Secondary metabolites, mycotoxins and mycobiome from Nepalese field maize and soil

by

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Abstract

Complex fungal communities (a.k.a. mycobiomes) can be found in maize ears. The origin of these mycobiome is not well understood. It could originate from spores in the soil, be transported by the air or rain; or be carried by insects or contaminated seeds, and its composition could be important in determining the secondary metabolites produced. A detailed understanding of the fungal community in maize seed and its influence on the secondary metabolites present remains unknown. I evaluated the mycobiomes in maize ears and the soil in which the plants were growing using ITS metabarcoding across 12 fields of two districts in Nepal with the goal of determining the contribution of the soil mycobiome to the mycobiome of the maize ear. The working hypothesis was that maize ears and soil harbor overlapping mycobiomes, but that these mycobiomes also differed by geographic location. In addition, we analyzed secondary metabolites with Liquid Chromatography-Mass Spectroscopy (LC/MS/MS) and coupled the chemical analysis with the mycobiome evaluation to assess the relationship between the maize ear mycobiomes and the secondary metabolites and mycotoxins present. The hypothesis was that the metabolites present, and their quantities, varied with the composition of the mycobiome. MiSeq ITS2 metabarcode data from the fungal communities indicated that mycobiomes from maize ears and soil were distinct as were mycobiomes from different locations, with mycobiomes of soil much larger, in terms of number of genera represented, than those from maize ears. Genera found frequently in maize were less frequent in the soil. *Fusarium* was usually the dominant genus from both the grain and the soil at all locations. Fungal richness and diversity were greater in soil than in the maize ears. Secondary metabolite profiles and mycobiome profiles did not always align 1:1. Aflatoxins, ochratoxin and *Aspergillus* spp. were recovered from only two ears. Fumonisins were recovered from 10/12 ears. A toxin-producing genus could be present even if the toxins associated with the genus were not

detected. In conclusion, maize ear mycobiomes play an important role in determining the secondary metabolites present. As members of the soil and maize ear mycobiomes were distinct and even the OTUs were in common often varied widely in relative frequency, soil is unlikely the sole source of the maize ear mycobiomes.

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Chapter 1 - Background

Microbiomes, first defined by Joshua and McCray (2001), are highly specific and complex microbial communities, consisting of hundreds of species of microbial lineages including bacteria, archaea, fungi, and protists. The fungal component of the total microbiome, i.e., the fungal microbiome, is the mycobiome. This term was coined by Ghannoum et al. (2010). Maize grains and soil are occupied by complex microbial communities, including mycobiomes, that have significant roles in plant health. These roles may be either beneficial or detrimental to the plants with which they are associated (Nadarajah and Rahman, 2021; Sarraf, et al., 2023). Complex fungal communities interacting with each other in maize and soil can offer advantages to the plant host including stress tolerance and resistance to pathogens.

The maize seed mycobiome is affected by biotic and abiotic factors in the soil, environment and host plant (Rajala et al., 2012; Trivedi et al., 2016). The fungi comprising these mycobiomes could originate in the soil the plant is growing in, the seed that was planted, from airborne contamination, or inoculum carried by insects. Microbiomes can be transmitted to plants either from the surrounding environment or acquired directly from the seed (Jonkers et al., 2022). A wealth of information is available for plant and soil microbiomes in agricultural systems, studies of only the soil mycobiome i.e., only fungi and no bacteria and their contributions to understanding maize ear mycobiomes are limited (Kandasamy et al., 2021; Schlatter et al., 2017).

In Chapter 2, I evaluate mycobiomes from maize ears and the soil in which the plant from which the ear was harvested was growing to estimate the degree to which the soil mycobiome could be contributing to the mycobiomes in the maize ear. My working hypotheses were that (i) maize ears and soil harbor overlapping mycobiomes and that (ii) the composition and diversity of the mycobiomes differed by geographic location. I tested these hypotheses by evaluating maize grain

and soil from 12 individual plants growing in two districts. The first was assessment of the relative frequency of metabarcoded DNA sequences from the ITS2 (rRNA Internal Transcribed Spacer) region, which was followed by a comparative study of mycobiome composition and diversity.

Plant microbiomes influence secondary metabolite production, and plants influence the microbiome by secreting various compounds that are consumed or modified by one or members of the microbiome (Pang et al., 2021). The mycobiome, i.e., the fungal portion of the microbiome in the plant, shaped by host genotype, endophytic seed microbiome, soil-microbe interactions, secondary metabolites, and environmental factors like soil, rain, agricultural practices and climate (Bintarti et al., 2022; Latz et al., 2021; Wagner et al., 2020). Endophytes are an important part of the maize mycobiome that can colonize host plants, alter plant growth, and enable resistance or susceptibility to diseases (Eaton et al., 2011; Tao et al., 2024).

Some of the fungi in the mycobiome can produce mycotoxins under conducive conditions either in the field before harvest or in storage afterwards. These toxins when present can cause a variety of adverse health effects in humans and domesticated animals that consume contaminated materials (Rahim Khan, 2024). Many fungi can produce secondary metabolites in maize. Toxin produced by fungi in three mycotoxigenic genera, *Aspergillus*, *Penicillium* and *Fusarium*, are the usual major culprits, with the risk evaluation often on the quantity and the carcinogenicity of the metabolites produced (Mafe and Busselberg, 2024; Sweeney and Dobson, 1998). However, metabolites produced by these fungi may be nephrotoxic, teratogenic and hepatotoxic as well (Berndt et al., 1980; Dilkin et al., 2003; Francis et al., 2024; Gelineau-van Waes et al., 2012, Ruan et al., 2022).

An overlooked topic is the relationship under field conditions between the maize ear mycobiome and the presence and amount of contaminating secondary metabolites, especially those other than

known mycotoxins. The source(s) of the fungi in the maize ear mycobiome responsible for mycotoxin and secondary metabolite production are not known (Kudjordjie et al., 2019).

In the third chapter, I examine the fungal communities from individual maize ears, and their association with the secondary metabolites found in grain from the ears. I wanted to know whether a fungal genus could be present even when its secondary metabolites were not detected, and vice-versa. I also wanted to know whether strains from genera known to interact with one another either synergistically or antagonistically under the lab conditions co-occurred in the field.

Chapter 2 - Maize Ear and Soil Mycobiomes

Abstract

Maize ears are colonized by multiple fungi. These fungi could originate in the soil the plant is growing in, within the seed that was planted, through airborne or water splash contamination, or from inoculum carried by insects. I evaluated the mycobiomes in maize ears and the soil the plant was growing in to determine the degree that the soil mycobiome could be contributing to the plant mycobiome. The hypotheses were that maize and soil harbor overlapping mycobiomes and that these mycobiomes also differed by geographic location.

Paired soil and maize ear samples were collected from the USAID Zone of Influence (ZOI) two districts in Nepal. DNA was extracted from maize kernels and soil and extracted DNA was Illumina sequenced to characterize fungal communities. I used alpha and beta diversity analyses to study richness, diversity and composition for mycobiome from soil and maize ears. The results suggested distinct mycobiomes in maize ears and soil in both districts. Maize ear and soil mycobiomes differed in many ways. Genera abundant in maize ears were not common in the soil and vice versa. Consequently, it seems unlikely that the soil reservoir is serving as the principal inoculum for the mycobiomes in the maize ears. *Fusarium* was the most common genus found in both soil and maize ears and *Sarocladium* was most frequent in maize ears. The contributions of the soil mycobiome to the maize ear mycobiome are not fully understood, and further research is required to understand the soil maize ear mycobiome relationships.

Introduction

Diverse, complex microbial communities, i.e., microbiomes, occur in agricultural soil. Fungi, the second most abundant living biomass after bacteria (Bar-On et al., 2018), also are common in soil, where the community they form is known as the mycobiome (Ghannoum et al., 2010). These fungi could be decomposers, and degraders, mutualists that colonize plants, to increase soil aggregation or improve plant health, or be pathogens on plants growing in the soil.

Many biotic and abiotic factors directly or indirectly influence the activity and diversity of fungi in the soil. Key abiotic factors include temperature, light, nutrient and water availability, soil composition, pH, moisture, organic carbon and nutrients in soil (Fierer, 2017; Newsham et al., 2011; Schimel et al., 2018). Soil fungi have three significant ecological activities: (i) Decomposition and nutrient cycling, (ii) Alter of plant/soil interactions, and (iii) Regulation of the soil ecosystem and its physiochemistry (Santos and Olivares, 2021). Beneficial fungi have fundamental roles in biocontrol, bio-stimulation and fertility of plants, whereas pathogenic fungi can cause disease in plants and alter their decomposition (Delaeter et al., 2024; Nazarov et al., 2020). Mycorrhizal fungi are well known fungi that have symbiotic relationships with many crops (van der Heijden et al., 2015).

Mycobiomes are strongly influenced by the diversity and composition of the plant populations that occur in the soils where they exist (Hannula et al., 2017). Agricultural intervention can reduce soil-inhabiting fungal diversity and result in the development of fungal communities that are quite different from those in soils that have never been used for production agriculture (Oehl et al., 2003; Turley et al., 2020; Wagg et al., 2018).

Fungal communities in native soils are complex. They consist of multiple, diverse species ranging from saprotrophs, endophytes, pathogens, and mycorrhizae (Pérez-Izquierdo, et al., 2021).

Mycobiomes in soil persist and evolve depending upon their environment and the plants available to associate with (Onet et al., 2025; Suman et al., 2022). Hyper-diverse fungal communities exists in forest soils where few very common and many less common species cooccur.(Buee et al., 2009). In bulk forest soil, Basidiomycetes usually dominate, whereas in plant, Ascomycetes usually are the most common (Terhonen et al., 2019). Prominent endophytic Ascomycetes in forest materials usually have a dark septate appearance (Jumpponen and Trappe, 1998), and could be decomposers, mutualists that colonize plant roots and help soil aggregation, or pathogens. Fungal decomposers have a significant role in nutrient cycling and are commonly found in the litter layer (Asplund et al., 2019).

Microbiomes in seed commonly are determined by the soil in which the plant is growing. Plants also acquire microorganisms through exposure to environmental factors such as rain, wind and insects (Abdelfattah et al., 2019; Lundberg et al., 2012). Only a small proportion of the diverse soil microbiome can colonize plant tissues (Rochefort et al., 2021). Many fungi are associated with seeds (Links et al. 2014, Rodriguez et al., 2009). These fungal genera often are found in soil and may be transmitted horizontally to plants.

Vertical transfer of seed endophytes from the parent plant to next generation, depends on the plant genotype, and the plant's health, and the environment in which the seed is produced and germinated (Johnston-Monje et al., 2021). Endophytic entomopathogenic (Zimmermann, 2007) fungi, such as *Metarhizium* and *Beauveria* can promote growth and provide insecticidal activity in maize, tomato, and potato (Ahmad et al., 2019; Bing and Lewis, 1992; Elena et al., 2011; Krell et al., 2018).

Fungi associated with maize: Pathogenic and beneficial

Maize is susceptible to fungal pathogens that cause different diseases and reduce crop yields. Some fungi also produces mycotoxins in maize that are detrimental to human and animal health (Awuchi et al., 2021). Soil pathogens in genera such as *Bipolaris*, *Fusarium*, *Penicillium*, and *Trichoderma* commonly infect maize. Many *Aspergillus* species, e.g., *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus niger*, and *Aspergillus tamarii*, as well as *Fusarium verticillioides* (Blacutt et al., 2018), *Penicillium citrinum*, and *Rhizopus stolonifera* are pathogenic to maize (Goko et al., 2021). *Aspergillus* species in maize can cause ear rots and produce aflatoxins, ochratoxin or other secondary metabolites (Fountain et al., 2014). *Aspergillus* spores are carried by wind or rain that land on the maize plant, especially pollen silks. Insects can carry *Aspergillus* spores and deposit them internally within the plant, ears or grain as the insects can burrow through the plant tissues that the fungus itself cannot penetrate. *Aspergillus* spp. in soil can be transmitted to maize plants after they germinate and are growing. *Fusarium* spp. may be soilborne including trash and stubble, airborne, rain-borne or insect-vectored, as well as being endophytic and transmitted through the seed from one generation to the next. *Fusarium* species can cause many diseases, including root, stalk, ear and kernel rots, and contaminate grain with various mycotoxins depending on the species that colonizes the plant (Leslie and Summerell,2006). *Penicillium*, like *Aspergillus* and *Fusarium*, can cause Penicillium ear rots in maize infested by insects (Liu et al., 2022). Toxic species of *Penicillium* also are soilborne and may be found in freshly harvested and stored maize (Mansfield and Kuldau, 2007). Some *Trichoderma* species from agricultural soil can cause ear rots in maize (Pfordt et al., 2025).

Along with fungal pathogens, soil also contains avirulent fungi, such as some *Trichoderma* spp., and symbiotic fungi, such as arbuscular mycorrhizal fungi (AMF). Avirulent *Trichoderma*

symbionts can be exploited as cost-effective biocontrol agents (Sood et al., 2020). AMF can be used as biofertilizers that improve maize yield (Fan et al., 2024).

Maize in Nepal is affected by a wide range of fungal pathogens, and soilborne pathogenic fungi cause significant losses. Manandhar (1997) identified 75 fungal maize pathogens in Nepal. The most common and economically important fungal maize diseases were gray leaf spot (*Cercospora*), northern corn leaf blight (*Exerohilum turcicum*), southern corn leaf blight (*Bipolaris maydis*) (Pattanayak et al., 2025), banded leaf and sheath blight (*Rhizoctonia*), ear and stalk rot (*Fusarium*), head smut (*Ustilago*), common rust (*Puccinia*), and brown spot (*Physoderma*). (Subedi, 2015). Fungi commonly recovered from asymptomatic preharvest maize include *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. (Ekwomadu et al., 2018; Katati et al., 2023). These fungi can produce aflatoxins, fumonisins, deoxynivalenol, nivalenol, ochratoxins, zearalenone, and other secondary metabolites (Chapter 3).

Maize is the second most common staple food consumed in Nepal (Choudhary et al., 2025) and is prone to contamination by aflatoxins and fumonisins (Desjardins and Busman, 2006; Joshi et al., 2022). When contaminated maize is consumed, the after effects can include mycotoxic, stunted growth, immunosuppression, liver and oesphageal cancer, and even, perhaps, death (Baral and Nakazawa, 2022; Joshi et al., 2022; Kamala et al., 2018; Tong et al., 2021). Women in the ZOI in western Nepal who tested positive for aflatoxin exposure often had smaller preterm babies with stunting issues (Trevino et al., 2022). Aflatoxin and fumonisin contamination in maize severely affected these women and children in western Nepal (Trevino et al., 2019) But this exposure was not just from contaminated maize, as exposure levels remained detectable even during the off season for maize consumption. Presumably, other foods in the diet also were contaminated with mycotoxins. These studies left unanswered questions related to the contamination of maize that

could be at least partially answered with an empirical study of the mycobiome in maize paired with a study of the soil sample where the plant was growing.

Historically, studies of fungal communities and diversity in soil and maize have been limited due to complexities associated with culturing. Culturing fungi in the lab allows identification of only a small fraction of the diversity present, as many fungi are difficult or impossible to grow under standard laboratory conditions (Rama and Qandt, 2021). Culture-independent metagenomic analyses with DNA obtained directly from the source material enable more comprehensive studies of fungal communities (Daniel, 2005).

To date, microbiomes studies tend to study soil mycobiomes and plant mycobiomes as separate entities. Many studies have focused on the plant and the soil microbiome's impact on plant yield, stress tolerance and resistance to pathogens (Arif et al., 2020; Qu et al., 2020). Microbiome composition depends on environmental and agricultural management systems, and the phenotype and genotype of the plant. Studies of fungal communities in paired samples remain overlooked.

Understanding taxonomic composition of the mycobiome and interactions between the members of the complex component network of beneficial and harmful fungi should increase our understanding of current problems in sustainable agriculture and help identify strains and interventions to help solve the detected problems.

The objective of this study was to evaluate fungal communities from maize ears and soil and determine if the degree of overall between soil and maize mycobiomes. My working hypotheses were that maize ears and soil harbor distinct overlapping mycobiomes and that these mycobiomes differed by geographic location. I tested these hypotheses by evaluating mycobiomes from maize flour from individual ears and soil in which 12 individual plants growing in two districts. Mycobiome members were identified with ITS2 metabarcode data at genus level and then

compared for fungal community composition and diversity. Results from this study provide a more complete picture of the fungal soil and maize ear communities at the genus level. This picture can be used to identify beneficial, antagonistic, and suggest a few functional roles for individual fungi within them.

Materials and Methods

Sampling sites. Paired soil and maize ear samples were collected from 6 fields in each of the two districts [Kailali and Dadeldhura (Fig. 2.1)]. Dadeldhura is a district in a hilly region of Nepal of 1,538 km^2 at an average elevation of 1,848 m (Department of Hydrology and Meteorology, 2020). The climate is temperate with annual temperatures ranging from 3–15º C (Weather and Climate Nepal, 2026). Annual rainfall in Dadeldhura is 1400-1640 mm (ADB, 2020). Monsoon rainfall begins in June and continues until September. October through May is the dry season in both the districts.

In Dadeldhura, soil pH is acidic ranging from 5.2-6.4. Organic matter and N content are low, and P and K content are high (Devkota et al., 2019). In the mid-hills, soils are cambisols, geologically young and without defined soil horizons. In the hills clay-loam, sandy loam and silty loam soils are found (Paudyal et al., 2001).

