Field Fungal Diversity in Freshly Harvested Maize

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Authors' contributions

This work was carried out in collaboration among all authors. Author XDS conceived and designed the experiments. Authors HS, LL, PS and JL performed the experiments. Authors XDS and HS analyzed the data. Authors LZ and HCY contributed analysis tools. Authors XDS and HS prepared the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Maize is a major crop in China and maize production in Heilongjiang Province ranks No.1 in the country in annual maize production in the whole country. Maize is prone to invasion by fungi and mycotoxins produced by these fungi are proven to be serious threats to animals as well as human health. Through high through-put sequencing we detected the dominant phylum to be *Ascomycota; Dothideomycetes, Sordariomycetes, Eurotiomycetes* and *Tremellomycetes*, *Saccharomycetes* were the dominant classes; *Hypocreales, Eurotiales, Capnodiales, Saccharomycetales, Tremellales*, and *Pleosporales* were the main orders; *Nectriaceae, Trichocomaceae, Cladosporiaceae, Debaryomycetaceae, Tremellaceae,* and *Pleosporaceae* were major families; *Gibberella, Cladosporium, Papiliotrema, Penicillium, Scheffersomyces, Talaromyces,* and *Epicoccum* were the most abundant phylotypes at the genus level. *Epicoccum_nigrum, Gibberella_zeae, Papiliotrema_flavescens,* and *Scheffersomyces_shehatae* were the dominant fungal species. Great fungal diversity was observed in the maize samples harvested in the five major maize-growing regions in Heilongjiang Province. Maize-1 in Nenjiang County was observed to have the greatest

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fungal diversity and abundance among the five regions. Since some of the fungal species are mycotoxin producing, it is necessary to take precautions to ensure the maize is stored under safe conditions to prevent the occurrence of mycotoxins and the growth and reproduction of other fungi which results in deterioration in the quality of maize.

1. INTRODUCTION

Maize (*Zea mays L*.) is an important foodstuff, feed, and raw material in China. Heilongjiang province produced 37 million tons of maize in 2017, ranks No.1 in total annual maize production in China, and accounts for 14.3 percent of the total maize production in the country [1]. In 2018, the total yield of maize in Heilongjiang province reached 39.82 million tons [2]. However, due to the shorter frost-free period in Heilongjiang province, maize may not be completely dry when harvested in October. The average moisture content in these kernels was recorded to be as high as 26.88% [3], the moisture content in most maize varieties often determined between 30-40% in this province [4]. The maize produced in this province frequently suffers from early frost and low temperature inhibits moisture evaporation from the maize kernels. Maize is prone to invasion by fungi and contamination by the mycotoxins secreted. Consequently, maize harvested in Heilongjiang Province under such high moisture contents are highly probable to be infected by various fungi, especially without drying treatment after harvest and storage under improper conditions. In addition, most small scale farmers dry their maize on polyethylene sheets or mats while others spread it on the bare ground in their yards or along countryside roads (give citation). These drying methods are slow and cause growth and development of fungi which in turn increases the potential for mycotoxin production. Some farmers also store or heap maize on the ground or in simple maize shelters, which leaves the maize vulnerable to rain or snow.

Fungi plays a key role in maize safety, and understanding the fungi community structure is of great importance in order to take appropriate measures to ensure maize safety. Fungi infects maize crops early in the field and may produce mycotoxins during this period. Consumer are concerned about this issue and consequently it is necessary to investigate the state of fungi contamination of maize in Heilongjiang province (add reference). Maize is widely cultivated in China under different climatic conditions and extensively contaminated by various fungi. However, little information is currently available on the fungal diversity of field fungi, especially aflatoxigenic fungal contamination of maize in the main growing region of Heilongjiang province. The objective of this study are to investigate fungal diversity of freshly harvested maize in the five main growing regions of Heilongjiang province through high-throughput sequencing and FUNGuild and try to find the abundance difference of dominant fungi among the five regions in the freshly harvested maize samples.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Five maize samples were harvested from five regions in the major maize growing areas in Heilongjiang as indicated in Fig. 1. Daxijiang Farm in Nenjiang County (maize-1), Rongjun Farm in Nenjiang County (maize-2), Heitai town in Mishan County (maize-3), Wanfa town in Bayan County (maize-4), and Jiangwan town in Yilan County (maize-5). Around 10 maize cobs were cracked from maize fields, placed into plastic bags, and immediately tightly sealed during 20-22 October 2017. After arriving at the lab, the maize cobs were hand-threshed from each maize sample and three 50 g maize kernels were weighed from each sample into 1000 ml Erlenmeyer flasks with 500 mL PBS buffer (KH_2PO_4 0.27 g, NA_2HPO_4 1.42 g, NaCl 8 g, KCl 0.2 g, diluted with 800 mL distilled water, pH value adjusted to 7.4, volume held constant at 1 L, then sterilized). They were labelled as three replicates of one sample. These samples were shaken with a vibrator for 30 minutes, then were subjected to suction filtration. They were filtered through 0.45 um water membranes and the residue was collected using medicinal ladles and transferred into 1 mL microcentrifuge tubes. They were then preserved by cryopreservation using liquid nitrogen. All the operations were conducted in a sterile room and masks and gloves were worn to guarantee the samples would not be contaminated by environmental fungi. These samples were sent to Guangzhou Gene Denovo Bio-Tech Ltd. Co. (Guangzhou, China) under dry

ice transportation conditions to perform high through-put sequencing of the PCR products. The obtained data was assembled into sequence tags and subject to BLAST in GenBank for classification of the microbes, followed by OTU, diversity, and inter-sample comparative analyses.

