing that the growing region has larger effects than variety on fungal assemblages associated with sugarcane leaf, stalk, root, and rhizosphere soil (Hamonts et al. 2018).

In the regreening stage, the HEEI values increased from rhizospheres to endophytes, demonstrating an increasing host effect, with the strongest effect in the leaf endosphere (Table 1 and Supplemental Table S1). This implied that only a small number of microbes has the ability to colonize inside plants, owing to the intensive selections caused by the host immune system and host exudates (Guttman et al. 2014; Zhalnina et al. 2018). Conversely, soil and environmental cues rather than host genetic variation have stronger effects on root endosphere and rhizosphere fungal populations (Almario et al. 2017). However, the contributions of location increased in the leaf and root endospheres and decreased in the rhizosphere samples at the heading stage.

The interplay effects between location and variety on wheat-associated fungal communities were more significant than the sole variety effect (Table 1). Additionally, growth-stage patterns of varietal effects on leaf endosphere and rhizosphere fungal communities were location specific (Fig. 3). This is possibly because host genetic variants influence only certain fungal populations, and the species pool of host-associated mycobiomes varies in different locations and growth stages. Another possible reason is that genotypes have phenotypic plasticity, which causes location-specific and stage-specific patterns of host genetic variations in plant traits that in turn affect plant-associated fungal communities. Therefore, these stage patterns of effects between location and variety on fungal communities in different wheat-associated compartments need further verification.

Field-grown wheats of different varieties share a core mycobiome

The core plant taxa co-evolve with terrestrial plants (Yeoh et al. 2016), which could ensure plant health and productivity (Lemanceau et al. 2017). In this study, some fungal taxa were consistently associated with leaf endosphere, root endosphere, and rhizosphere of wheat from five varieties and three locations at the same growth stage, with the exact same ITS sequence variants detected in plants and soils sampled hundreds of kilometers apart (Fig. 4). Even though we did not set a relative abundance threshold to identify core fungal OTUs, the highly prevalent core fungi were abundant. Only 0.8–1.7% of all fungal OTUs accounted for 55.1–86.3% of all fungal sequences in different samples (Fig. 4). Previous large-scale microbiome studies revealed that a small number

of the core taxa of plants (e.g., *Arabidopsis thaliana* or sugarcane) accounts for a majority of the microbial community (Hamonts et al. 2018; Lundberg et al. 2012). The core fungal taxa of wheat include well-known plant-associated taxa and some poorly characterized and as yet uncultured taxa. This study revealed the core fungal taxa associated with different wheat compartments, and we can target these to study their potential functions (plant growth promoters, symptomless colonizers, or pathogens) and agricultural applications.

At the heading stage, *M. tassiana* and *A. alternata* accounted for high proportions in the core mycobiomes of wheat-associated compartments, while this obviously increasing trend at the heading stage was not observed in bulk soil samples (Fig. 4). This indicated that the sharp increase of *M. tassiana* and *A. alternata* at the heading stage was not driven by the bulk soil species pool. Reportedly, *A. alternata* could produce ethyl acetate and bioactive compounds to suppress a wide number of fungi (Sudharshana et al. 2019). Thus, *A. alternata* may gain more competitive advantages in root endospheres and rhizospheres at the heading stage (Fig. 4 and Supplemental Fig. S4). Additionally, *A. alternata* could be used as a protective agent because of its ability to inhibit mycotoxin production. In this study, *M. tassiana* had the highest abundance among core taxa in the leaf endosphere, root endosphere, and rhizosphere at the heading stage and is predicted to be a pathotroph (Fig. 4). The common hypothesis is that many fungal pathogens could experience a common phase, living as an endophyte in their life cycle (Joshee et al. 2009; Pawłowska et al. 2014). However, it remains elusive why *M. tassiana* accounted for a higher proportion in wheat-associated compartments at the heading stage and what is its potential role in plant growth and health.

Even though these core fungal taxa were shared in five wheat varieties and three locations, based on their abundances in different wheat compartments at the regreening or heading stage, they could be grouped mainly into three clusters associated with location in the leaf and root endosphere at the heading stage (together with lower variety effects) and in rhizospheres at the regreening stage (Supplemental Fig. S6). Compared with the regreening stage, core taxa at the heading stage were low in diversity but high in abundances in the leaf and root endospheres and in the rhizosphere. Together, this suggests that different wheat varieties share more abundant fungal taxa in each compartment niche, thus lowering the variety effects on leaf and root endosphere fungal communities at the heading stage. Conversely, core fungal taxa in rhizosphere samples were not clearly clustered at the heading stage based on their abundances. More significant varietal effects on the rhizosphere fungal community were observed at the heading stage. The growth stage patterns of the fungal community in the rhizosphere are likely linked to root exudates. Young plants exude

sugars that could be used by a wide diversity of microbes, whereas plants release more specific exudates (e.g., phenolic compounds) with plant growth, which possibly select more specific microorganisms in the rhizosphere (Schlemper et al. 2017). Thus, wheat varieties exert much stronger selections (both taxonomy and quantity) on the fungal community in the rhizosphere at the heading stage. Our results also verified that the stage patterns of variety effects were location specific, highlighting the importance of the interactive effects of multiple factors (e.g., growth stage, location, variety) on the assembly of wheat-associated fungal community.

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Author contribution YZ, MY, DH, and XL conceived and designed research. YZ, HC, LL, and XZ conducted experiments. YZ analyzed data. YZ wrote the original manuscript. XL, MY, and JW critically revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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