Kailali is a district in the Terai region with an area of 2,742 km^2 at an average elevation of 140 m. The climate is tropical to subtropical (FAO UNDP, 2003) with annual temperatures ranging from 17-39º C. The annual rainfall in Kailali is 1650-1915 mm per year (Department of Hydrology and Meteorology, 2024).

In Kailali, soil in the terai are alluvial with low to high sand content, and are mostly sandy loam (Paudyal et al., 2001). These soils have a loamy texture, neutral pH and moderate levels of N, P, and K (Bhatta et al., 2026).

Figure 2.1. A map of Nepal showing with sampling sites. Six fields were selected for maize and soil collection in Dadeldhura and Kailali districts (GoN, 2000).

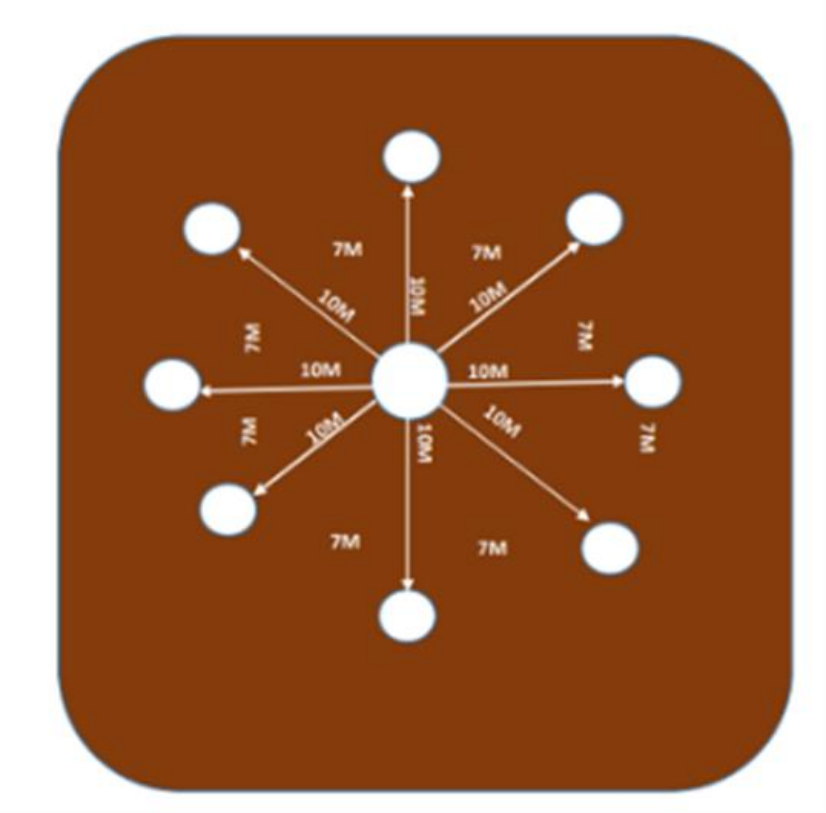

Figure 2.2. Map used for field soil collection.

Soil and maize sample collection.

Paired soil and maize ear samples were collected from six fields in each district. Soil was collected from the fields where the maize plant was growing. The soil was collected from nine equidistant points from the center of the plant (Fig. 2.2). Approximately 200 g of soil was collected from each point. To obtain the soil sample, a piece of PVC pipe 5 cm in diameter and 30-50 cm in length was used. One end of pipe was sharpened so it could be pushed into the ground. A mark at 10 cm was made on the pipe and a second piece of pipe slightly smaller in diameter was used to push the soil out of the pipe once the sample had been taken. The sharpened pipe was pushed into the soil up to the 10 cm mark on the outside of the pipe. The pipe with the soil was removed from the ground and soil pushed into a bucket with the second piece of pipe. The soil volume from one sample was approximately 200 g for a total of 1.5-2.0 kg across the ten subsamples for each field.

Twelve soil samples, one from each field, were collected in separate polythene bags.

Each field soil sample was transferred to a separate sterile steel container and mixed well to ensure uniformity. The homogenized soil was stored at 4°C for two weeks until DNA was extracted.

Maize sample collection.

A single stored maize ear with husk intact, was taken from those harvested from the fields in Dadeldhura and Kailali in the second week of February 2019 up to six months post the harvest.

Maize from Dadeldhura was collected near Sandanee village (Lat: 29° 6.6′ N, Long: 80° 14.4′ E., Alt: 276 m, GPS precision: 6.07 m). Maize was harvested in the 4th week of Bhadra (August) and the 1st week of Asoj (September) and then stored for up to six months before analysis. Soil was semi-dry/moist when the maize was harvested from the field.

Maize from Kailali was collected from Motinagar (Lat: 28° 32' N , Long: 81° 5′ W, Alt: 100 m, and GPS precision: 4.55 m) and Himmatpur village (Lat: 28° 32' N, Long: 81° 5′ W, Alt: 99 m GPS precision: 4.55 m). The maize was harvested in the fourth week of Jesttha (June), the fourth

week of Bhadra (August), and the 1st week of Asoj (September). Maize ears were stored on farm for 5-9 months prior to sample collection. The soil was mostly semi-dry and dry when the maize was collected from the field.

Maize grain was dried, ground to flour, and shipped to University of Nebraska-Lincoln and then forwarded to Kansas State University.

DNA extraction, PCR amplification and Illumina MiSeq sequencing.

Total DNA was extracted from 500 mg soil in triplicate (36 soil DNA samples) from each field by Intrepid Nepal, (Kathmandu, Nepal). using Nucleospin® Soil kits (Machery Nagel, Pennsylvania) following the manufacturer's instructions. 36 soil DNA samples (12 fields × 3 technical replicates) were used for amplicon PCRs and sequencing.

Total DNA was extracted from 1500 mg maize flour with Nucleospin® Plant II Maxi kits in triplicate (36 maize DNA samples: 12 ears × 3 technical replicates) according to the manufacturer's instructions for June 2019/Rev.11. and stored in elution buffer (5mM Tris HCL, pH 8.5) at – 20°C until PCR amplification. DNA concentration was measured with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Carlsbad, CA) and normalized to 5 ng/µL.

DNA samples (72) were stored at -20°C in elution buffer until further processing.

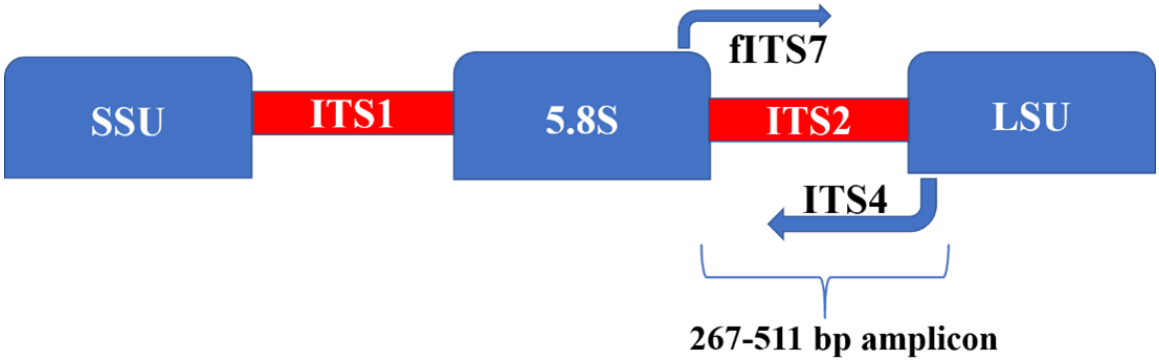

Figure 2.3. ITS2 region of fungal rDNA with fITS7 and ITS4 region targeted for sequencing

Molecular identifications were based on the metabarcode sequence of the ITS2 rRNA region (Taylor et al., 2016).The first stage PCR amplifies DNA by using ITS 4 and fITS7: fITS7 (5′-GTGARTCATCGAATCTTTG-3′) (Ihrmark et al., 2012) with a 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′ overhang adapter, and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990) with a 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′.

First step primers targets ITS2 region (Fig.2.4) and contain heterogeneity spacers and Illumina sequencing primers

The PCR reactions included an initial denaturing step for 3 minutes (95°C) followed by 25 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds; extension at 72°C for 30 seconds; and concluding with a 5 min final extension (72°C) (Schmidt et al., 2013).

First stage PCRs were performed in 25 µl reaction volumes using: 12.5 µl 2× KAPA HiFi HotStar ReadyMix [buffer containing dNTPs (0.3 mM each at 1×),DNA Polymerase (0.02 U/µL reaction), $MgCl_2$ (2.5 mM at 1×) with stabilizers], 2.5 µl fungal DNA (5 ng/µl in 10 mM Tris pH 8.5), 5 µl forward primer (1 µM), and 5 µl reverse primer (1 µM).

After the PCR amplification of the targeted ITS region with overhang adapters attached, a second set of PCRs (8 cycles), with the same program as the first set of PCRs was done to add indexes. Barcodes and flow cell linkers are introduced in this second round of PCR by using an Illumina sequencing primer as a target.

For the second set of PCR reactions, the full-length dual indexes and the Illumina sequencing adaptors (P5 Index 2 adapter and P7 Index 1) were added. The second PCR was performed in 50 µl reaction volumes containing: 5 µl Clean PCR1 Library, 5 µl Nextera XT Index Primer 1

(N7xx), 5 µl Nextera XT Index Primer 2 (S5xx), 5 µl CD Index Primer 1 (24), 5 µl CD Index Primer 2 (24), and 25 µl 2× KAPA HiFi HotStart Ready Mix. (Illumina , 2019).

The resulting libraries were validated with the DNA 7500 chip on the Agilent 2100 Bioanalyzer and subsequently pooled in equimolar amounts. The final pooled library, including water as the negative control, was sequenced by using a 500 cycle (2 × 250 bp) protocol with an Illumina Nano sequencing kit (1 million read pairs output) (Caporaso et al., 2012; Holm et al., 2019).

Sequence data analysis

Paired end sequencing output was retrieved from a Base Space Sequence Hub (Illumina). Sequence data were processed with the bioinformatic program mothur pipeline following MiSeq standard operating protocol (v.1.47.0; Schloss et al., 2009).

In brief, the paired end fastq sequences were contigs, with primer sequences trimmed and screened to cull sequences with ambiguous bases (≥ 8). Fungal sequences were truncated to the shortest sequences in the dataset (245 bp) to generate the shortest high-quality reads. The high-quality sequences were truncated to 245 bp to allow for pre-clustering and vsearch clustering. The CLUSTER command in mothur clustered clean and non-redundant sequences that were ≥ 97% similar to OTUs (Kozich et al., 2013). OTUs that were detected in the negative control. Fungal sequences were screened for chimeras using VSEARCH (Rognes et al., 2016) and sequences presumably chimeric were removed. The remaining sequences were assigned to fungal taxonomic groups at genus level by using the mothur implemented Naïve Bayesian Classifier (Wang et al., 2007) and the UNITE reference database (UNITE_public_mothur_10.05.2021), (Abarenkov et al., 2024). Sequences without matches in the UNITE database and these for non-target taxa such as Metazoan, Rhizaria, and Viridiplantae were removed.

Data Analyses

RStudio (version 2025.05.0, Build 496) was used for statistical analyses and graphical data representations of community composition. Fungal communities were analyzed at the genus level. Technical replicates of each sample were pooled into one experimental replicate, reducing the number of data sets from 72 to 24 (12 maize ear and 12 soil samples). The fungal community composition in soil and maize was determined based on the relative abundance of sequences assigned to each genus.

We rarefied the sequence data to 6000 sequences per sample based on the sample with the lowest number of reads to analyze α and β diversity/compositional differences of the fungal community in maize ear and soil. We used the function "rarefy_even_depth_()" in phyloseq [v.1.46.0; McMurdie and Holmes (2013)] to avoid unequal sequence bias while comparing estimators in samples with unequal numbers of sequences (Gihring et al., 2012).

Alpha diversity (within-sample community diversity) was measured with four metrics: observed number of OTUs (Sobs-observed species richness), Chao1 (extrapolated species richness), Shannon's Diversity Index (H), and Shannon's evenness (J) (Schloss et al., 2009) and visualized in box plots (Fig. 2.7). The observed number of genera, i.e., the number of observed OTUs, was used to estimate the community richness. Chao 1 extrapolates and estimates species richness after accounting for undetected rare genera based on observed data (Gotelli and Chao 2013). Shannon's Diversity Index is combined measure of species richness and evenness. This index provides the relative abundance. It increases with richness and evenness, but is weighted heavily towards richness. A numerical estimate of evenness is Shannon's Evenness, which is calculated as $J = H / \log(S)$ where H is Shannon's index and S is the number of species observed (Magurran, 2004). Evenness is 1 when all genera have the same relative abundance. It decreases as differences in the

relative abundances of species in the community increases. The functions “estimate_richness” and “plot_richness” were used to create a graph of richness estimates for each of the 12 samples in the phyloseq datasets (McMurdie and Holmes, 2013). Differences in all four fungal α diversity metrics across both sites were evaluated with a two-way ANOVA in R Studio. To avoid violating the assumptions of parametric tests, we tested group differences with a Kruskal-Wallis rank test.

Beta diversity (between-sample diversity) was estimated by calculating the pairwise Bray-Curtis distances which were visualized by using Principal Coordinate Analysis (PCoA) with the function “ordinate ()” (method = "PCoA") (distance = "bray") in the phyloseq package (Bray and Curtis, 1957). To test compositional differences among the sampling sites (Kailali vs. Dadeldhura) or sample types (soil vs maize), a non-parametric permutational analysis of variance (PERMANOVA) was done on the Bray-Curtis distance matrix. Function adonis2 was used to calculate PERMANOVA in the vegan package in R (Oksanen et al., 2020). A permutation test for homogeneity of multivariate dispersions were performed on Bray-Curtis distance matrices (Yan et al., 2020). Homogeneity of group dispersion was evaluated by using the ‘betadisper’ function and group differences inferred by adonis.

To determine if any OTUs were disproportionately abundant in soil or maize ears in either district, we used indicator species analyses with the “multipatt()” function in R package “indicspecies” (v. 1.8.0; De Cáceres and Legendre, 2009). Indicator value is an index to measure association between species and a site group (Dufrêne and Legendre, 1997). We calculated indicator values for the 100 most abundant OTUs across all samples and inferred differences by using a permutation test. In order to account for multiple testing, we calculated false discovery rate (FDR) by using the function “p.adjust()” in R. Indicator values for OTUs in each sample ranged from 0-1. The higher

the indicator value, the stronger the association of the OTU with a sample. A lower indicator value and the lack of significant *p* value could both be used to suggest that the associated OTU was not a good indicator.

Results

Community description and OTUs in soil

We analyzed DNA from maize ears and soil from 12 fields in two districts of Nepal. 395,747 sequences were retained after quality control; 213,3004 high quality sequences from soil and 182,743 from maize ears communities. There were 2,458 OTUs in the combined soil and maize ear communities. The number of sequences per sample ranged from 6,169 to 25,450 with a mean of 16,489 ± 4,929.

The sequences clustered into 2,458 OTUs. The overall fungal community was dominated by Ascomycetes (78% of sequences and 60% of OTUs), followed by Basidiomycetes (14% of sequences and 21% of OTUs), Mortierellomycetes (4% of sequences and 2% of OTUs), and Chytrids (2% of sequences and 5% of OTUs). A small proportion of the sequences could not be assigned to a taxon (2% of sequences and 7% of OTUs).

Soil community

Soil community sequences were assigned to Ascomycetes (75% of sequences, 59% of OTUs), Basidiomycetes (10% of sequences, 21% of OTUs), Mortierellomycetes (7% of sequences, 2% of OTUs), Chytrids (4% of sequences, 2% of OTUs) and unclassified fungi (3% of sequences, 7% of OTUs). Members of several phyla such as Glomeromycota, Mucoromycota, Kickxellomycota,

Basidiobolomycota, etc. were infrequent and represented by < 1% of the sequences from soil. High-quality DNA sequences from soil clustered to 2,350 OTUs of which 1601 (68%) were assigned to 630 known fungal genera of which 571 could be placed in known/ classified genera. The remaining 32% of the OTUs were assigned to more inclusive taxa. In soil, 608 OTUs and 82 genera were unique to Dadeldhura and 836 OTUs and 163 genera were unique to Kailali. The number of sequences from a soil community ranged from 10,412 to 22,175, with a mean of 17,750 ± 3,079.

The genus level assignment for all of the sequences from the soil samples can be found in the Appendix Supplementary Table 1.

Fungi in soil from Dadeldhura were in 452 known genera and from Kailali there were 532 known genera.357 genera were shared between the locations, whereas 95 genera were unique to Dadeldhura soil and 177 were unique to Kailali. The ten most abundant genera in soil represented 40% of the soil community: *Fusarium* (9%), *Mortierella* (6%), *Curvularia* (4%), *Cyphellophora* (3%), *Pseudothielavia* (3%), unclassified fungi (3%), *Acremonium* (3%), *Colonostachys* (2%), *Chorodomyces* (2%), and *Lectera* (2%). *Fusarium* and *Mortierella* were present in all the soil samples (Fig. 2.4).