2.2 Protocol

2.2.1 DNA extraction and PCR amplification

Microbial DNA was extracted from stool samples using the E.Z.N.A. stool DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.) according to manufacturer's protocols. The ITS region of the Eukaryotic ribosomal RNA gene was amplified by PCR ($95°C$ for 2 min, followed by 27 cycles at $98°C$ for 10 s, $62°C$ for 30 s, and $68°C$ for 30 s and a final extension at $68°C$ for 10 min) using primers ITS3_KYO2F 5'- GATGAAGAACGYAGYRAA -3' and ITS4R 5'- TCCTCCGCTTATTGATATGC-3', where the barcode is an eight-base sequence unique to each sample. PCR reactions were performed in triplicate 50 μL mixture containing 5 μL of 10 × KOD Buffer, 5 μL of 2.5 mM dNTPs, 1.5 μL of each primer (5 μM), 1 μL of KOD Polymerase, and 100 ng of template DNA.

2.2.2 Illumina Hiseq2500 sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, U.S.). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina platform according to the standard protocols.

2.2.3 Bioinformatics analysis

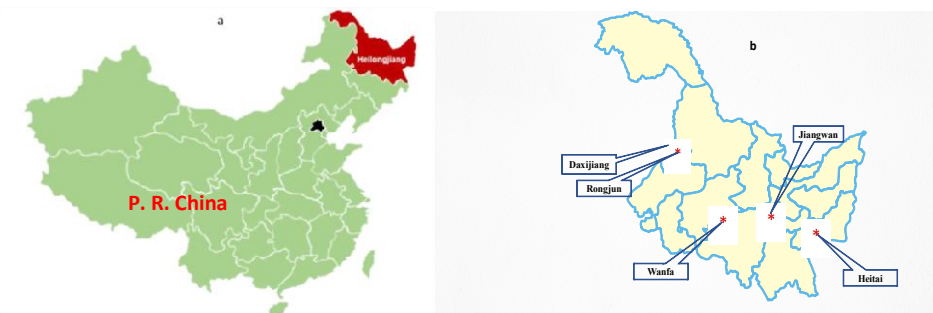The Bioinformatics analysis procedure was conducted according to Fig. 2.

Fig. 1. Distribution of samples collecting locations in Heilongjiang province of China. a. Illustration of the geographical location of Heilongjiang province in China. b. Distribution of samples collecting locations in Heilongjiang province
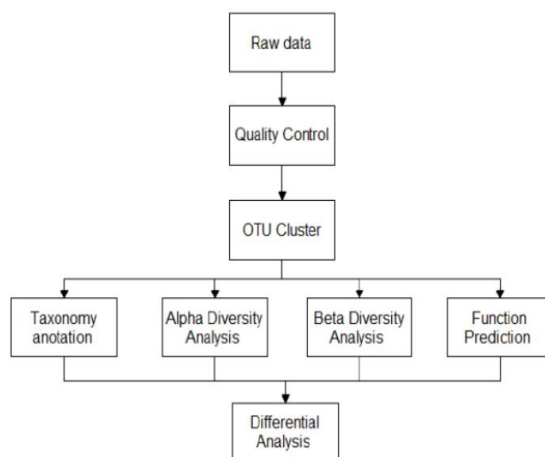

Fig. 2. ITS analysis flow chart

2.2.4 Quality control and reads assembly

Reads filtering: Raw data containing adapters or low-quality reads would affect the following assembly and analysis. Therefore, to get high-quality clean reads, raw reads were further filtered according to the following rules:

1) Removing reads containing more than 10% of unknown nucleotides (N);
2) Removing reads containing less than 80% of bases with quality (Q-value)＞20.

Reads assembly: Paired-end clean reads were merged as raw tags using FLSAH [5] (v 1.2.11) with a minimum overlap of 10 bp and mismatch error rates of 2%.

Raw tag filtering: Noisy sequences of raw tags were filtered by QIIME [6] (V1.9.1) pipeline under specific filtering conditions [7] to obtain high-quality clean tags.

Chimera checking and removal: Clean tags were searched against the reference database (http://drive5.com/uchime/uchime_download.html) to perform Reference-based chimera checking using UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html). All chimeric tags were removed and finally obtained effective tags for further analysis.

2.2.5 OTU cluster

The effective tags were clustered into operational taxonomic units (OTUs) with greater than 97% similarity using UPARSE [8] pipeline. The tag sequence with the highest abundance was selected as a reprehensive sequence within each cluster. Between groups, Venn analysis was performed in R to identify unique and common OTUs.

2.2.6 Taxonomy classification

The representative sequences were classified into organisms by a naive Bayesian model using a RDP classifier [9] (Version 2.2) based on UNITE [10] Database (https://unite.ut.ee/). The abundance statistics of each taxonomy and a phylogenetic tree was constructed in a Perl script and visualized using SVG. Biomarker features in each group were screened by Metastats and LEfSe software.