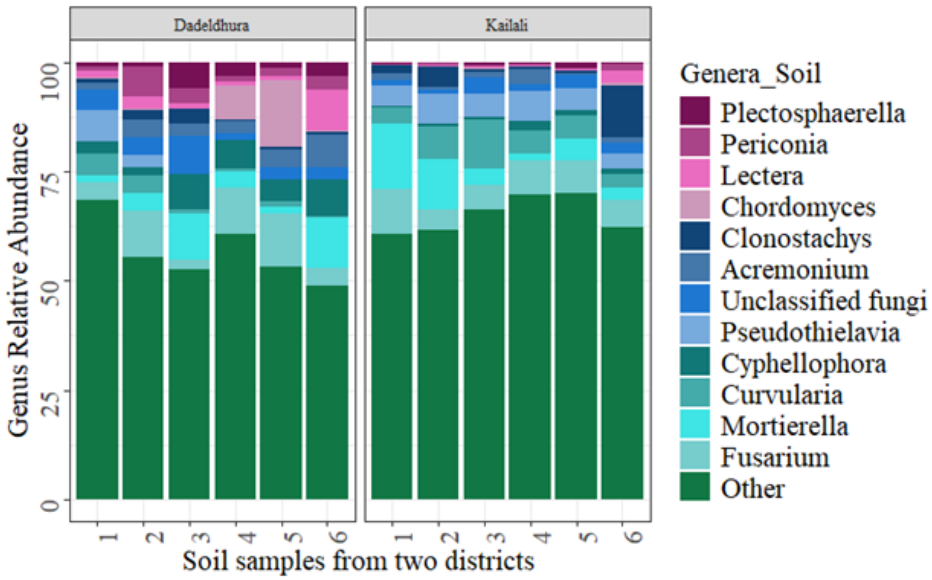

Figure 2.4. Relative abundance in soil of genera with a relative abundance > 1.5% of the total sequences. Genera with an individual relative abundance < 1.5% are pooled together and listed as 'Other'. Each colored segment corresponds to a genus in the legend, and bar heights represent the relative abundance of the displayed genus within each soil sample.

Maize ear community

The maize ear community sequences were assigned to Ascomycetes (81% of sequences, 71% of OTUs), Basidiomycetes (18% of sequences, and 20% of OTUs) and unclassified fungi, Mucoromycetes, Chytridiomycetes, Basidiobolomycetes, Glomeromycetes and Kickxellomycetes collectively (1% of sequences, and 9% of OTUs). The high-quality sequences from maize clustered to 366 OTUs, 310 of which were assigned to 179 known genera whereas 27 were assigned only to more inclusive taxa. The number of sequences from a maize ear sample ranged from 6,169 to 25,450, a mean of 15,229 ±6,152. Maize ears from Dadeldhura had 29 unique OTUs and maize ears from Kailali had 49 unique OTUs. Of the 206 genera recovered from maize ears, 179 genera were known, classified to specific genera, seven were unknown/ unclassified genera and 20 were unclassified (Appendix Supplementary Table 2).

At the genus level, 90% of the sequences from maize ears at both locations belong to *Sarocladium* (33%), *Fusarium* (19%), *Ceratobasidium* (10%), *Neodeightonia* (7%), *Waitea* (5%), *Trichoderma* (5%), *Lasiodiplodia* (4%), *Bipolaris* (3%), and *Aspergillus* (3%). *Sarocladium*, *Fusarium* and *Ceratobasidium* were present in all samples (Fig. 2.5).

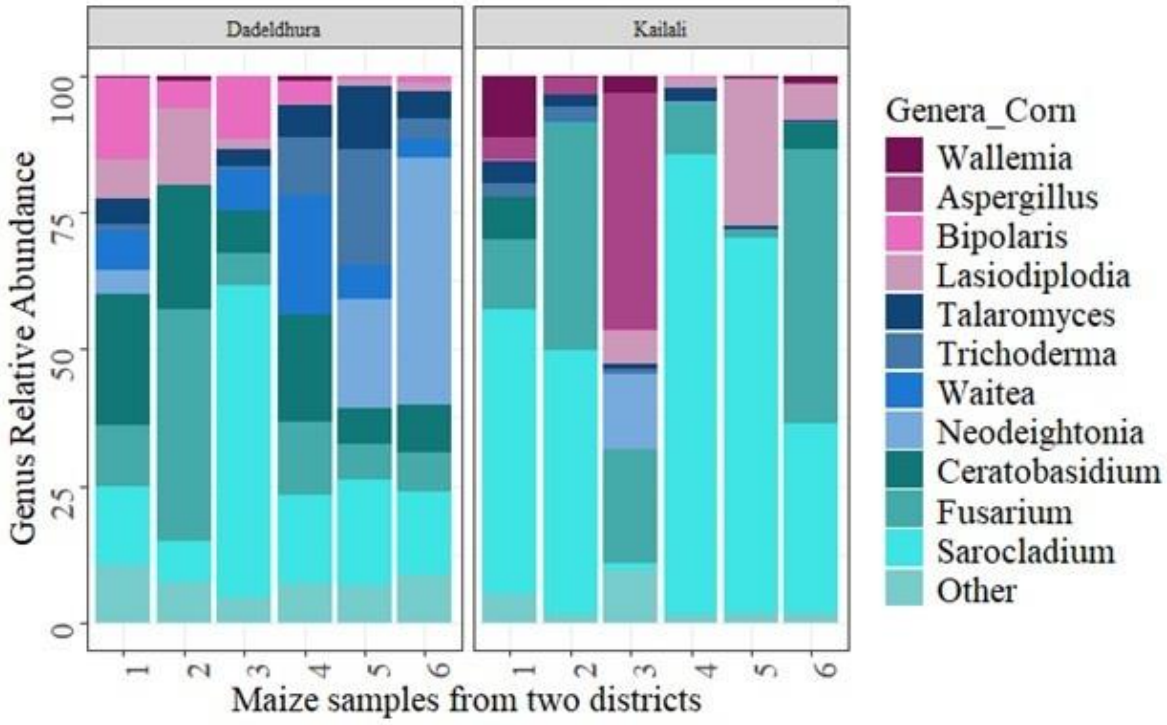

Figure 2.5. Relative abundance in maize ears of genera with relative abundance >1.5% of total sequences, genera with relative frequency < 1.5% are pooled together and listed as 'Other'. Each colored segment corresponds to a genus listed in the legend, and bar heights represent the relative abundance of the displayed genus within each sample.

Maize ears from Dadeldhura had 127 known genera, of which 104 also were found in soil and 8 were unique to these maize ears as indicated in the fig.2.6. Maize ears from Kailali had 124 known genera of which 96 also were observed in soil, and 14 were unique to these maize ears. Seventy-two genera (72/179) 40% were present in maize ears from both locations.

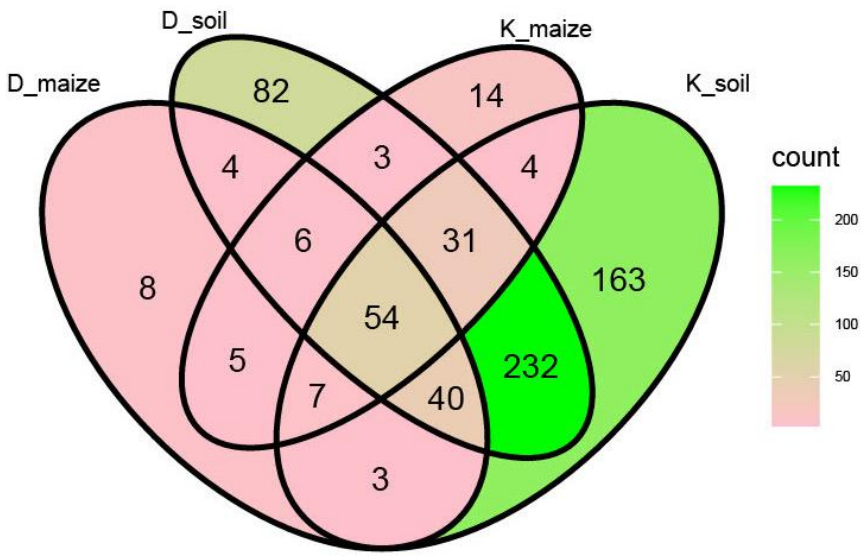

Figure 2.6. Four set Venn diagram showing number of genera shared in soil and maize ears community from two districts. Ellipse "D_maize" in the Venn diagram represents number of genera from maize ears in Dadeldhura. Ellispse "D_soil" represents number of genera from soil in Dadeldhura. Ellipse "K_maize" shows number of genera from maize ears from Kailai. Ellipse "K_soil" represents the number of genera from soil in Kailali.

Richness and diversity in maize ears and soil

Fungal communities were diverse within maize ear and soil samples. The Sobs were higher in Kailali maize ears and Kailali soil than in Dadeldhura maize ears and soil (Fig .2.7). Shannon diversity and evenness were higher in Dadeldhura maize and Kailali soil. (Fig.2.7). Calculations for α diversity metrics to describe richness and diversity of fungal communities in soil and maize ears can be found in Appendix Supplementary Table 3.

Both observed richness (Sobs) and extrapolative richness (Chao1) estimates were higher for soil than for maize ears and differed between the sample types but not between districts.

The number of unique OTUs observed in maize ears was estimated to range from 44 to 80 with a mean of 58±13. The number of unique OTUs in soil samples was estimated to range from 419-599 with a mean of 449±61. Maize ears and soil had significantly different Sobs/observed species richness (Kruskal-Wallis: χ^2= 19, df = 3, p = 0.0002). The Sobs/observed species for soil is significantly larger than that for maize ears. Species richness for combined samples from Dadeldhura (232±175) and Kailali (275±220) were not significantly different (Kruskal-Wallis: χ^2= 0.85, df = 1, p =0.35).

Chao1 was estimated to range from 50-91 with a mean of 72±14 in maize ears. In soil, Chao1 ranged from 479-737 with a mean of 557 ±79. (Chao1) differed between maize ears and soil: sample types ($F_{1,20}$= 653, p <0.0001). Chao1 for soil is significantly larger than that for maize ears. Extrapolative richness estimate for combined samples from Dadeldhura (289±216) and Kailali (340±276) were not significantly different. Kruskal-Wallis test confirmed that Chao1 of soil and maize ear did not differ in two sites (Kruskal-Wallis: χ^2= 0.4, df = 1, p = 0.5).

Shannon diversity was tested with a two ANOVA and it differed between maize ear and soil communities ($F_{1,20}$ = 358, p < 0.001) but not between sites ($F_{1,20}$ = 4, p = 0.05). The effect of sample types on Shannon diversity was not the same in both districts. Pairwise post-hoc tests indicated that Shannon diversity in maize ears from the districts differed (p < 0.05), but that there was no significant difference between the soils at the two sites (p > 0.05).

Shannon evenness was tested with a two-way ANOVA and differed between maize ears and soil ($F_{1,20}$= 118, p < 0.001). Overall ANOVA detected the site effect ($F_{1,20}$ = 12, p = 0.002). Thus, the pairwise post-hoc tests indicated that Shannon evenness differed in maize ears from two districts. (p < 0.001). But in case of soil, tukey-adjusted post-hoc pairwise comparisons found no statistically

significant differences between soil from two sites (p = 0.98). Thus, although overall there was evidence for overall heterogeneity among sites, evenness in the soil communities from the two sites did not differ.

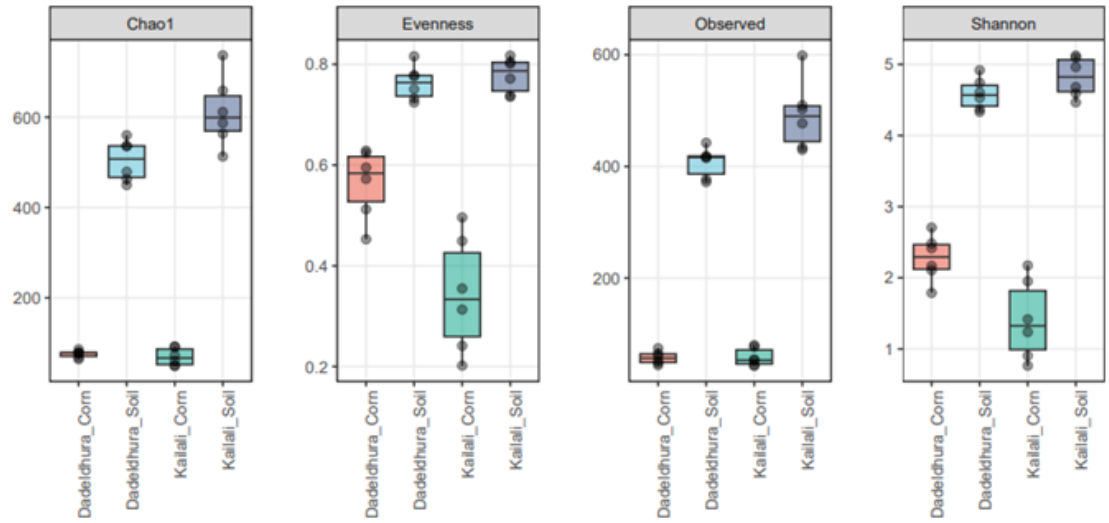

Figure 2.7.Fungal diversity measure metrics in soil and maize ears from two districts. Boxplot shows Chao1, evenness, richness and Shannon diversity.

Community Composition/ Beta diversity

We used PCoA and PERMANOVA to visualize and test community differences. We observed significantly different fungal community profiles between soil and maize in the two districts. Site and sample type shaped the distinct fungal community compositions (Fig. 2.8). In this analyses, PCoA 1 explains 37% of the observed variation, and PCoA 2 explains 12% of the variation. Strong separation of maize ear and soil is clear on the first PCoA axis (37%). To confirm statistical significance of variation in mycobiome composition, separate permutational multi variate analyses of variance

(PERMANOVA) were done on Bray-Curtis distance. Sample type explained higher percentage of variation in mycobiome composition than di the sampling sites. PERMANOVA confirmed the significance differences in community composition in soil and maize ears ($F_{(1,20)} = 3.83$, $R^2 = 0.36$, $p < 0.001$). The two districts differed but this effect was small as indicated by R^2 ($F_{(1,20)} = 15.8$, $R^2 = 0.08$, $p = 0.003$).Sampling site ($R^2 = 0.087$, ≈ 9%), sample type ($R^2 = 0.36$, 36%), and interaction of sampling sites and sample ($R^2 = 0.10$, 10%) explained 55% of the variation in community composition and 45% was unexplained residual in PERMANOVA. The PERMANOVA (55%) and PCoA (49%) estimates are not the same because PCoA axes represents variation represented in two-dimensional plot where as PERMANOVA statistically shows how much variation can be statistically attributed to the groups being compared.

To test if one group was more dispersed or clustered relative to the other, a β dispersion analysis was used (Appendix Supplementary Fig A. 2). There were differences in dispersion ($pr > F > 0.05$) among sites or sample types.

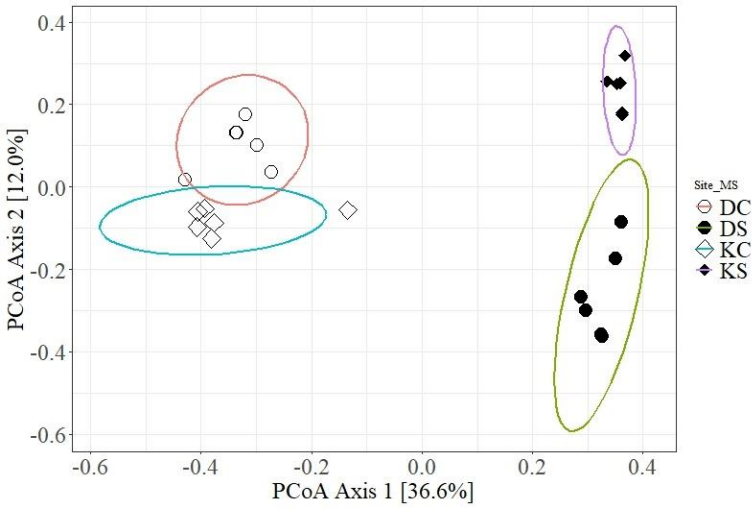

Figure 2.8. Principal Coordinate Analyses (PCoA) of fungal communites from maize ears and soils from two districts. 'DC' indicates an individual maize ear from Dadeldhura and 'DS' represents an individual soil samples from Dadeldhura. 'KC' represents an individual maize ears from Kailali and 'KS' represents soil samples from Kailali.

Indicator species analyses were used to identify OTUs disproportionately associated with fungal communities in maize ears and soil. False detection rate (FDR) correction was applied, significant indicator remained for soil communities, but not in maize communities (Appendix Supplementary Table 4). Prior to FDR, I identified 12 indicator OTUs in maize ears that were assigned to *Ceratobasidium*, *Neodeightonia*, *Waitea*, *Bipolaris*, *Talaromyces*, *Curvularis*, *Nigrospora*, unclassified *Cyphellaceae*, and unclassified *Trichosphaeriales* at $\alpha = 0.05$.

In soil, there were 56 OTUs for 46 unique indicator genera (Appendix Supplementary Table 5), for example, *Acremonium*, *Curvularia*, *Fusarium*, *Mortierella*, and *Talaromyces* at $\alpha = 0.05$ statistical significance was retained for all the OTUs after the FDR correction.

Discussion

Comparison of Phyla/genera in Soil and maize ears

1. Phyla in soil

Comparative study of fungal communities in soil and maize ears was done to understand the degree of overlap of the soil mycobiome with the maize ear mycobiome. ITS2 metabarcode DNA sequences from 12 soil samples and 12 maize ears from two districts clustered into 2458 OTUs.