2.2.7 Alpha diversity analysis

Chao1, Simpson and all other alpha diversity indices were calculated in QIIME. OTU rarefaction curve and Rank abundance curves were plotted in QIIME. Statistics of between-group Alpha index comparison was calculated by a Welch's t-test and a Wilcoxon rank test in R. Alpha index comparisons among groups was computed by a Tukey's HSD test and a Kruskal-Wallis H test in R.

2.2.8 Beta diversity analysis

The weighted and unweighted unifrac distance matrix was generated by QIIME. Multivariate statistical techniques including PCA, principal coordinates analysis (PCoA) and NMDS of (Un)weighted unifrac distances were calculated and plotted in R. Statistics of Welch's t-test, Wilcoxon rank test Tukey's HSD test, Kruskal-Wallis H test, Adonis (also called Permanova) and Anosim test was calculated using R.

2.2.9 Functional prediction

The functional group (guild) of the OTUs was inferred using FUNGuild [11] (v1.0).

3. RESULTS AND DISCUSSION

3.1 Fungal Diversity and Richness in Single Maize Samples and Comparison of These Indexes among the Five Samples

Total fungal ITS tags (103283, 89719, 96884, 92409, and 129746) were recovered from 5 samples (maize-1, maize-2, maize-3, maize-4, and maize-5, respectively). The library samples were then clustered into fungal Operational Taxonomic Units (OTUs) at 97% similarity (Table 1).

Maize-1 was determined to have the highest number of OTUs out of the the five samples (regions), while Maize-5 obtained the minimum value. No significant difference was found in OTU values among Maize-2, Maize-3, and Maize-4. An OTU is usually recognized as a genus of microorganism. Consequently, a total of 608, 367, 408, 386, and 257 fungal genera were observed in the 5 maize samples, respectively.

The good coverage value indicates the depth of sequencing. The sequencing good coverage in the five samples all reached 99.8%, which indicated that almost all fungi have been detected. As seen in the table, no significant

Table 1. Community richness, diversity and coverage indexes in the five samples*

Region	OTU	Chao 1	Ace	Good coverage (%)	Shannon	Simpson
Maize-1	608 ± 24^a	729 ± 67^a	729 ± 50^a	99.8 ± 0.0^a	4.66 ± 0.06^a	0.90 ± 0.00^a
Maize-2	367 ± 24^b	532 ± 35^b	539 ± 45^{bc}	99.8 ± 0.0^a	4.02 ± 0.08^b	0.90 ± 0.01^a
Maize-3	408 ± 11^b	590 ± 53^b	593 ± 21^{ab}	99.8 ± 0.0^a	3.93 ± 0.17^b	0.87 ± 0.01^a
Maize-4	386 ± 13^b	567 ± 29^b	568 ± 44^{ab}	99.8 ± 0.0^a	3.83 ± 0.37^b	0.85 ± 0.06^a
Maize-5	257 ± 64^c	374 ± 120^{bc}	378 ± 132^c	99.8 ± 0.0^a	3.80 ± 0.20^b	0.88 ± 0.01^a

The data represents mean ± SD. Data followed by the same superscript letter in the same column is not significantly different

difference was found in the values of Good coverage. Maize-1 and Maize-2 were collected in Nenjiang County which is located to the north of the other three regions and whose temperature ranges between -4°C and 9°C in October. In comparison, the southern location of the other three areas result in a warmer climate and recorded temperatures of 0-12°C, 1-12°C, and 0-12°C in October in the regions of Mazie-3 (in Mishan County), Maize-4 (in Bayan County), and Maize-5 (in Yilan County), respectively. It is reasonable to assume that the numbers of fungal genera in a warmer area should be greater than that of in a cooler one. However, the rain from the day before sample collection in Nenjiang County made the maize cobs wet and likely resulted in greater numbers of fungal genera, and consequently higher numbers of OTU. Other factors such as air humidity, fungal diseases, birds, insects, wind, etc. can comprehensively affect the fungal community in maize cobs.

To compare fungal diversity and richness among the five maize samples (regions), data was statistically analyzed and presented in Table 1. The Chao 1 and ACE are abundance indexes; greater values of Chao 1 (richness estimate) and ACE indicate more community richness. As seen in Table 1, the greatest Chao 1 and ACE values in Maize-1 indicate the highest fungal abundance, whereas the smallest Chao 1 and ACE values in Maize-5 represent the lowest fungal abundance.

The Shannon and Simpson are diversity indexes. The Shannon index assesses the general biodiversity, accounting for both the abundance and evenness of the fungal species present, while the Simpson diversity index is a measure of diversity which also takes into account both richness and evenness of the fungal community. A greater Shannon and Simpson value indicates greater community diversity. However, the Shannon index emphasizes richness whereas Simpson pays more attention to dominant species. The greatest Shannon value of Maize-1

(4.66) indicates the greatest community diversity while the Shannon values of the other four maize samples are not significantly different. A significant difference exists between Maize-1 and the other four maize samples. No significant difference was statistically observed for Simpson values in the five samples (regions). This is inconsistent with the result of Shannon values, likely due to the different equations applied for the two indices.

As stated above, Maize-1 and Maize-2 were collected in Nenjiang County which is located to the north of the other three regions and whose temperature range was lower in comparison with the other three regions in October. Consequently, it is reasonable to expect a relative smaller community diversity. It is most likely the rain from the day before sample collection in Nenjiang County made the maize cobs wet and probably resulted in greater numbers of fungal genera thus the greatest community diversity in Maize-1 and Maize-2.

3.2 Fungal Community Composition

For the 5 maize samples, fungal community compositions were determined at seven levels: Domain, Phylum, Class, Order, Family, Genus, and Species. Fig. 3 demonstrates the taxonomy stack distribution of the Phylum, Order, Genus, and Species of the identified fungi. Of the classifiable sequences, two Phyla were identified as seen in Fig. 3a: *Ascomycota* and *Basidiomycota*, in which *Ascomycota* is absolutely dominant. At the Class level, *Dothideomycetes*, *Sordariomycetes*, *Tremellomycetes*, *Eurotiomycetes*, and *Saccharomycetes* were identified, where *Dothideomycetes* and *Sordariomycetes* are absolutely dominant. At the Order level, *Capnodiales, Pleosporales, Hypocreales, Tremellales, Sporidiobolales*, and *Eurotiales* were determined, in which *Capnodiales, Hypocreales*, and *Eurotiales* are dominant (Fig.

3b). At the Family level, *Cladosporiaceae, Pleosporaceae, Nectriaceae, Tremellaceae, Debaryomycetaceae, Cordycipitaceae, Saccharomycodaceae,* and *Bulleraceae* were detected. *Cladosporiaceae, Trichocomaceae*, as well as *Nectriaceae,* are the dominant families (data not shown in Fig. 3). As seen in Fig. 3c, *Cladosporium Epicoccum, Alternaria, Gibberella, Penicillium, Papiliotrema, Talaromyces, Meyerozyma, Aspergillus, and Scheffersomyces* were detected at the genus level in these maize samples. From Fig. 3d, it can be observed that unclassified species account for large portions of the whole bars. Due to a limitation of the UNITE Database, large amounts of species were unable to be classified. *Papiliotrema_flavescens, Gibberella_zeae, Scheffersomyces_shehatae*, and *Penicillium_polonicum* are the dominant fungal species found in the determined samples. A relatively high percentage of *Meyerozyma_guilliermondii* and *Penicillium_polonicum* was detected in two samples (Maize-2 and Maize-5). *Fusarium_proliferatum,Penicillium_adametzioides*, and *Gibberella_fujikuroi* were also detected at a relatively low percentage.

Gibberella zeae, usually known by the name of its anamorph *Fusarium graminearum*, is identified as a plant pathogen which causes *Fusarium* head blight and can produce toxins, particularly deoxynivalenol (DON). *Fusarium spp.* produces a number of diverse secondary metabolites, including some fatal mycotoxins [12] and they are attributed to the most important toxigenic fungi in the Northern temperate areas [13]. *F. proliferatum* is a fungus distributed worldwide that has been associated with many diseases in a number of economic plants including maize, and produce mycotoxins such as fumonisin B_1, moniliformin, beauvericin, and fusaproliferin [14].

Through a naive Bayesian model using RDP classifier based on UNITE Database analysis of the assembled sequences, three species of yeasts, *Papiliotrema_flavescens, Scheffersomyces _shehatae,* and *Meyerozyma_guilliermondii* were found in the maize samples. In addition, *Penicillium_adametzioides* was reported to be a potential biocontrol agent for ochratoxin - producing fungus in grapes [15].
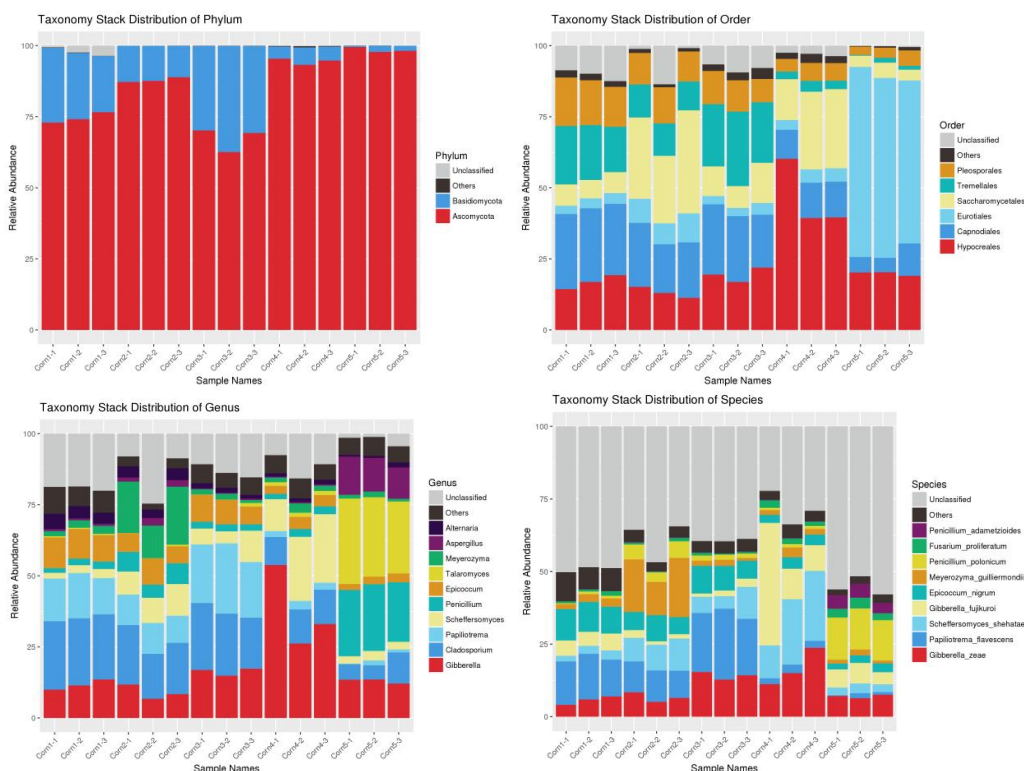