These sequence data from soil belonged to Ascomycetes, Basidiomycetes, Chytridiomycetes, Mortierellomycetes and Mucoromycocetes (Fernandes et al., 2022; Lourenco et al., 2020) consistent with other soil analyses. The analyzed soil mycobiome contained all previously reported abundant fungal phyla in soil worldwide (Egidi et al., 2019; Muturi et al., 2024; Philippot et al., 2013). The presence of these dominant phyla int the current samples is consistent with the presence of a typical fungal community int the soil that can decompose organic matters and recycle nutrients (Lourenco et al., 2020; Sindhu et al., 2024)

2. Phyla in maize ears

Maize ears in this study were dominated by Ascomycocetes (71%) and Basidiomycocetes (20%) followed by Chytrids, Mortierellomycetes, Mucoromycetes, Glomeromycocetes, Basidiobolomycocetes and Kickxellomycocetes (< 3%). These results are consistent with those from Abe et al (2015) that documented abundant Ascomycetes and fewer Basidiomycetes in maize ears. The fungi such as *Aspergillus, Penicillium*, and *Fusarium* with potential to secrete secondary metabolites all belongs to common Ascomycetes (Chapter 3).

3. Most common genera in soil and maize ears

Mycobiomes in maize ears and soil overlap because soil contributes to the microbiome of the plant while it is growing (Li et al., 2014). 725 fungal genera were identified from 2340 OTUs in soil and 206 genera identified from 356 OTUs were detected in maize ears (Fig.2.6). 477 genera from 2092 OTUs were unique to soil fungal communities and 27 genera from 258 OTUs were detected. Based on 72% of the maize ear communities overlap with the soil communities and 11% of the soil communities overlap with maize ear communities. With 72% of the OTUs in the maize ear communities also present in soil communities, there is a strong and clear connection in terms of OTUs present between the soil and maize ear mycobiomes. Thus, 25-30% of the maize ear mycobiomes originates from a source other than the soil the plant is growing in.

3.1. Fusarium

Fusarium, an ascomycete was the most abundant genus in both soil and maize ears in my study. It is distributed worldwide Asia, Antarctica, Arctic ocean, Atlantic ocean, Australia, Europe, Indian ocean, North America, Pacific ocean and South America in water, soil, air, coral, deadwood, dust, lichen, litter, rhizosphere soil, shoot, topsoil, etc. (Vetrovsky et al., 2020). *Fusarium* species survive in soil, different plants and seeds (Leslie et al., 1996; Leslie and Summerell, 2006; Meyer et al., 2014; Nikitin et al., 2023). It is commonly detected in roots, shoots, stems and seeds at pre- and post-harvest stages of maize growth (Katati et al., 2023; Logrieco et al., 2002; Pfordt et al., 2020). *Fusarium* spp. are best known from tropical climates (high temperature, precipitation and humidity), hot arid climates, and temperate climates (Gordon, 1960; Summerell et al., 2003) but also found in Artic settings (Vetrovsky et al., 2020).

Fusarium spp. cause diseases in plants and most of the propagates over season in soil that survives or diseased plant debris. *Fusarium* spp.in soil often face intense competition with other saprophytic fungi there (McKeen and Wensley, 1961; Gordon, 2017). *F. verticilloides* can be found on seed surfaces as well as within internal tissues of maize (Xing et al., 2018). *Fusarium* species are the primary causes of maize ear, kernel and stalk rot (Li et al., 2019). They also are capable of producing mycotoxins such as fumonisin, DAS, deoxynivalenol (DON), nivalenol, T2, and zearalenone, with potentialy devastating impacts on maize consumers (Munkvold et al., 2021).

Higher relative abundance of *Fusarium* in maize ears could be attributed to: (i) *Fusarium* may move endophytically from the planted seed to the maize ear, (ii) fungal growth may have occurred during storage, or (iii) spores may have been transferred directly from the soil or other plants via wind, rain splash or insect's movement. Thus, while soil is an important contributor to the maize ear mycobiome , it is not the only contributor.

The maize ears analyzed were stored for several months before collection, and *Fusarium* could have grown on the ears while they were in storage if temperature and moisture conditions were appropriate for proliferation. The high abundance of *Fusarium* spp. capable of causing soil borne fungal diseases and producing mycotoxins in maize and other food plants grown in these fields.

3.2 *Sarocladium* and *Acremonium*

Sarocladium, an ascomycete, is one of the most common fungi in maize ears in this study but was not common in soil. *Sarocladium* was found in all the maize ears. The relative abundance of *Sarocladium* was 32% in maize ear communities and 0.35% in soil communities. *Acremonium*, another ascomycete contains species that are morphologically similar to some species in *Sarocladium*. The relative abundance of Acremonium in soil was 2.7% and 0.06% in maize ears. *Acremonium* and *Sarocladium* are closely related but are in distinct genera. *Acremonium* and *Sarocladium* have had taxonomic revisions in which species have been moved from one genus to another. The maize endophyte *Acremonium zeae* is an older name for *Sarocladium zeae.* The genus *Acremonium*, previously known as *Cephalosporium*, is common in soil and on dead plants.

Pyrrocidines can be produced by some *Acremonium* spp. and are antagonistic to kernel rotting and mycotoxin production by *A. flavus* and *F. verticillioides* in preharvest maize kernels (Wicklow et al., 2005). Pyrrocidine A also had activity against the causative agent of Goss's bacterial wilt of maize and other ear rot pathogens of maize, including *Fusarium graminearum* and *Nigrospora oryzae*. *Nigrospora* spp. was detected in both the soil and maize communities evaluated in this study.

Sarocladium was abundant in all the maize ears in this study, a finding consistent with previous reports from maize (Abe et al., 2015; Wang et al., 2019). In addition, the relative abundance of

Sarocladium was higher than that of *Fusarium* in 8/12 of the maize ears evaluated. This differential abundance could be attributed to antagonistic activity by *Sarocladium* towards *Fusarium*, as previously reported (Katati et al., 2023; Wicklow and Poling, 2008) although there was no evidence of a negative correlation between the abundance of *Fusarium* and *Sarocladium i*n this study.

Sarocladium, like other genera in this study, contains multiple species that can not be readily resolved in ITS metabarcode data. Thus, the *Sarocladium* spp. present in the three ears dominated by *Fusarium* might be one or more *Sarocladium* species other than *S. zeae.*

The high relative abundance of *Sarocladium* and *Fusarium* in maize ears is a clear indication

That these fungi both can have an important role in this ecological niche and that better understanding of the interaction(s) is needed to understand maize ear and kernel rots and mycotoxin profiles.

Genera abundant in soil but not in maize and vice-versa

Other than *Fusarium*, the genera abundant in soil were not abundant in maize ears and vice versa. Even though there were overlapping OTUs between soil and the maize ears, their relative abundance in the two sources varied. 146 genera were recovered from both soil and maize. There were 13 OTUs capable of colonizing a maize ear while 6 were unable to colonize. Other than *Fusarium*, the ten most common genera in soil were not found amongst the ten most common genera in maize ears (Rochefort et al., 2021). These differences can be attributed to ecological factors and to host filtering.

Mortierella is the most abundant genus in soil after *Fusarium*. The relative abundance of *Mortierella* in soil was 6% and it was < 0.1% in maize ears. *Mortierella* is common in soil samples

in which multiple crops, including wheat, potato, maize, beans, and citrus fruits have been grown (Nguyen et al., 2019; Ozimek et al., 2018). *Mortierella* was recommended as a potential indicator of sensitivity to long-term maize cultivation (Wolińska et al., 2022). It's relative abundance is associated with the intercropping. Its relative abundance declined in monoclulture field. High abundance of *Mortierella* in the soil indicates that intercropping, rather than monocropping, had been adopted as the management strategy in the fields of maize from which my soil samples were derived. Had these fields been managed through monocropping, then *Exophiala* would have been more abundant (Wolińska et al., 2022). The relative abundance of *Mortierella* DNA sequences in the soil (6.1%) relative to *Exophiala* (0.04%) is consistent with an intercropping management strategy as well (Wolińska et al., 2022). *Mortierella* in the soil also enriches for beneficial microorganisms, provides resistance to soil-borne pathogens, and can increase crop yields (Ozimek et al., 2018). The presence of *Mortierella* in my results is consistent with the hypothesis that some of the fungi in these soil might be used as biocontrol agents or to increase plant growth (Ozimek et al., 2020).

Curvularia, an ascomycete, was the third most common fungal genus in my soil samples. Its relative sequence frequency in the soil was 3.8% and in the maize ears it was 0.75%. *Cuvularia lunata* is responsible for *Curvularia* leaf spot in maize (Cui et al., 2023; Henrickson and Koehler, 2022). Secondary metabolites of *Curvularia* spp. have been suggested for use in weed management and the control of plant diseases (Ribeiro et al., 2025).

Chytrids are known in Nepal in unvegetated soil from high elevation sites, e.g., the Annapurna Conservation Area (Freeman et al., 2009). My results extend the range of these fungi to lower altitude areas that are suitable for farming, as 4% of the total DNA sequences were attributed to phylum Chytrids and 1.2% of the total DNA sequence reads were for unclassified Chytrids. The

chytrid DNA sequences detected in my soil samples suggest that additional species in this group from Nepal remain to be described.

Genera common in maize

Sarocladium and *Fusarium* have been previously discussed. *Ceratobasidium* and *Waitea*, both basidiomycetes, were the next most common genera in maize, with the relative abundance of *Ceratobasidium* sequences at 10% in maize ears and 0.13% in soil, while the frequencies for *Waitea* were 5% in maize ears and 0.1% in soil. The binucleate *Ceratobasidium* and multinucleate *Waitea* are both teleomorphs of anamorph genus *Rhizoctonia* (Blanco, et al., 2018), which has been associated with plant diseases of many crops including beans, cabbage, oilseed rape and maize (Kammerer, 2011; Leiner and Carling, 1994). Basidiomycetes were relatively less common in soil in this study than in maize ears, which is not consistent with the previous studies that in some cases reported basidiomycetes were dominant in soil where maize was cultivated (Jose et al., 2018). The results of my study builds on previous studies where soil-borne *Ceratobasidium* and *Waitea* were plant pathogens. The abundance of these fungi in maize ears could provide a source of fungi that results in plant diseases in the host plant, or result from a plant defense that aborted a fungal attack and resulted in an accumulation of these fungi in the maize ears.

Trichoderma, an ascomycete, was also relatively abundant in maize ears (4.8%) and relatively rare in soil (0.4%). Some *Trichoderma* spp. can cause ear rot of maize (Pfordt et al., 2024; Sanna et al., 2026). While species of *Trichoderma* have been exploited as biocontrol agents and plant growth promoters (Benitez et al., 2004; Fu et al., 2021; Manandhar et al., 2018; Mitrovic et al., 2025; Pandya et al., 2011). Since ITS2 metabarcodes cannot differentiate species of *Trichoderma*, the *Trichoderma* spp. present in the maize ears and soil could be a mixture of both beneficial and pathogenic fungi species.

Bipolaris, an ascomycete, was relatively abundant in maize ears (3.5%) and relatively rare in soil (1%). *Bipolaris zeicola* can cause maize ear rot (Naumann et al., 2009), and *Bipolaris maydis* causes Southern corn leaf blight (Aregbesola et al., 2019). Given the limitations of the ITS2 metabarcode analyses, *Bipolaris* spp. may be relatively common in maize fields in Nepal and interventions to control it should be developed.

Aspergillus, an ascomycete, was relatively common overall in maize ears (3%) and surprisingly rare in soil (< 0.1%). Some *Aspergillus* is a complex genus that contains numerous soilborne opportunistic plant pathogens that can infect crops in the field, but also can infect crops while in storage. A major concern with *Aspergillus* contamination is that the crop also could be contaminated with aflatoxins or ochratoxins (Probst et al., 2011), and that the toxin contamination could increase post-harvest due to improper storage and transportation conditions (Mahuku et al., 2019). The disparity in the abundance of *Aspergillus* DNA in the maize ears suggests that the initial inoculum might not arrive passively from the soil and that other inoculum sources, e.g., contaminated planting material or insect transport could be very important.Most (42%) of the OTUs for *Aspergillus* spp. were unique to maize ears. Use of AflaGaurd which has atoxigenic spores of *Aspergillus* spp. that flood the germinating plants, leaves no space in the niche, or outcompetes the native toxigenic *Aspergillus* spp. A competitive biocontrol strategy, e.g. AflaGuard/AflaSafe (Bandyopadhyay et al., 2021; Cotty, 1989; Sweany et al., 2022), could be quite effective in reducing aflatoxin contamination since the resident population in the soil against which the biocontrol strain must compete appears to be limited.

Unclassified sequences in the soil sample

A large portion, 20%, of the fungal DNA sequences (fungi_unclassified: 3%, glomeromycota: 0.3%) from the soil mycobiome in this study could not be assigned to a genus, whereas only 1.3% of the sequences from the maize ears could not be classified.(Porter et al., 2008) unified unclassified sequences from soil as soil clone group I and has been classified as Archaerhizomycetes but still failed to include all of the unknown soil fungi with sequences in this study. More detailed identification of soil-borne fungi would have given a more detailed picture of the soil mycobiome and its composition in my soil samples.

DNA sequences from Glomeromycetes i.e. AMF were detected very low in my soil samples. AMF fungi occur in Nepal as 22 species in eight genera have been resolved based on morphology (Baral et al., 2021).The ITS2 metabarcodes used in this study are not optimal for AMF due to poor representation and poor curation of AMF sequences in ITS2 metabarcodes sequence databases (Buee et al., 2009; Stockinger et al., 2010).In addition, the abundance of AMF in soil in this study were low.

Fungi with low abundance and antagonistic activity in soil and maize

Metarhizium (Ahmad et al., 2019) and *Beauveria* (Behie and Bidochka et al., 2014) are insect pathogenic fungi observed in both maize ears, relative abundance 0.5% and < 0.1%, respectively, and in soil both with relative abundance, < 0.1% and < 0.1% respectively. *Metarhizium* and *Beauveria* found in soils could be exploited for plant growth promotion and also are available commercially as biopesticides. Further research into the entomopathogenic properties of these fungi in maize seeds or maize ears, both pre-harvest and post-harvest, could be important in determining the best strategy for using them to reduce insect damage and to increase yields.

Talaromyces spp. have been used for plant growth promotion and to reduce the impact of plant pathogens. Endophytic *Talaromyces* spp. occur in maize plants in most Asian countries (Nicoletti et al., 2023). The presence of *Talaromyces* spp. in soil with relative abundance 1.4% and in maize ears with relative abundance 4%, could indicate some of these fungi are already functioning as a natural biocontrol and biofertilizer.

Alpha and beta diversity analysis of the mycological community

Alpha/α diversity indices, were used to evaluate fungal diversity and abundance. The number of unique OTUs were higher in soil from Kailali than Dadeldhura. Without data for soil physiochemical properties, differentiating α and β diversity due to abiotic factors is difficult. Fungal α diversity (Kingsbergen et al., 2025) increases in soil in a grazed natural system.

Species richness was higher in maize from Dadeldhura than from Kailali. The species richness detected in the maize ears (206 genera) in this study was higher than in a comparable Chinese study (Xing et al., 2018) that relied on rDNA- ITS1-5.8S-ITS2 metabarcoding sequences but found only 16 species and 10 genera when they cultured fungi under laboratory conditions. Maize producing higher yields has been associated with higher fungal OTU richness and α diversity (Kandasamy et al., 2021).

The composition of the mycobiome in the maize ears also may depend on the genetic, physiological and physical characteristics of the plant (Moore et al.,2023; Lanubile et al., 2017). This information is lacking for the ears evaluated in this study, but the plant was most likely an open-pollinated local variety that was maintained by the farmers. Each farmer may have maintained their own preferred line. Abiotic factors, such as climate and soil conditions also could be important. where maize is grown. The hot and humid weather conditions in Kailali could be a

reason that *Sarocladium* and *Fusarium* were more common there than in Dadeldhura where the temperature is usually cooler.

PCoA ordinations were used to visualize the compositional variation of the fungal communities in both soil and maize ears. Sample type (maize ear vs. soil) was the main source of variation with significant differences between the maize ear and soil mycobiomes. There were, however, no differences between Kailali and Dadeldhura when the soil and maize ear mycobiomes at each site were merged for comparison even though 55% of the variation in mycobiome composition coul be explained by sample type and site location. The reasons underlying for remaining 45% of the variation remains unknown. The β diversity data do not support the hypothesis that the soil and maize mycobiomes differ between these two geographic locations. More rigorous tests of differences due to geographic location are needed, than a simple comparison of two adjacent districts. If these analyzed locations had had greater environmental differences then the impact of the environmental contributions to differences in fungal mycobiome composition might have been detectable. As previously reported, there was no clearly distinguishable single factor shaping these maize-associated mycobiomes (Kandasamy et al., 2021).

Conclusions

In summary, the maize ear and soil mycobiomes in this study are unlike and diverse, but in different ways. The soil mycobiome had more genera, most at lower frequencies, with 20% of the DNA sequences recovered not associated with a known genus. Meanwhile the maize ear mycobiome was dominated by fewer genera that were present at higher relative frequencies. With the exception of *Fusarium*, genera abundant in maize ears were not common in the soil and vice versa. It is unlikely that the soil is serving as the sole inoculum source for the mycobiome of the maize ears as some of the more common fungi in the maize ear e.g. *Aspergillus* spp. were present at a frequency of 1% or less in the soil mycobiome. The relatively high frequency of *Mortierella* sequences in the soil samples suggests that the farmers in both Dadeldhura and Kailali are practicing intercropping. Since *Fusarium* was an indicator taxon and the most abundant taxa in the soil community, maize and other crops grown in these fields may be prone to *Fusarium* diseases and to contamination with mycotoxins produced by one or more *Fusarium* species. As *Aspergillus*, *Fusarium* and *Penicillium* appear to be established in both soil and maize ear mycobiomes, they are likely to persist for the long term. Thus, the major question is how to manage these toxigenic species rather than how to get rid of them. Mycotoxins and mycotoxigenic fungi appear to be part of the agricultural landscape in Nepal for the foreseeable future.