Fig. 3. Taxonomy stack distribution of the 5 corn samples at different levels in Heilongjiang province. a. at phylum level; b. at order level; c. at genus level; d. at species level

Penicillium_polonicum is an endophytic fungus that produces secondary metabolites including bioactive compounds which can be used as antibiotics [16,17]. *Gibberella fujikuroi* is a fungal plant pathogen that produces a wide variety of secondary metabolites including gibberellins, carotenes, bikaverins, and hydroxylated anthraquinones (mainly pigments). *Epicoccum nigrum* is a plant pathogen and endophyte which produces metabolites such as colored pigments that can be used as antioxidant, anticancer, antibacterial and antifungal agents against other pathogenic fungi [18,19].

As seen in Table 2, for the five samples (regions), the dominant fungi at the Genus level are different. For Maize-1 *Cladosporium, Papiliotrema, Gibberella*, and *Epicoccum* are the four dominant genera, for Maize-2 *Cladosporium, Meyerozyma, Papiliotrema,* and *Scheffersomyces are the* dominant genera, for Maize-3 *Papiliotrema, Cladosporium, Gibberella*, and *Epicoccum* are the dominant genera, for Maize-4 *Gibberella, Scheffersomyces, Cladosporium,* and *Epicoccum* are the dominant genera, and for Maize-5 *Talaromyces, Penicillium, Gibberella*, and *Aspergillus* are the dominant genera.

Cladosporium is recognized as a psychrophile hence it is more adaptable to cool temperature condition, it is the most abundant genus in Maize-1 and Maize-2, since the growing regions of Maize-1 and Maize-2 were Nenjiang County located around 49 degrees north latitude, whereas the growing areas of Maize-3, Maize-4, and Maize-5 are located south of 46.5 degrees north latitude. Consequently, the temperatures in Mishan, Bayan, and Yilan Counties are a little bit warmer than that of in Nenjiang County. *Cladosporium* has proven to be a mycotoxin-producing fungus and potentially pathogenic one frequently occurring in outdoor environments [20].

Talaromyces is a genus of fungi in the family *Trichocomaceae*, it is identified as a sexual state of *Penicillium* that produces soft walled ascomata covered with interwoven hyphae [21]. Some of the *Talaromyces* species are medically important that produce mycotoxins such as Rubratoxin which is a potential hepatotoxin in food products [22]. *Talaromyces marneffei* is an emerging fungal pathogen resulting in a fatal mycosis in especially immunocompromised persons from Asian countries such as China [23]. *Talaromyces islandicus* is one of the most harmful and destructive fungi that result in the yellowing of rice during storage [24,25] and also produces mycotoxins including cyclochlorotine, erythroskyrine, islanditoxin, as well as luteoskyrin which exhibit hepatotoxic and carcinogenic characteristics [21].

In addition, the proportion of *Talaromyces* (27.80%), *Penicillium* (22.54%), and *Aspergillus* (12.07%) in Maize-5 were significantly greater than those of in the other four maize samples (regions). It seems like fungal diversity in warmer regions would be richer than those of in cooler ones.

3.3 Cluster Analysis of the 5 Maize Samples

As seen in Fig. 4, the fungi of the 15 maize treatments (three replicates for each sample) from Heilongjiang province were cluster analyzed.

Table 2. Fungal diversity and abundance (%) of maize samples at genus and species levels collected from the five regions*

Levels	Fungal strains	Maize-1 Daxijiang	Maize-2 Rongjun	Maize-3 Heitai	Maize-4 Wanfa	Maize-5 Jiangwan
	Gibberella	11.64±1.79[a]	8.940±2.56[a]	16.35±1.28[a]	37.64±14.39[b]	13.02±0.81[a]
	Cladosporium	23.51±0.55[a]	18.25±2.56[b]	21.10±2.86[a]	11.29±1.28[b]	7.14±3.35[b]
	Papiliotrema	14.60±1.67[a]	10.36±0.74[b]	21.63±2.67[c]	2.51±0.46[d]	0.97±0.73[d]
	Penicillium	2.18±0.51[a]	6.28±1.45[b]	2.38±0.09[a]	2.46±0.52[a]	22.54±1.41[c]
	Scheffersomyces	2.62±0.62[a]	9.38±1.57[a]	6.93±3.57[a]	19.33±7.00[b]	2.89±0.41[a]
Genus	*Talaromyces*	0.46±0.03[a]	0.26±0.28[a]	0.50±0.69[a]	1.37±0.13[a]	27.80±2.39[b]
	Epicoccum	10.18±0.78[a]	7.23±1.74[a]	8.13±1.70[a]	3.68±0.76[b]	2.63±0.53[b]
	Meyerozyma	2.40±0.58[a]	16.64±4.56[b]	1.62±0.57[a]	2.29±0.89[a]	1.43±0.53[a]
	Aspergillus	0.74±0.05[a]	2.12±0.65[a]	0.38±0.08[a]	0.44±0.06[a]	12.07±1.20[b]
	Alternaria	4.49±0.77[a]	3.71±0.64[a]	1.63±0.27[b]	1.33±0.24[b]	1.05±0.68[b]
	Unclassified	19.12±0.82[a]	13.78±9.41[a]	13.34±2.34[a]	11.34±4.12[a]	2.36±1.80[b]