Chapter 3 - Maize Mycobiome and Mycotoxins

Abstract

Mycotoxins are secondary metabolites produced by fungi that contaminate human food and animal feed. They also are important components in the unseen microbial warfare that determines which fungi can colonize a substrate. In maize, *Aspergillus* spp. and *Fusarium* spp. are common fungal toxin-producing contaminants, but they are not the only fungi present. The relationship between the fungal mycobiome and mycotoxin levels in maize is not well understood. So, I evaluated grain from 12 maize ears from two locations for the presence of up to 660 secondary metabolites. This evaluation was coupled with a mycobiome assessment of these ears with ITS 2 (rRNA Internal Transcribed Spacer) metabarcode sequences. The objective was to dissect fungal communities on individual maize ears and analyze their association with the secondary metabolites present. The working hypothesis was that metabolite presence and quantity depended upon the composition of the mycobiome.

The maize ears were contaminated with 34-59 fugal classified genera and 10-51 secondary metabolites. *Aspergillus* and *Fusarium* metabolites were found above the regulatory limit in two or more samples. Maize ear mycobiomes are diverse and complex, with interactions amongst the fungi likely responsible for the mycotoxins produced. Further mycobiome characterization and exploration at the species level could be used to identify toxin-producing species present and assist in their management. Managing the toxin producers and reducing the amount of toxin produced will increase the value and safety of the crops

Introduction

Maize plants and seeds are naturally inhabited by many microorganisms. Maize seeds interact with diverse microorganisms including bacteria, fungi, viruses, protists, and nematodes. Seeds are inhabited by diverse communities, internally by endophytes and on the surface by epiphytes (Nelson, 2018). The seed-inhabiting organisms interact with each other in a complex microbiome community of which fungi are an integral part. The fungal community in maize seed is termed a mycobiome (Ghannoum et al., 2010). Mycobiome members may have mutualistic or pathogenic activities. Pathogenic plant/fungal interactions may trigger plant diseases whereas beneficial interactions can provide protection from pests and diseases and promote plant growth.

Plant pathogenic fungal genera, such as *Aspergillus*, *Fusarium*, and *Penicillium,* are genera commonly recovered from maize (Katati et al., 2023). *Aspergillus* and *Fusarium* are fungi that commonly contaminate maize after the harvested grain is stored. These fungi can secrete low molecular weight secondary metabolites or mycotoxins while the grain is in the field before harvest or post harvest during storage. These secondary metabolites can cause diseases and present health hazards to animals, including humans that consume contaminated plants. Mycotoxins may be hepatotoxic, nephrotoxic, neurotoxic, immunotoxic and carcinogenic (Bennett and Klich, 2003; Yokoyama et al., 2018).

Different samples of feed and feed raw materials including maize from Europe, Hungary, Austria, Denmark, USA, and Canada contained between seven to 69 metabolites (Streit et al., 2013). Thousands of maize, maize silage and finished feed samples collected from 44 countries globally each had 16-35 of 57 screened metabolites present at the same time (Kovalsky et al., 2016).

Masked mycotoxins, mycotoxin conjugates and unmodified mycotoxins, e.g., aflatoxins, ochratoxins, and fumonisins, all were detected (Karlovsky, 2011; Rychlik et al., 2014).

The most important class of mycotoxins produced by *Aspergillus* fungi, *e.g.*, *A. flavus* and *A. parasiticus*, are aflatoxins (Bennett and Klich, 2023). The most important aflatoxins are B_1, B_2, G_1, G_2, M_1 and M_2. Aflatoxin B_1 is a well-known carcinogen (Cao et al., 2022). Aflatoxin M_1 also occurs in milk and is a biotransformation of aflatoxin B_1 that results when cows are fed aflatoxin B-contaminated feed (Min et al., 2021). The level of aflatoxin contamination varies, depending on substrate, environmental conditions in the field and in storage, and the fungal strain present and its ability to produce aflatoxin (Diener et al., 1987; Manouras et al., 2026).

Fumonisins are mycotoxins ususally produced by *F. verticillioides* and *Fusarium proliferatum* which are both the major maize pathogens (Picot et al., 2010). There are 28 known analogs of fumonisin, with FB_1, FB_2, FB_3 and FB_4 the most commonly observed (Rheeder et al., 2002). *Fum1*, which encodes the first step in fumonisin biosynthesis, is a plant virulence factor but is neither necessary nor sufficient for virulence on maize (Desjardins et al., 1995). Multiple environmental conditions, such as pH, temperature, water availability, etc., can influence fumonisin production in maize from pre-to-post-harvest (Keller et al., 1997; Samapundo et al., 2005). Fumonisins are associated with health risks in humans and domesticated animals. In humans, the consumption of fumonisin-contaminated food is associated with birth defects, childhood stunting, partial immune system suppression, and cancer (Missmer et al., 2006; Marasas et al., 2004; Tessema et al., 2021).

Maize is the second most widely grown staple crop in the hills and Terai of Nepal (MoALD, 2024). Maize is grown from March/April to July/August in the hilly region. The crop matures in the hills in 140-150 days. In the Terai, most of the maize is grown from April-July but it is planted in summer, winter, and spring. Maize in summer is planted in April and May and is harvested in

August and September. Winter maize is grown from November/December to March/April. Similarly, spring maize is planted in March/April and harvested in June/July (Nayava and Gurung, 2010).

Maize, peanuts and milk are foods commonly consumed in Nepal that can be contaminated with aflatoxins and fumonisins (Desjardins and Busman, 2006; Kafle et al., 2024). Since mycotoxins are proven to be hepatotoxic, carcinogenic, nephrotoxic, and immunosuppressive in experimental models (Creppy, 2002), maize consumers in Nepal are at high risk. The risk of liver cancer in Nepal was estimated at 0.38 cases/year/100,000 people assuming average maize consumption (Joshi et al., 2022). Exposure to mycotoxins also is associated with stunting and weight loss in children in Nepal (Trevino et al, 2022). Health problems that are widespread in the country according to the Nepal Demographic and Health Survey (Baral and Nakazawa, 2022). Preliminary analysis of a cohort study in Bhaktapur indicated that chronic aflatoxin exposure was common in the general population (Mitchell et al., 2017). Mycotoxin exposure in children was high in Banke and in the USAID defined Zone of Influence (ZOI) in western Nepal. Exposure to aflatoxin and other mycotoxins in young children was associated with impaired growth, increased stunting, and weight loss (Andrews-Trevino et al., 2022). Nearly 94% of pregnant women in Banke were exposed to aflatoxin in the previous six months, but this exposure was not just from contaminated maize as exposure levels remained positive even when maize was not available for consumption. Women who tested positive for aflatoxin exposure often had smaller preterm babies with stunting issues (Andrews-Trevino et al., 2019). Thus, mycotoxin contamination of the food supply adversely affects the health of mothers and children in western Nepal.

USAID's Feed the Future program defined a ZOI in 16 districts in the terai and mid-hills of western Nepal (U.S. Embassy in Nepal, 2023). Dadeldhura and Kailali in the Sudurpaschim and Kailali

provinces are in the ZOI and were the districts from which the maize samples analyzed in this study were collected. A multi-mycotoxin analysis of multiple foods (maize, rice, complementary food, peanut, and chilis) and animal feed was conducted in the ZOI. Maize in ZOI contained aflatoxins and fumonisins at levels far above the regulatory limits (Feed the Future PHLIL Nepal Report, 2020).

Maize in Nepal can be contaminated with *Fusarium* species that produce fumonisins (Desjardins and Busman, 2006). In addition to fumonisins, maize samples were found to be contaminated with either aflatoxins, ochratoxins, zearalenone or deoxynivalenol in 45 districts of Nepal (Joshi et al., 2022). Aforementioned multiple mycotoxins also were co-occurring in maize, food and feed globally (Ibanez-Vea et al., 2012; Streit et al., 2013).

Traditionally, mycotoxin risks are assessed for a single mycotoxin at a time. But maize is inhabited by multiple fungi capable of secreting very different metabolites. Many fungi, e.g. *Aspergillus* spp. and *Fusarium* spp., can produce several mycotoxins simultaneously, and maize can be contaminated by multiple fungi simultaneously. Two or more mycotoxins often are detected together in samples. Co-contamination of grain with aflatoxin and fumonisin is common and contamination with just a single mycotoxin is rare (Borutova et al., 2010). Mycotoxin analysis of tons of maize at industrial scale identified aflatoxins, beauvericin, deoxynivalenol, fumonisins B_1 and FB_2, ochratoxin A, and zearalenone in the same batch (Pascale et al., 2022). Maize collected from 300 households in Tanzania exhibited high levels of aflatoxins, fumonisins and deoxynivalenol (Kamala et al., 2015). The expected occurrence of multiple toxins and secondary metabolites in maize raises questions of their interactions and whether these interactions enhance or reduce the composite toxicity (Alassane-Kpembi et al., 2016). As humans are exposed to multiple fungal metabolites when contaminated food is consumed, the analysis of maize for

contaminants with multiple metabolites and an understanding of the physiological significance becomes important.

The occurrence of multiple toxins also indicates the presence of multiple fungi probably with different roles. These fungi can have roles in biocontrol, mycotoxin production and plant diseases or defense. Some endophytes in maize promote plant growth by suppressing the virulence of pathogenic fungi (Wicklow et al., 2005). Secondary metabolites produced by non-pathogenic fungi may act as antifungal agents against pathogenic fungi (Rodrigo et al., 2022). Many bacterial are effective, under laboratory conditions, as biocontrol agents against fungi like *F. graminearum* can reduce contamination with deoxynivalenol (Mousa et al., 2015). Filamentous fungi and yeasts, both ascomycetes and basidiomycetes, can also be associated with seeds (Links et al., 2014). These fungi can be carried on the seed or transferred to the seed from the soil or other components of the local environment. Fungal endophytes of maize, *e.g.*, *Sarocladium*, are not well studied and might be utilized for biocontrol against disease-causing microorganisms (Xing et al., 2018).

Mycotoxin contamination is not homogeneously distributed within a bulk sample of maize grain, and sampling is one of the greatest sources of variation in mycotoxin analyses (Miraglia, 2005; Whitaker and Johansson, 2005). Mycotoxin contamination in maize grain samples usually isanalyzed at the bulk level. When individual kernels are analyzed, one may be highly contaminated and the next kernel may show no contamination at all (Lee et al., 1980). If only a relatively small numbers of kernels are contaminated, improper sampling may result in a non-representative sample and the results obtained will not accurately reflect the overall contamination of the batch. If contaminated kernels are not common or sort to one portion of the bin, e.g., a bottom corner, then obtaining a representative sample becomes even more difficult (Whitaker and Johansson, 2005).

Even though the seed mycobiome plays an important role in plant health and secondary metabolites produced, limited data are available on how the maize fungal community members affect the secondary metabolites present or vice-versa. The seed and ear mycobiomes are an overlooked topic in the maize microbiome studies. A detailed understanding of the fungal community in maize seed and how it influences the secondary metabolites present remains unknown. With the advent of high throughput DNA sequencing and more sophisticated chemical analytical methods, a study that pairs DNA sequencing with a LC/MS/MS analysis to enable simultaneous identification of fungi from and secondary metabolites present in the same sample becomes doable.

The objective of this study was to identify the members of the mycobiome and the secondary metabolites present in individual maize ears. The working hypothesis was that metabolite presence and quantity varied with the composition of the mycobiome. In particular, we asked the following overarching questions (i) Could a fungus be present without producing its associated secondary metabolites? (ii) Could a species that might function as a biological control agent reduce or prevent secondary metabolite production by one or more species? and (iii) Did the relative abundance of a fungal DNA correlate with the level of its secondary metabolite(s) in the samplse. The results of this study advance the field by providing an inventory of the fungal genera and mycotoxins present in individual maize ears, and by demonstrating that variation within the mycobiome and the secondary metabolites produced in individual ears is not unlike the variation observed in bulk samples of grain.

Methodology

Maize sample collection, DNA extraction, sequencing and analysis

Maize flour from maize ears collected for pairwise comparison study of soil and maize ear mycobiomes was used in this study. DNA extraction from maize flour and subsequent Illumina sequencing of fungal ribosomal internal transcribed spacer (ITS2) metabarcodes were used to detect genera in these fungal communities. Secondary metabolites in flour from maize ears were analyzed with Liquid chromatography-tandem mass spectrometry (LC/MS/MS) at the University of Natural Resources and Life Sciences, Vienna (BOKU).

Multi-Mycotoxin Analysis

A ground maize flour sample was used for analyzing the secondary metabolites by LC/MS/MS. Approximately 10g of each maize flour were shipped to Michael Sulyok at the Laboratory Center for Analytical Chemistry, Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU). Five (5) g of each 10 g sample was extracted with 20 mL of extraction solvent (acetonitrile/water/acetic acid, 79:20:1, v/v/v). Extracts were diluted with same volume of dilution solvent (acetonitrile/water/acetic acid 20:79:1).The diluted extract was mixed well and 5 µl injected into the LC-MS/MS (Sulyok et al., 2020).

Chromatographic separation was performed at 25°C with a Gemini® C18 column (Phenomenex, Torrance, CA, USA). Then a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo Ion Spray electrospray ionization (ESI) source was used to detect and quantify the fungal metabolites.

Mycotoxins were quantified by external calibration (1/*x* weight) by using a multi-component standard prepared from authentic standards (see Sulyok et al., 2020) to quantify > 500 mycotoxins

and other secondary metabolites from fungi in cultures, cereals, maize flour, food and feed products.

Bioinformatics Analysis and Data Analysis

ITS fastq sequencing data from Illumina were processed through the mothur pipeline (v.1.47.0, Schloss et al., 2009) as reported in detail in Chapter 1. RStudio (version 2025.05.0 Build 496) was used for statistical analyses and graphical data representations of fungal community composition as described in Chapter 1. Technical replicates of each sample were pooled into a single dataset, reducing the number of data sets from 36 to 12. Fungal community composition analysis done in RStudio (version 2025.05.0, Build 496) is described in Chapter 2.

Heat maps were generated with R Studio using ggplot2 and geom_tile () function to indicate presence or absence of secondary metabolites. Samples containing mycotoxins or secondary metabolites at concentrations ≥ LOQ (Limit of Quantification) were classified positive and < LOD (Limit of Detection) were recorded as not detected. Values between LOQ and LOD were noted as detected.

Results

Overall results of the mycobiome analysis in maize are presented in Chapter 1.

Mycotoxins and secondary metabolites detected in flour from maize ears

LC/MS/MS detected sixty-six secondary metabolites (Table 3,1) among 660 possible metabolites (Appendix SupplementaryTable 6) screened in maize flour from ears from both districts.

Some major mycotoxins – aflatoxin, ochratoxin, nivalenol, and zearalenone – were detected in maize flour from fewer than 3/12 maize ears. Fumonisins B_1 and B_2 were detected in maize flour of 11/12 maize ears. Other secondary metabolites produced by *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. coexisted in the maize flour samples. Fifteen other metabolites, including asperglaucide, asperphenamate, beauvericin, bikaverin, citrinin, cyclo-(L -Pro-L-Val) dihydroxymellein, flavoglaucin, pestalotin, hydroxypestalotin, iso-rhodoptiometrin, kojic acid, moniliformin, questiomycin A, and quinolactin A, were detected in maize flour from more than five maize samples (Fig. 3.1).

All the maize flour samples contained at least 10 secondary metabolites. The number of metabolites detected in a maize flour ranged from 10-51 (Fig. 3.2). Maize flours from both locations contained an average of 24 metabolites. The number of metabolites detected in flour from grain maize ears from Dadeldhura ranged from 16-29 and from 10-51 in ears from Kailai. The relative abundance of fungi in each sample is shown in Fig. 2.5. *Sarocladium, Fusarium, Ceratobasidium, Neodeightonia, Waitea, Trichoderma, Talaromyces, Lasidiplodia, Bipolaris, Aspergillus*, and *Wallemia* composed > 90% of the fungi recovered from all 12 maize ears.

Table 3.1. Sixty-six metabolites detected in flour from maize ears of two districts.