* Values followed by the same superscript letter in the same row are not significantly different

Three treatments of Maize2, Maize-3, and Maize-4 can be clustered into three groups, respectively. Three treatments of Maize-1 can be clustered into one group. Only Maize-5 cannot be clustered into one group. This is probably a result of near geographical proximity among the 5 spots where the maize samples were collected leading them to have a similar fungal community. Maize fields in one region may vary in soil types, water resources, types of fertilization, maize seed varieties, and other environmental factors. These might increase the possibility of fungal diversity and make it difficult to cluster the fungi in maize samples from the same region into one group. Nevertheless, most of the fungi in maize samples from the same region are able to be clustered into the same group.

3.4 Fungal Communities and Functional Guilds Analysis

Fungal communities and functional guilds of the 5 maize samples are shown in Fig. 5. As seen in this Figure, an open environment enables the maize to be a plant host to a wide range of environmental fungi. The most abundant phylotypes are seen to be plant pathogens and undefined saprotrophs, followed by fungal parasites and endophytes as well as animal pathogens, soil saprotrophs, and wood saprotrophs. For all the maize samples, plant pathogens and undefined saprotrophs are absolutely dominant. For the mycotoxigenic fungi species, they are in the category of a plant pathogen.

It is estimated that around 70% of all major crop diseases were induced by fungal plant pathogens. In addition, through yield losses and mycotoxin contamination, 15% of global agricultural production was destroyed [26]. By far, plant pathogens and especially mycotoxigenic fungi are considered to be the most harmful class of plant pathogens. As a cosmopolitan genus of filamentous ascomycete fungi, *Fusarium* includes a number of toxin-producing plant pathogens of agricultural importance [27]. *Gibberella zeae* is plant pathogen which causes *Fusarium* head blight and produces toxins, deoxynivalenol (DON) in particular. For the maize freshly harvested in Heilongjiang province, the *Fusarium_proliferatum* identified probably include mycotoxigenic species. Other fungal genera and species such as *Talaromyces*, *Penicillium*, and *Aspergillus,* etc., possibly pose a potential threat to maize quality and safety through quality destruction and mycotoxin production, although a fungitoxicity test has not been conducted yet.
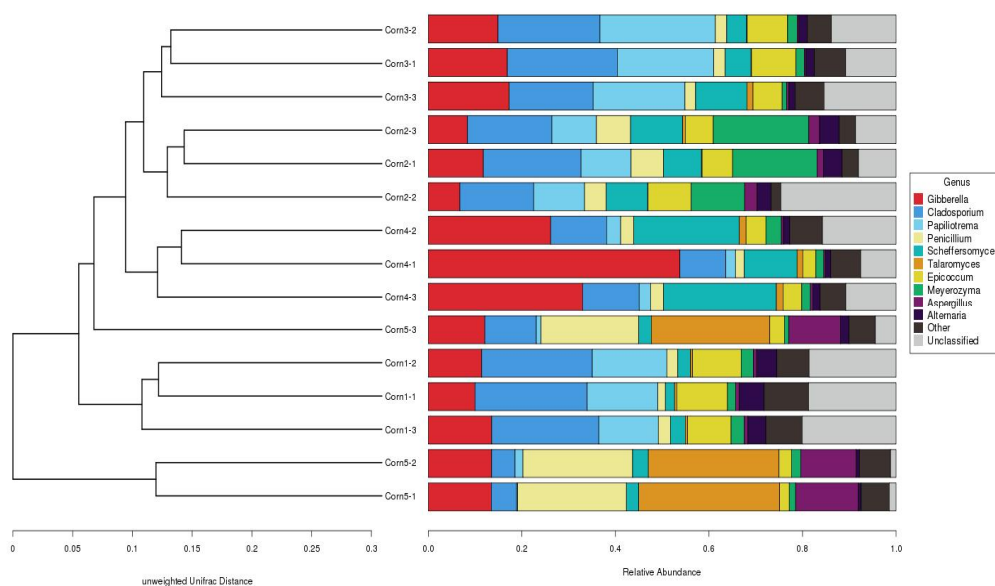

Fig. 4. Unweighted pair-group method with arithmetic means (UPGMA) analysis of microbial community structure based on ITS gene amplicon sequencing data
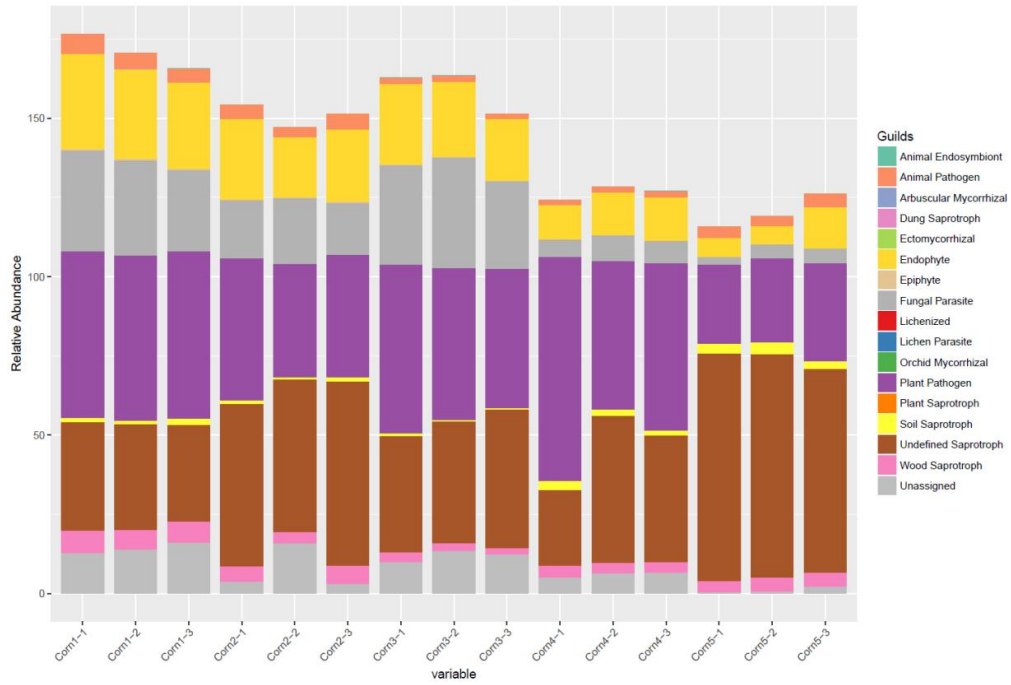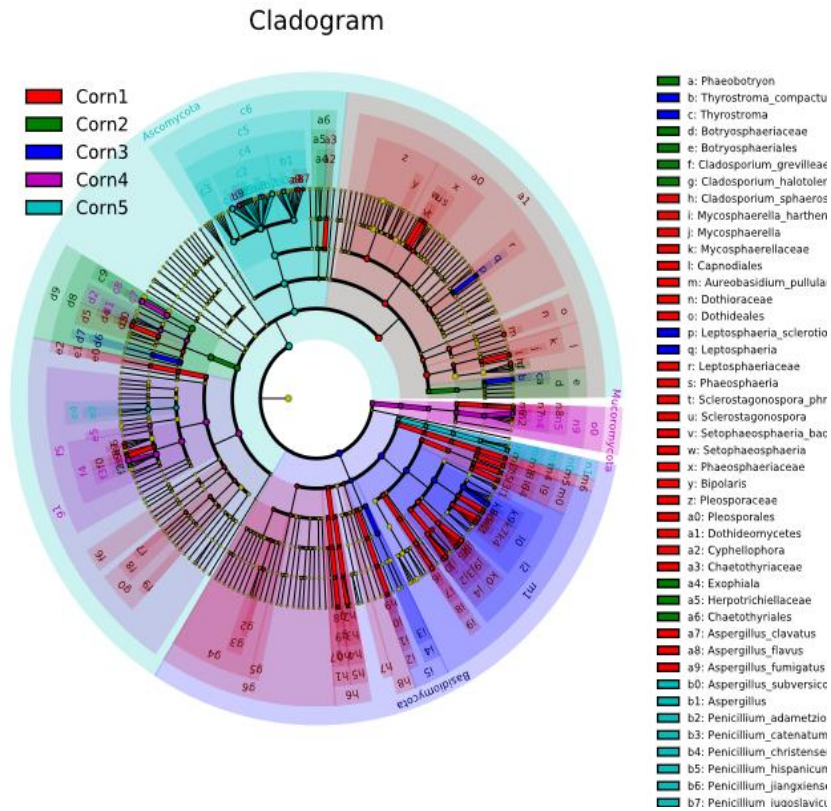
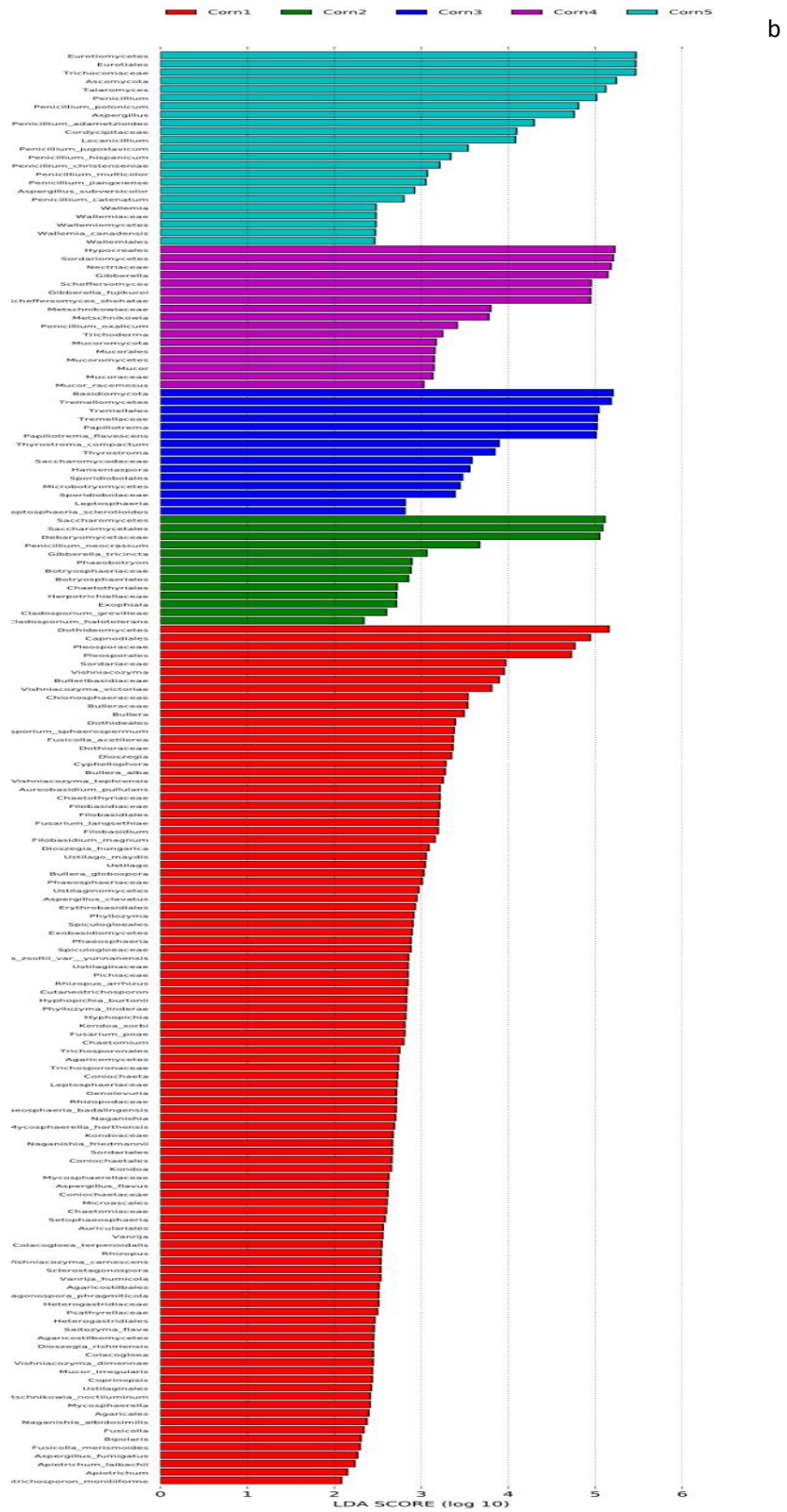**Fig. 5. Stacks of guilds of the 5 corn samples from Heilongjiang province**