Values expressed as µg/kg, corrected for apparent recoveries

	LOD	LOQ	D1M	D2M	D3M	D4M	D5M	D6M	K1M	K2M	K3M	K4M	K5M	K6M
[1]Aflatoxin B_1	0.22	0.72							< LOQ	57.49	428			
[1]Aflatoxin B_2	0.06	0.21								8.19	50.9			
[1]Aflatoxin M_1	0.13	0.44								3.46	27.9			
[1]Aflatoxicol	0.23	0.76								14.33	15.5			
[1]Ochratoxin A	0.46	1.52								25.04	9.49			
[1]Ochratoxin B	0.11	0.36								2.22				
[1]Fumonisin B_1	2.10	7.00	301	161	100	283	244	62.2		637		1840	32.1	
[1]Fumonisin B_2	2.10	7.00	61.2	21.1	18.9	54.8	61.9			104	41.7	437.8		
[1]Fumonisin B_3	2.10	7.00	52.4		22.2	51.4	31.8			86.8		196	11.2	
[1]Fumonisin B_4	2.40	8.00	33.5	9.34	<LOQ	15.9	19.5			61.9	12.8	115	<LOQ	
[1]Fumonisin A_1 Precursor	0.60	2.00					<LOQ			<LOQ		6.46		
[1]Fumonisin A_2	2.40	8.00										11.3		
[2]Asperglaucide	0.07	0.22	4.22	8.39	5.43	13.5	2.59	14.8	17.7	158	32.5	1.02	1.88	3.00
[2]Flavoglaucin	0.03	0.10	3.99	3.27	2.80		1.74	47.0	6.45			13.8	5.51	4.63
[2]Kojic acid	21.0	68.0	258			194			124	5234	15393			

Table 3.1 continued

	LOD	LOQ	D1M	D2M	D3M	D4M	D5M	D6M	K1M	K2M	K3M	K4M	K5M	K6M
[3]Asperphenamate	0.39	1.32	< LOQ	10.4	2.48	12.5	2.95	8.11	< LOQ	78.5	4.50	3.02	1.43	3.40
[3]Citrinin	0.75	2.50	2245	10.0	17.3		499	5.88		1929	70.5	2.68		
[3]Iso-Rhodoptilometrin	0.03	0.11	1.34		0.33	0.73	0.73	0.14		18.8	0.15			
[4]Quinolactacin A	0.01	0.04	114	0.31	4.77	0.52	13.5	1.05	1.59	206	15.4	0.04	0.21	<LOQ
[4]Dihydroxymellein	0.51	1.71	2.70	3.08	3.12		2.72	6.23	2.17	3.69	36.9	22.4	4.97	11.2
[4]Questiomycin A	0.80	2.70	17.9	11.5	38.0	27.2	22.4	16.0	5.10	72.6		41.03	12.1	4.36
[4]cyclo(L-Pro-L-Val)	1.17	3.88	4.76	6.76	4.62	8.17	3.93	<LOQ	15.4	71.8	43.7	<LOQ	<LOQ	4.44
[4]Hydroxypestalotin	0.50	1.60	4.20		4.61	4.80	3.38	2.09		16.1		3.58		
[4]Pestalotin	1.0	3.0	4.2		<LOQ	<LOQ	6.0	3.7		12.6		4.2		

Table 3.1 continued

	LOD	LOQ	D1M	D2M	D3M	D4M	D5M	D6M	K1M	K2M	K3M	K4M	K5M	K6M
[5]Bikaverin	3.00	10.0	44.8	18.5	23.2	72.8	36.4	21.9		159	< LOQ	52.1	< LOQ	< LOQ
[5]Moniliformin	1.50	5.00	156	113	9.78		194	18.6			8.91	19.2		
[5]Beauvericin	0.03	0.10	2.57	0.23		5.60	<LOQ	<LOQ	0.18	19.9				
Secondary metabolite in less than 5 samples														
Nivalenol	1.14	3.79												43.5
Zearalenone	0.20	0.60									37.15			
Dihydrocitrinone	1.10	3.75	265				35.2			177				
Butenolid	3.00	11.0	125				95			1138				
Chrysogin	0.56	1.86						2.00			<LOQ			
W493	NA	NA				3.52		23.3						
O-Methylsterigmatocystin	0.07	0.23								0.67	9.78			
3-Nitropropionic acid	0.74	2.47							14.1	1785	544			
Sterigmatocystin	0.08	0.25								0.30	1.49			
Versicolorin A	0.05	0.16									10.5			
Versicolorin C	0.15	0.50								1.90	66.2			

Table 3.1 continued
Secondary metabolites in less than 5 samples

For metabolites in red nembers denote LC-MS peak area (no quantitative standard available)

	LOD	LOQ	D1M	D2M	D3M	D4M	D5M	D6M	K1M	K2M	K3M	K4M	K5M	K6M
Averufin	0.02	0.07								85.8	138			
Asperfuran	6.44	21.5								547				
Aspulvinone E	NA	NA								770800				
Isosulochrin	NA	NA								11.02				
Nigragillin	NA	NA	301040			503600		271680			1861600			
Phenopyrrozin	0.39	1.29								1.71				
Pyranonigrin	5.63	18.76									387			
Pyrophen	0.41	1.38								<LOQ	4.08			
Quinolactacin B	0.41	1.38	7.03				<LOQ			9.26	<LOQ			
Aspochracin	NA	NA								3483				
Aspterric acid	18.4	61.4								414				
NP1793	NA	NA								983				
Rugulovasine A	7.64	25.5				<LOQ	44.3	<LOQ		75				

Table 3.1 continued

	LOD	LOQ	D1M	D2M	D3M	D4M	D5M	D6M	K1M	K2M	K3M	K4M	K5M	K6M
Sclerotin A	NA	NA	29.2				28.3			23.8				
Koninginin E	0.63	2.09					11.9							
Lecanoic acid	NA	NA					3.36							
Macrosporin	0.13	0.44				4.50								
Monocerin	0.06	0.20			1.23	3.45								
Brevianamid F	1.00	3.34							<LOQ	10.4	5.60			
Citreorosein	0.75	2.50	9.39		<LOQ	<LOQ		<LOQ		17.1	4.01			
cyclo(L-Pro-L-Tyr)	8.49	28.30				<LOQ			<LOQ	37.9	<LOQ			
[6] Emodin	0.11	0.35	1.69		<LOQ	0.91	0.42	0.53		15.7	3.33			
[6] Endocrocin	15.0	50.0								433	172			
[6] N-Benzoyl-Phenylalanine	0.15	0.50		<LOQ	<LOQ	3.07	<LOQ	1.55		31.99	1.50	<LOQ		
[6] Rugulusovin	0.67	2.25								23.84	<LOQ			
[6] Skyrin	0.08	0.26				21				11.60				
[7] Tryptophol	3.00	1.00	4.07	9.51	10.1	31.1	< LOQ	5.33	17.1	21.9	18.5	16.2	6.28	10.0

1. Regulated mycotoxin in Fig. 3.3
2. *Aspergillus* secondary metabolites
3. *Aspergillus* and *Penicillium* secondary metabolites
4. *Penicillium* metabolites
5. *Fusarium* metabolites
6. Unspecific metabolites.
7. Saccharomyces metabolites
8. Black boxes: < LOD

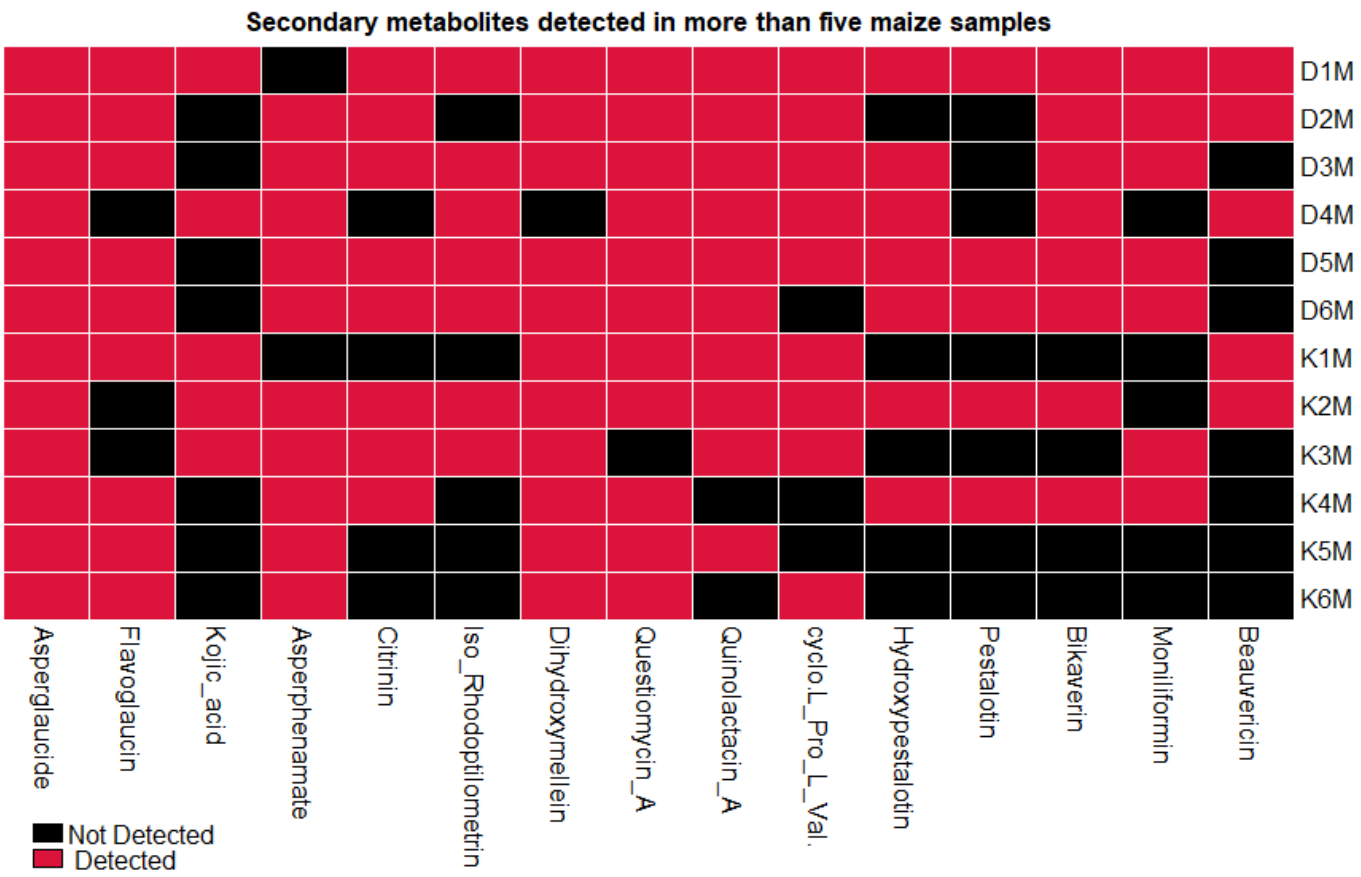

Figure 3.1. Heatmap showing secondary metabolites detected in maize flour from more than 5 maize ears. D1M-D6M: Maize ears from Dadeldhura. K1M-K6M: Maize ears from Kailali.

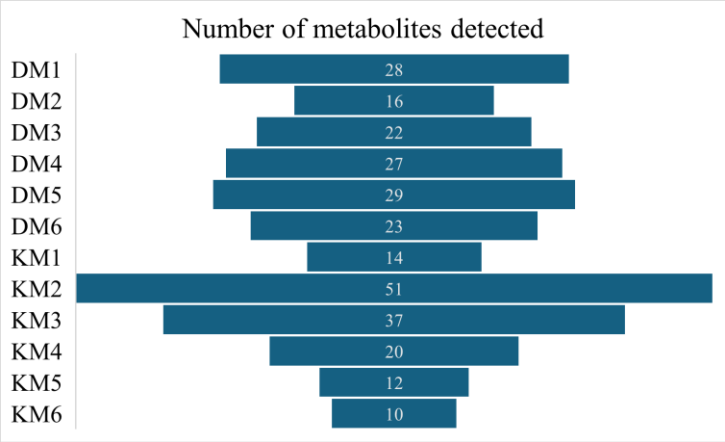

Figure 3.2. Number of metabolites detected in maize flour from each maize ears from Dadeldhura and Kailali.

Table 3.2. Range of recovered levels of regulated secondary metabolites across maize flour from all 12 ears.

Metabolites	Regulatory limit	Amount detected
[1]Total Aflatoxins	20 µg/kg (US, FDA)	< LOD-478 µg/kg
Total Fumonisins	2000-5000 µg/kg (US, FDA)	< LOD-2590 µg/kg
[2]Ochratoxin A	2-10 µg/kg (EU, 2023)	9.5 & 25 µg/kg
[3]Nivalenol	2 µg/kg (TDI, Opinion of scientific committee, regulatory limit undefined.)	2044 µg/kg
[4]Zearalenone	100 µg/kg (EU Commission regulation 2023/915)	37 µg/kg

[1]Aflatoxin detected only in ears K2M and K3M

[2]Ochratoxin detected only in ears K2M and K3M

[3]Nivalenol detected only in ear KM3.

[4]Zearalenone detected only in ear K6M.

LODs (µg/kg): Aflatoxin B_1 – 0.14; Total Fumonisins – 2.1; Ochratoxin A – 0.5; Nivalenol – 1.1; & Zearalenone – 0.2.

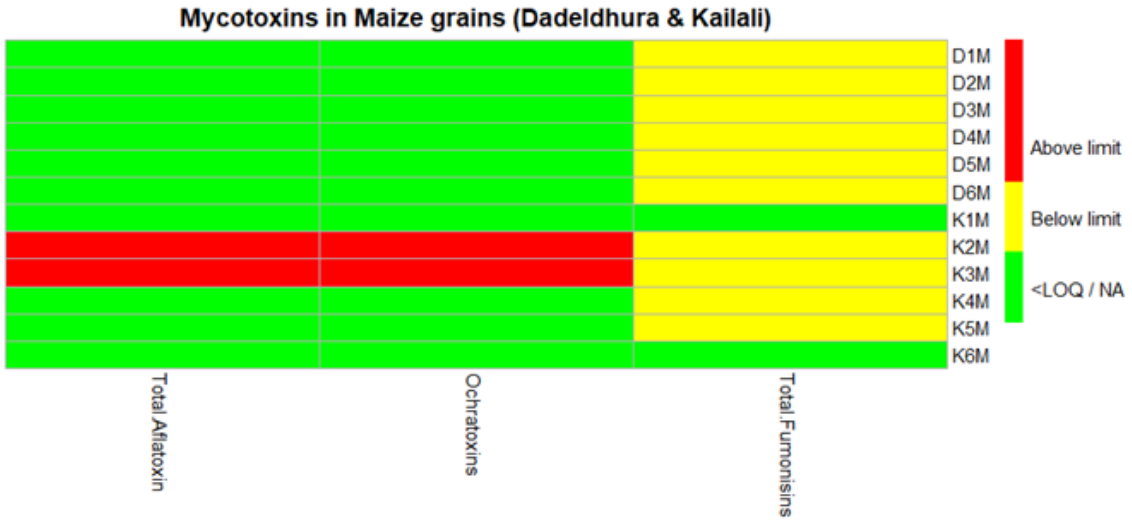

Figure 3.3. Heatmap showing levels of selected regulated mycotoxins in maize ears. D1M-D6M are maize ears from Dadeldhura and K1M-K6M are maize ears from Kailali.

In addition to the six regulated mycotoxins, other secondary metabolites from *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. were detected in more than five maize samples (Fig. 3.1). Metabolites produced by *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. could all be detected simultaneously in maize flour from the same ear.

Based on the distribution of the regulated mycotoxins detected in maize flour from the ears, the ears were divided into three groups:

1. Ears with high levels of aflatoxin and ochratoxin (K2M, K3M)
2. Ears containing fumonisin (D1-D6M, K2M-K5M)
3. Ears containing no regulated mycotoxins (K1M,K6M)

Group 1. Ears with high levels of aflatoxin and ochratoxin.

Maize flour from two maize ears (K2M, K3M) from Kailali had high levels of aflatoxin > 20 µg/kg. Aflatoxins and ochratoxin were below the detection level in the other 10 maize ears. The aflatoxin levels detected in the maize flour of two ears was proportional to the percentage of *Aspergillus* DNA sequences found in the corresponding mycobiomes (Table 3-2). The aflatoxin concentration in K3M was higher than in K2M.

Group 2. Ears containing fumonisins.

Maize flour from ten of 12 ears were contaminated with Fumonisin B. The ears with fumonisins also had DNA with metabarcodes assigned to from *Fusarium* species. The concentration of *Fusarium* metabolites and the absolute number of *Fusarium* DNA sequences were not correlated. For example, 50% of the DNA sequences in K6M were assigned to *Fusarium* spp., but no fumonisin was detected. For comparison, only 11% of the DNA sequences from D1M were attributed to *Fusarium* spp. yet 449 µg/kg of total fumonisins was observed. We assume that these maize ears were colonized by multiple species of *Fusarium* spp. because there were a large number of OTUs assigned to *Fusarium* spp present in the ears. No single species in the genus *Fusarium* synthesizes all of the secondary metabolites produced by the genus. ITS data and ITS metabarcodes in particular, are insufficient to distinguish species within the *Fusarium* from one another. Thus, it is possible that variation in the *Fusarium* species present on an ear could lead to lack of correlation between number of *Fusarium* DNA sequences present and the amount of fumonisin detected.

Table 3.3. Percentage of fungal DNA for selective genera in maize ears and the amounts of(µg/kg) of their corresponding metabolites

Genus present	D1M	D2M	D3M	D4M	D5M	D6M	K1M	K2M	K3M	K4M	K5M	K6M
Sarocladium	15%	7.6%	57%	16%	19%	15%	52%	48%	1.1%	84%	69%	35%
Fusarium	11%	42%	6.2%	13%	6.7%	7.4%	13%	42%	21%	9.3%	1.4%	50%
Total Fumonisins (µg/kg)	449	192	141	405	244	62	ND	891	54	2590	43	ND
Aspergillus	0.1%	0.1%	< 0.1%	0.3%	< 0.1%	0.1%	4.0%	3.3%	41%	0.1%	< 0.1%	0.2%
Total Aflatoxins (µg/kg)	ND	ND	ND	ND	ND	ND	ND	69	538	ND	ND	ND

D1M-D6M – Maize ears from Dadeldhura.

K1M-K6M – Maize ears from Kailali.

ND – Not detected, i.e. amounts were less than the limit of detection.

Group 3. Ears lacking regulated mycotoxins.

Maize flour from ears from Dadeldhura (D1M-D6M) and from four ears of Kailali (K1M & K4M-K6M) lacked detectable aflatoxins and flour of two ears from Kailali (KM1& KM6) also lacked detectable fumonisins. Maize flour from all the ten ears lacked aflatoxin although they contained DNA from fungi in *Aspergillus*. Presumably some of the *Aspergillus* strains represented were capable of producing those metabolites.

K1M and K6M had *Fusarium* DNA sequences, 13% and 50%, respectively, but lacked detectable fumonisins B_1-B_4. K1M had 50% of the total DNA sequences attributed to *Sarocladium*. KM6 had 35% of total sequences attributed to *Sarocladium* and 50% of the total sequences attributed to *Fusarium.* Fungi in the genus *Sarocladium* could be the reason for the lack of fumonisins in these samples (Katatai, et al., 2023).

Samples with numerous detected secondary metabolites.

Maize flour from Ears K2M (51) and K3M (37) had the highest number of secondary metabolites detected. Ninety-three percent of the DNA sequences from K2M were attributed to fungi in *Sarocladium* spp., *Aspergillus* spp. or *Fusarium* spp. In K3M, almost 60% of the DNA sequence reads were attributed to *Fusarium* spp. or *Aspergillus* spp. Metabolites from *Penicillium* spp., including aspochracin, aspterric acid, dihydroxymellein, NP1793, rugulovasine A, pestalotin, hydroxypestalotin, questinomycin, quinolactin and sclerotin A were detected in K2M maize grains along with *Aspergillus* spp. metabolites including aflatoxins, ochratoxins, kojic acid, methylsterigmatocystin, sterigmatocysin, nitropropionic acid, citrinin, and dihydroxycitrinin (Birkelund, et al., 2021), veriscolorin, averufin, asperfuran, and aspulvinone E, and *Fusarium* spp. metabolites including fumonisins B_1-B_4, beauvericin, bikaverin, and butenolide and *Aspergillus* spp. metabolites: citrinin and dihydroxycitrinin .Given the relative rareness of *Penicillium* spp. reads0.98 % of the total DNA sequences) in K2M, relatively little DNA reads can still indicate that there is enough of a genus present to produce a significant amount of genus-specific secondary metabolites.

Metabolites found in flour of more than five maize ears.

Many metabolites other than regulated toxins were present in the maize flour from the analyzed ears (Table 3.1). Some of these metabolites are better known than others. A partial list of these metabolites, all of which were present in at least 5/12 ears includes:

- Citrinin was detected in flour from eight maize ears is a mycotoxin produced by *P. citrinum*, *Penicillium expansum*, and *Penicillium verrucosum (*Hetherington and Raistr, 1931; Alborch et al., 2012; Schmidt et al, 2011). Citrinin produced by fungi in the genus *Monascus* has nephrotoxic, hepatotoxic, immunosuppressive, and carcinogenic effects in humans and laboratory animals (Margo et al., 2016). Based on the latest phylogen, the genus *Monascus* has been placed in family Aspergillaceae, showing a close relationship with the well-known genera *Aspergillus* and *Penicillium* (Visagie et al., 2025). There were sequences assigned to family Aspergillaceae, but no DNA attributable to the *Monascus* genus in my maize ears samples.
- Moniliformin (MON) was detected in flour from seven maize ears and is a mycotoxin produced by at least 30 *Fusarium* species, *e.g.*, *Fusarium. avenaceum*, *Fusarium. subglutinans*, *F. proliferatum*, *F. graminearum*, and *Fusarium fujikuroi*, in maize and other crops (Schutt et al., 1998; Chelkowski et al., 1990). Moniliformin contaminated food is acutely toxic to animals such as rats and birds such as ducks (Kreik et al., 1977; Knutsen et al., 2018).
- Beauvericin was detected in flour from five maize ears and is a mycotoxin produced by many species in the genus *Fusarium*, *e.g.*, *F. proliferatum*, *Fusarium semitectum*, and *F. subglutinans* (Logrieco et al., 1998; Caloni et al., 2020) and occurs naturally on maize. Beauvericin is cytotoxic and has antimicrobial activity and insecticidal properties against many insects including mosquito larvae, blowflies, and the Colorado potato beetle (Sinha et al., 2016).

- Bikaverin was detected in flour from eight maize ears and is a red pigment produced by at least 18 *Fusarium* species including *F. verticillioides*, *Fusarium. sacchari*, *F. proliferatum* and *Fusarium oxysporum* (Leslie and Summerell, 2006; Chelkowski et al., 1990). It has antimicrobial activity against bacteria and fungi (Al-Saady et al., 2023; Lu et al., 2025).
- Kojic acid was detected in flour from five maize ears and is an organic acid secreted by some members of the genus *Aspergillus* including *Aspergillus oryzae*, *A. tamari*, *A. flavus* and *A. parasiticus*. It was first isolated by Saito in 1907 (Bentley, 2006). Kojic acid is utilized commercially as a depigmenting agent and its derivatives have been proposed to act as antioxidants, antimicrobials and anti-inflammatory agents (Owolabi, et al.,2020; Wang, et al., 2019).
- 7-hydroxy-pestalotin was detected in flour from seven maize ears. This fungal metabolite can be isolated from *Penicillium* but also is produced by the endophytic fungus *Pestalotiopsis microspora* (McGahren et al., 1973; Rathnayake et al., 2019; Labuda et al., 2021). Hydroxy-pestalotin from *Pestalotiopsis microspora* was active against murine leukemia and P388 cells (Riga et al., 2019). No *Pestalotiopsis* DNA reads were detected in any of the maize ears analyzed, but *Penicillium* spp. were present in all maize samples when hydroxy-pestalotin was detected.
- Questiomycin A was detected in maize flour from 11 ears. It is a secondary metabolite produced by *P. expansum* with antimicrobial activity against bacteria, yeasts, and fungi (Kozlovsky et al., 2004). Questiomycin was found in 11 ears and co-occurred with *Penicillium* spp. and fumonisins in 8 ears.
- Quinolactin A was detected in flour from 11 maize ears and can be produced by the *P. citrinum* (Liu et al., 2023) and by *Aspergillus ochraceus* (Moore et al., 1972). Quinolactin A can inhibit

tumor necrosis factor (Kakinuma et al., 2000) and has antioxidant activity and neuroprotective efficacy following H_2O_2 insult to SH-SY5Y cells *in vitro* (Tong et al., 2021).

- Flavoglaucin was detected in flour from eight maize ears. It can be produced by *Aspergillus ruber* and has antiviral activity against Herpes Simplex Virus type 1 (HSV-1) (Liang et al., 2018). HSV-1 causes oral herpes with small painful blisters on lips, mouth or chin (WHO 2025) Flavoglaucin from *Aspergillus* spp. (reported as teleomorph name *Eurotium herbariorum* syn. *Aspergillus glaucus*) also inhibited tumor promotion in mouse skin (Miyake et al., 2010).
- Asperglaucide was found in flour from all twelve maize ears. It can be produced by metabolite of *A. glaucus* and *Aspergillus penicilloides* with strong anti-inflammatory properties in rat (Cox et al., 1976; Isshiki et al., 2001). Asperglaucide was detected with *Aspergillus* DNA reads in all 11/12 the maize ears analyzed. D3M is only ear lacking *Aspergillus* spp. DNA that has asperglaucide. Thus, fungi other than *Aspergillus* spp. may be capable of synthesizing this metabolite.
- Asperphenamate was detected in flour from ten maize ears and is a metabolite of *Penicillium brevicompactum*, *Penicillium buchwaldii*, *Penicillium spathulatum*, *Aspergillus terreus* and *Aspergillus aculeatus* (Li et al., 2018). Asperphenamate has anticancer properties (Frisvad et al., 2013). *Penicillium* DNA reads were present whenever asperphenamate was detected.
- Isorhodoptilometrin was detected in flour from seven maize ears, and can be produced by *Penicillium oxalicum* (Kim et al., 2022). It has potential anti-inflammatory activity for the treatment of neurodegenerative diseases. Quinolactin A and asperglaucide also were detected in the ears in which isorhodoptilometrin was detected.

- Cyclo-(L-Pro-L-Val) was detected in flour from nine maize ears and is a metabolite of *Bacillus amyloliquefaciens* capable of inhibiting *F. graminearum* (Jamal et al., 2017). Cyclo-(L-Pro-L-Val) is a known diketopiperazines found in organic extracts obtained from solid phase cultures of *Penicillium bilaii*. Repeated HPLC fractionation yielded pure samples of diketopiperazine cyclo (L-pro-L-val) (Capon et al., 2007). It was detected in all nine ears in which *Penicillium* DNA sequence reads were present.

Discussion

Major mycotoxin prevalence

The aim of the study was to identify fungal communities in maize ears and determine if with the number and abundance of secondary metabolites detected in flour of matched expectations from *in vitro* studies. The ears were co-contaminated with regulated mycotoxins, *e.g.*, aflatoxins and fumonisins, and other known fungal secondary metabolites.

Group 1 with high aflatoxin, ochratoxin and its relationship with *Aspergillus* spp.

Maize ears with high aflatoxin.

Maize is an important grain crop susceptible to aflatoxin contamination when environmental conditions are favorable both in the field and in storage. Two maize ears in Kailali were heavily contaminated with aflatoxin, although four other ears from this province were not. These ears were sufficiently heavily contaminated that if their grain was mixed with non-contaminated grain from the other four ears, the resulting bulk would have been contaminated at a level above the regulatory limit. The ten maize ears without detectable aflatoxins usually had some DNA sequences from fungi in the genus *Aspergillus.* This expectation underlies the effectiveness of the AflaGuard/AflaSafe biocontrol for the reduction of aflatoxin contamination (Bandyopadhyay et

al., 2016). AflaGaurd is a biocontrol agent that contains the spores of atoxigenic *Aspergillus* species that swamp out the native toxigenic aflatoxic *Aspergillus* species when AflaGaurd is applied to a maize field. Therefore, aflatoxin negative kernels could be loaded with atoxigenic *Aspergillus* from AflaGaurd treatment.

Finding that maize in Nepal is contaminated with aflatoxins is not new. Aflatoxin B_1 has been detected in maize samples from the Kathmandu valley (Gautam et al., 2009) and from the eastern part of Nepal (Koirala et al., 2005), with cornflakes regularly contaminated with aflatoxin above the regulatory limit. Aflatoxins, fumonisins, zearalenone and DON (deoxynivalenol) co-occurred in five provinces in Nepal, but neither Kailali nor Dadeldhura were part of that study (Joshi et al., 2022).

Results from the present study support earlier findings that aflatoxin alone can contaminate maize by itself, but it is more likely to be found in concert with other mycotoxins. As not all of the ears were contaminated with aflatoxin, sorting out contaminated ears before combining grain into a bulk could reduce the level of contamination in maize available for human consumption or sale.

Two of twelve maize ears I examined were heavily contaminated with aflatoxin. The remaining ten maize ears had no detectable aflatoxins although they usually had some DNA sequences from fungi in the genus *Aspergillus*. In ears containing a high level of aflatoxin, aflatoxin negative kernels could be next to kernels colonized by *A. flavus* (Lee et al., 1980).

Maize ears with high ochratoxin A.

Maize in Nepal can be contaminated with multiple mycotoxins (Joshi et al., 2022). Results from this study are consistent with previous reports that co-contamination of maize with aflatoxin and ochratoxin is worldwide and also occurs in Nepal (Pfohl-Leszkowicz, et al., 2007; Reddy et al., 2009). Ochratoxin A at levels exceeding the regulatory limit was detected together with aflatoxin in two ears from Kailali. Ochratoxin A in dried stored foods, cereals and feeds can be produced by multiple species, with *A. ochraceus* and *P. verrucosum* the most prominent producers in different parts of the world (Callaghan et al., 2006; Jorgensen and Jacobsen, 2002). Results in the present study are consistent with those previously reported in that the two maize ears were contaminated with ochratoxin also were contaminated with *Aspergillus* and *Penicillium*. Samples lacking ochratoxin A also had DNA sequences from fungi in *Aspergillus* and *Penicillium.*

Relationship between presence/absence of aflatoxin/ochratoxin and the fungi potentially producing them

Detection of DNA from the genus *Aspergillus* but not detecting aflatoxin could be due to limiting conditions in seed or variation in the *Aspergillus* species present (Lee et al,1980). Both *Aspergillus* and *Penicillium* with *Fusarium* share the "multiple species in the genus that do not produce toxins" problem (Greeff-Laubscher et al., 2019; Phoku, et al., 2017). In *Aspergillus*, there is a class of strains within *A. flavus* that does not produce aflatoxins and has been exploited for biological control (Bandyopadhyay et al., 2016).

ITS2 metabarcode sequences are insufficient to discriminate all of the fungal species recovered from the maize ears. Based on the detection of aflatoxin in two of the maize ears, the *Aspergillus* spp. present in those ears probably include one or more of the toxigenic species such as *A. flavus*

and *A. parasiticus.* The data in my study are insufficient to determine whether the lack of aflatoxins is due to lack of adequate fungal biomass to synthesize a detectable amount of the toxin or if the *Aspergillus* strains present belong to a species that cannot produce aflatoxin. A third possibility is that one, or more other fungi in the mycobiome may inhibit either the growth of a toxin-producing strain(s) or limit the ability of these strains to produce the toxin. It also is possible that DNA detected is from isolates that are no longer alive and hence not producing toxins or any other secondary metabolites. Finally, the physiological conditions in the maize ear may not be conducive for toxin production.

The count of DNA sequences from the genus *Aspergillus* and the amount of toxins and secondary metabolites present were not consistently proportional. The absolute count of *Aspergillus* DNA sequences was highest in K2M and K3M as was the amount of aflatoxin, ochratoxin and multiple other *Aspergillus* related secondary metabolites which was not true in other ten samples. In samples lacking aflatoxins with sequences assigned to *Aspergillus* spp., there were other secondary metabolites such as kojic acid, sterigmatocystin, citrinin, etc. known to be potentially produced by *Aspergillus* spp.

Inhibition of Fusarium by *Aspergillus.*

A. niger can be common in newly harvested maize grain (Goher, et al., 2021). In ear K3M, there were more *Aspergillus* sequences than there were *Fusarium* sequences. Aflatoxin B_1 was greater than the regulatory limit and no fumonisin was detected. A similar result has been reported by Xing et al. (2018), where *Aspergillus* was more common than *Fusarium* in a maize sample. *A. flavus* also may inhibit the growth of *F. verticillioides* (Fakhrunnisa and Ghaffar, 2006).

Group 2 with fumonisin and its relationship with *Fusarium*

Fumonisin B_1 and its analogues FB_2, FB_3, FB_4, FA_1 and FA_2 as well as other *Fusarium* metabolites, *e.g.*, moniliformin, bikaverin, and beauvericin, were detected in maize samples from both Kailali and Dadeldhura. High levels of fumonisin contamination have previously been reported in maize from Nepal (Desjardins and Busman, 2006) as have *F. verticillioides*, *F. proliferatum*, *F graminearum*, *Fusarium equiseti*, and *Fusarium incarnatum* (Desjardins, 2000). The results of my study are consistent with these previous results. FB_{1-4} were detected within the regulatory range (1000-4000 μg/kg) in addition to fungi in the genus *Fusarium* in 10/12 maize samples, clearly demonstrating that fumonisin and related contaminants are a food safety issue in Nepal.

Relationship between presence/absence of fumonisin and and fungi producing it.

Numerous members of the fumonisin biosynthetic pathway (encoded by *FUM* genes are known in *F. fujikuroi*, *Fusarium globosum*, *F. proliferatum, Fusarium nygamai*, *F. oxysporum*, and *F. verticillioides*, but the gene sets are not always complete (Proctor et al., 2004). Species and strains lacking the genes for the entire biosynthetic pathway could account for the presence of *Fusarium* DNA sequences even when fumonisins were not present. Two maize ears in my study (K1M and K6M) had no detectable *Fusarium*-associated metabolites, i.e., fumonisins, moniliformin, bikaverin (Lu et al., 2025), beauvericin, etc. Fumonisin production is sensitive to environmental conditions, including light, moisture, and temperature (Cao et al., 2014; Fanelli et al., 2012; Matic et al., 2013; Zuniga et al., 2024). Thus, the absence of fumonisins in K1M and K6M could be attributed to abiotic factors, as well as the absence of species and strains capable of producing the toxins and secondary metabolites.

Co-occurrence of *Aspergillus* metabolites.

A. flavus has 56 known secondary metabolite gene clusters (Cary et al., 2018).

The L and S morphotypes within the species with vary in their potential to produce conidia, sclerotia and aflatoxins. L strain isolates produce either relatively few or no, large sclerotia (on average >400 μm in diameter), and copious quantities of conidia. In contrast, the S strain isolates produce copious amounts of small sclerotia (on average <400 μm in diameter), and very few conidia. Aflatoxin production by L strain isolates varies widely with some isolates highly toxigenic and others producing no aflatoxins (= atoxigenic), whereas S strain isolates consistently produce high levels (> 10,000 μg/kg) of aflatoxins (Cotty, 1989). On average, L strain isolates produce lower amounts of aflatoxins than S strain isolates (Probst et al., 2007; Probst et al., 2009).