Fig. 6. Cladogram, LDA score and relative abundance of fungi of corn samples

3.5 LEfSe Analysis

Key phylotypes of maize fungi microbiota representing the five samples (regions) identified using linear discriminant analysis (LDA) effect size (LEfSe) are shown in Fig. 6. As seen in Fig 6a, the Cladogram indicates that the numbers of 44 fungal orders are significantly different from those of the corresponding orders or classes in the other four maize samples (regions). There are 24, 8, 4, 0, and 8 orders in Maize-1, Maize-2, Maize-3, Mazie-4, and Maize-5 samples, respectively. The fungi count of these orders or classes are significantly different from one sample to the other four samples. Their LDA scores are greater than 2 (Fig. 6b) and they might be considered as specific fungi associated with different regions, especially those with the highest LDA scores in each maize sample (region) (around 13 orders or classes in total). For example, *Dothideomycetes* and *Capnodiales* for Maize-1, *Saccharomycetes* and *Saccharomycetales* for Maize-2, *Basidiomycota* and *Tremellomycetes* for Maize-3, *Hypocreales* and *Sordariomycetes* for Maize-4, and *Eurotiomycetes* and *Eurotiales* for Maize-5. Consequently, it might be possible to develop biomarkers through using the above fungi to distinguish maize from various regions.

4. CONCLUSION

To explore filed fungal diversity and the potential of fungal mycotoxin production in maize, freshly harvested maize samples in Heilongjiang province were collected and detected via high through-put sequencing. Our results indicate that the five major fungal genera in maize are *Cladosporium, Gibberella, Papiliotrema, Penicillium,* and *Talaromyces.* However, the dominant genera in different samples are different probably due to various climatic conditions, fertilizing manner, seed varieties, and etc. *Epicoccum_nigrum, Fusarium_proliferatum,* and *Gibberella_zeae* are identified harmful fungi species, in which *Fusarium_proliferatum* has the potential to produce mycotoxins such as fumonisin. Maize planted in these regions has the potential to produce Fumonisin, DON, and NIV. In addition, 44 fungal orders or classes have been identified as the specific fungal orders or classes which distinguish maize planted in one region from the other four regions, in which around 13 orders or classes in total are especially suitable for the development of biomarkers. Consequently, it is necessary to take adequate measures to prevent fungi growth and reproduction, especially mycotoxins production, as well as related damage induced by non-mycotoxins-producing fungi growth and reproduction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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