Therefore, production of some *Aspergillus* metabolites could depend on the expression of specific gene clusters as well as the type of strain in different environmental conditions in the field and in storage. Aflatoxin, ochratoxin, sterigmatocysin and kojic acid all co-occurred in the K2M and K3M ears. Sterigmatocystin is an intermediate in the aflatoxin biosynthesis pathway and kojic acid is commonly produced by *A. flavus* and *A. oryzae* (Keller et al., 1994; Sweany et al., 2024; Wang et al., 2019). Based on the detection of aflatoxin, ochratoxin, sterigmatocysin, and kojic acid in K2M and K3M, the *Aspergillus* species present in those samples could include *A. flavus*, *A. parasiticus*, *A. nidulans*, *A. oryzae* and *A. ochraceus* amongst others(Sweany et al., 2024).

Highly variable contamination of aflatoxin in two of the maize ears and absence of aflatoxin in rest of the ears is consistent with the results of Cotty(1989) where an L strain of *A. flavus* that was highly toxigenic was present in two of the maize ears while other ears lacking aflatoxin could contain atoxigenic strains that produced no aflatoxins.

Co-occurrence of fumonisins and other *Fusarium* metabolites.

Frequent co-occurrence of regulated toxins, such as fumonisins, with moniliformin is known from a global scale study of regulated and masked mycotoxins (Kovalsky et al., 2016). In my study as well, all maize ears contaminated with moniliformin, citrinin, and fumonisin also contained DNA from *Fusarium* and *Penicillium*. The amount of beauvericin detected in 5/12 positive maize ears was low (0.2-20 µg/kg) and probably too low to have an acute insecticidal effect. Co-occurrence of fumonisin and beauvericin has been previously reported (Suhajda et al., 2025). The co-occurrence of multiple metabolites raises the concerns regarding combined toxicological effects (Alassane-Kpembi et al., 2016).

Co-occurrence of *Penicillium*-associated metabolites.

Co-occurrence of multiple metabolites associated with *Penicillium* was evident in my results. Citrinin, questiomycin A, quinolactin, dihydroxymellein and flavoglaucin co-occurred in at least seven maize samples. Quinolactin A produced by *Penicillium* spp. was detected in 11 samples simultaneously with fumonisins whenever *Penicillium* spp. and *Fusarium* spp. were both present. Maize samples containing aflatoxins and ochratoxins were lacking flavoglaucin pointing to the presence of multiple *Aspergillus* species in maize ears in which both aflatoxins and flavoglaucin were detected. Ears containing high levels of aflatoxin might also carry species such as *A. flavus* capable of producing aflatoxin and the other 10 samples might have carried other species of *Aspergillus* capable of producing flavoglaucin. Citrinin production in *P. expansum* and *P. verrucosum* are stimulated and increased by white, red, yellow, green, and blue light (Schmidt-Heydt et al., 2011). In my study, the lack of citrinin in four ears could be attributed to species differences or unfavorable physiological conditions to produce citrinin.

The relative abundance of *Penicillium* in all of the maize ears is generally less than that of either *Aspergillus* or *Fusarium.* This difference may be due to *Fusarium* species inhibiting the growth of *Aspergillus* and *Penicillium* (Marin et al., 1998).

Sarocladium and its antagonistic activity towards *Fusarium* and fumonisin production.

DNA Sequences assigned to *Sarocladium* were abundant in all maize ears. This report is the first of this fungus from western Nepal. Higher levels of *Sarocladium* sequences than *Fusarium* sequences were found in 9/12 ears. *S. zeae*, previously known as *A. zeae*, is a seed-borne endophyte that can increase maize plant heath. *S. zeae* is antagonistic to *A. flavus* and *F. verticillioides in vitro* (Wicklow and Poling, 2008; Summerbell et al., 2011), and detection of high levels of *Sarocladium* in maize has been previously reported (Katati et al., 2023). The relatively low abundance of *Aspergillus* and *Fusarium* in the nine ears in which *Sarocladium* dominated may be due to inhibition of growth of *Aspergillus* and *Fusarium* by *Sarocladium.* Lower levels of fumonisin B_1 and other Fusarium secondary metabolites also could result from the reduced growth of *Fusarium* due to higher relative abundance of *Sarocladium* (Katati et al., 2023). *Sarocladium* was present in the other three ears evaluated in this study, but *Fusarium* sequences were more frequent in these ears. *Sarocladium*, like other genera in this study, contains multiple species that are not readily resolved with ITS2 metabarcode sequences. Thus, the *Sarocladium* species present in the ears dominated by *Fusarium* might be one or more species other than *S. zeae*.

S. zeae can produce dihydroresorcylic acid and pyrrocidine A. Pyrrocidine inhibits the growth of *F. verticilloides* and interacts with the *FvZBD1* gene, to turn off fumonisin biosynthesis (Wicklow and Poling, 2008). Pyrrocidine is not reported to be hazardous to humans or domesticated animals,

and instead has some anti-cancer activity and can induce apoptosis in HL60 cells growing *in vitro* (Gao et al., 2020; Uesugi et al., 2015). *S. zeae* can potentially be used as a biological or chemical control to reduce contamination by *Fusarium* spp. and their metabolites (Wicklow et al, 2005). Controlling fumonisin production with pyrrocidine could be an effective measure to reduce mycotoxin contamination and increase food safety.

Some *Trichoderma* spp. can cause ear rot of maize (Pfordt et al., 2024; Sanna et al., 2022). Other *Trichoderma* spp. has been exploited for their biocontrol potential against *Aspergillus* spp. and *Fusarium spp*. (Benitez et al., 2004; Pandya et al., 2011). Thus, *Trichoderma* spp. present in maize ears could be a mixture of beneficial and plant pathogenic fungi. Therefore, *Trichoderma* spp. in addition to *Sarocladium* spp. present in ears, could be synergistically reducing *Aspergillus* and *Fusarium* contamination in maize ears by inhibiting pathogen growth, reducing ear severity, and increasing grain yield (Mitrovic et al., 2025).

Trichoderma spp. have been popular as biocontrol agents and plant growth promoters in Nepal as well as other parts of the world (Manandhar et al.,2018; Mitrovic, et al., 2025).

Since partial ITS2 metabarcodes can not differentiate the species of *Trichoderma*. These data likely represent a mixture of both beneficial and pathogenic fungi. Controlling mycotoxin-producing fungi with *Trichoderma* could be one strategy for reducing aflatoxin and fumonisin contamination in maize in the ZOI.

Conclusion

Maize is the second most important staple food in Nepal. ITS2 metabarcodes combined with a LC/MS/MS analysis of the secondary metabolites enabled the simultaneous detection of fungi from and the secondary metabolites present in individual maize ear. Maize ear mycobiomes are diverse and complex at the genus species levels. Secondary metabolite production depends on the mycobiome and not only on the presence of fungi able to synthesize these metabolites *in vitro*. The large number of sequences assigned to *Sarcocladium*, for example, suggest a natural means of reducing contamination by species of *Fusarium* and *Aspergillus* the toxins they can produce.

Unfortunately, the LC/MS/MS protocol used in this study did not screen for pyrrocidines, which means the significance of this compound in these communities requires further investigation. Multiple metabolites were detected in all 12 ears, and fumonisins, aflatoxin and ochratoxins were all present in one or more ears at levels greater than that generally allowed in human food in Nepal. Contamination of bulk maize could be reduced if heavily contaminated ears were sorted out before the grain on ears was combined in a bulk lot.

If a metabolite was detected, then DNA sequences from one or more of the fungi that could produce the metabolite also were detected. The relative abundance of DNA sequences from potentially toxin producing fungal genera was not reliably correlated with the amount of a secondary metabolite detected. Future studies need to explore the details of the community composition and toxins present by using chemical techniques that allow the identification of more metabolites and genetic analyses that allow identification at the species level coupled with a study of expression of genes associated with the metabolites.

Chapter 4 - Conclusion

Soil mycobiomes and maize ears mycobiomes are very diverse, with composition and diversity determined by multiple biotic and abiotic factors. In my study, the soil mycobiome was not the only source contributing to the complex, distinct, and diverse maize ear mycobiomes. Composition, diversity, and interactions amongst members of the maize ear mycobiome define the quantity and presence of mycotoxin and secondary metabolites detected in maize.

In Chapter 2, I catalogued the fungi in soil and maize ears from two districts of the ZOI in Nepal and explored the extent to which the soil mycobiomes overlapped with the maize ears mycobiomes. There was significant overlap in OTUs between soil and maize mycobiomes, but numerous unique OTUs were in the maize mycobiome. Most of the unique OTUs belonged to the soil mycobiome. As expected, the richness and diversity of fungal communities in soil were higher than in maize ears, but did not differ significantly between the two districts. The fungal community composition from soil and maize ear differed in both districts. My results indicate that the maize ears have their own mycobiomes but those overlap with the soil mycobiomes. Therefore, our results support the prevailing conclusion that the soil mycobiome is a major contributor to the maize ear mycobiome. My study aligns with those previous studies where no clear single source was responsible for the content of the maize ear mycobiome (Kandasamy et al., 2021; Rochefort et al., 2021).

In Chapter 3, the focus was on fungi in the maize ear mycobiome and their relationship with secondary metabolites, including mycotoxins detected there. When LC/MS/MS analysis detected a secondary metabolites, the ITS2 metabarcode data included sequences from fungi with the potential to produce the metabolite. Multiple metabolites were detected in all ears along with major mycotoxins including aflatoxins, ochratoxins, and fumonisins at or above the regulatory level in

some ears. The genus *Sarocladium* is reported for the first time in maize from western Nepal, and it could antagonize growth and toxic production by *Aspergillus* and *Fusarium* (Wicklow et al., 2005). The present results highlight the potentially complex multiple species interactions in maize and multiple biotic and abiotic attributes that remain unexplored.

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Appendix A - Supplementary Data

Supplementary Tables

Supplementary Table A.1. Relative abundance of all the genera detected in soil sample from both districts.

Genus	Number of DNA sequences	% of Total
Fusarium	19183	9.01
Mortierella	13081	6.14
Curvularia	8186	3.84
Cyphellophora	7178	3.37
Pseudothielavia	7017	3.29
Fungi unclassified	6375	2.99
Acremonium	5779	2.71
Clonostachys	5134	2.41
Chordomyces	5066	2.38
Lectera	3775	1.77
Periconia	3768	1.77
Neocosmospora	3751	1.76
Plectosphaerella	3626	1.70
Talaromyces	3040	1.43
Bipolaris	2609	1.22
Sordariacea unclassified	2599	1.22
Chytridiomycota unclassified	2350	1.10
Setophoma	2342	1.10
Pleosporales unclassified	2279	1.07
Ochroconis	2162	1.02
Phoma	2011	0.94
Ascomycota unclassified	1987	0.93
Sordariomycetes unclassified	1959	0.92
Alternaria	1951	0.92
Pyrenochaetopsis	1931	0.91
Phaeosphaeria	1824	0.86
Gibellulopsis	1808	0.85
Chaetothyriaceae unclassified	1735	0.81
Didymocrea	1620	0.76

Genus	Number of DNA sequences	% of Total
Nigrospora	1586	0.74
Mortierellomycota unclassified	1528	0.72
Verticillium	1511	0.71
Stachybotryaceae unclassified	1398	0.66
Thanatephorus	1342	0.63
Rhizophlyctis	1339	0.63
Acrocalymma	1337	0.63
Clitopilus	1325	0.62
Spizellomyces	1291	0.61
Torula	1289	0.61
Xenomyrothecium	1285	0.60
Sordariales unclassified	1221	0.57
Rhizoctonia	1195	0.56
Tetraplosphaeriaceae unclassified	1190	0.56
Ascobolus	1179	0.55
Chaetothyriales unclassified	1174	0.55
Edenia	1115	0.52
Metarhizium	1087	0.51
Trichocladium	1073	0.50
Westerdykella	1055	0.50
Phialophora	1042	0.49
Arrhenia	1002	0.47
Rhizophlyctidaceae unclassified	1000	0.47
Chaetomiaceae unclassified	978	0.46
Podospora	944	0.44
Hypocreales unclassified	931	0.44
Kernia	930	0.44
Trichoderma	913	0.43
Emericellopsis	910	0.43
Fusidium	859	0.40
Ceratobasidiaceae unclassified	850	0.40
Paracremonium	823	0.39
Preussia	818	0.38
Sarocladium	763	0.36
Cyathus	760	0.36
Acrophialophora	716	0.34
Polyschema	707	0.33
GS13 unclassified	686	0.32
Atractiella	685	0.32

Genus	Number of DNA sequences	% of Total
Metacordyceps	682	0.32
Dothideomycetes unclassified	670	0.31
Conocybe	664	0.31
Chaetomium	661	0.31
Pterula	658	0.31
Papiliotrema	639	0.30
Trichomeriaceae unclassified	623	0.29
Geastrum	608	0.29
Mycothermus	576	0.27
Acrostalagmus	544	0.26
Marasmius	542	0.25
Striaticonidium	504	0.24
Phaeosphaeriopsis	495	0.23
Scolecobasidium	477	0.22
Cystofilobasidium	468	0.22
Poaceascoma	463	0.22
Psathyrella	457	0.21
Monocillium	447	0.21
Microdochium	441	0.21
Cirrenalia	429	0.20
Powellomycetaceae unclassified	427	0.20
Oliveonia	423	0.20
Aporospora	419	0.20
Clavicipitaceae unclassified	418	0.20
Scytalidium	415	0.19
Cumuliphoma	411	0.19
Hydropisphaera	406	0.19
Phaeosphaeriaceae unclassified	402	0.19
Xylomyces	390	0.18
Didymellaceae unclassified	387	0.18
Coniochaetaceae unclassified	382	0.18
Agaricomycetes unclassified	381	0.18
Ceratobasidiaceae unclassified	381	0.18
Coprinellus	380	0.18
Coniochaeta	379	0.18
Stachybotrys	369	0.17
Coprinopsis	367	0.17
Tricellula	365	0.17

Genus	Number of DNA sequences	% of Total
Dimorphiseta	364	0.17
Bullera	363	0.17
Paratrimmatostroma	350	0.16
Fimicolochytrium	348	0.16
Stachylidium	339	0.16
Cladophialophora	325	0.15
Sympoventuriaceae unclassified	318	0.15
Zopfiella	314	0.15
Pseudopithomyces	312	0.15
Plectosphaerellaceae unclassified	306	0.14
Lasiosphaeriaceae unclassified	301	0.14
Agaricaceae unclassified	292	0.14
Ceratobasidium	290	0.14
Sampaiozyma	289	0.14
Alfaria	284	0.13
Geminibasidium	282	0.13
Tubeufiaceae unclassified	278	0.13
Serendipita	271	0.13
Aaosphaeria	263	0.12
Remersonia	263	0.12
Auriculariales unclassified	257	0.12
Tetraplosphaeriaceae unclassified	257	0.12
Gloeoporus	255	0.12
Lycoperdon	251	0.12
Jahnula	249	0.12
Cordana	239	0.11
Ganoderma	239	0.11
Paramyrothecium	237	0.11
Basidiomycota unclassified	230	0.11
Thelephoraceae unclassified	227	0.11
Cercophora	227	0.11
Waitea	227	0.11
Spizellomycetales unclassified	226	0.11
Agaricales unclassified	223	0.10
Albifimbria	219	0.10
Entyloma	217	0.10
Trichothecium	215	0.10
Sporidiobolaceae unclassified	214	0.10

Genus	Number of DNA sequences	% of Total
Cladosporium	213	0.10
Agaricus	212	0.10
Wallemia	205	0.10
Filobasidium	204	0.10
Thermomyces	204	0.10
Dokmaia	200	0.09
Strelitziana	200	0.09
Russulales unclassified	198	0.09
Gliomastix	196	0.09
Filobasidiales unclassified	195	0.09
Tilachlidium	192	0.09
Symmetrospora	190	0.09
Microsphaeropsis	183	0.09
Lepiota	181	0.08
Remotididymella	178	0.08
Orbiliomycetes unclassified	175	0.08
Pseudallescheria	170	0.08
Cristinia	169	0.08
Arthroxylaria	168	0.08
Trichobolus	166	0.08
Cephalotrichum	165	0.08
Xylariaceae unclassified	162	0.08
Pezizaceae unclassified	161	0.08
Tremellomycetes unclassified	160	0.08
Perisporiopsis	158	0.07
Chytridiomycota unclassified	158	0.07
Magnaporthaceae unclassified	158	0.07
Gregatothecium	157	0.07
Sphaerobolus	156	0.07
Tetraploa	149	0.07
Bovista	148	0.07
Petriella	145	0.07
Microbotryales unclassified	145	0.07
Spizellomycetales unclassified	145	0.07
Rinaldiella	144	0.07
Sporobolomyces	142	0.07
Testudinaceae unclassified	140	0.07
Arnium	133	0.06

Genus	Number of DNA sequences	% of Total
Brunneomyces	133	0.06
Funneliformis	131	0.06
Tubeufiaceae unclassified	130	0.06
Claroideoglomus	128	0.06
Nectriaceae unclassified	125	0.06
Purpureocillium	124	0.06
Myrothecium	122	0.06
Geminibasidiales unclassified	120	0.06
Madurella	118	0.06
Russula	116	0.05
Sakaguchia	116	0.05
Pithomyces	114	0.05
Arizonaphlyctis	112	0.05
Bionectria	112	0.05
Corynascella	111	0.05
Melanotaenium	111	0.05
Coniochaetales unclassified	110	0.05
Branch06 unclassified	109	0.05
Echinocatena	106	0.05
Mariannaea	106	0.05
Xylogone	106	0.05
Onygenales unclassified	106	0.05
Rhizophagus	104	0.05
Magnaporthiopsis	102	0.05
Udeniomyces	102	0.05
Beauveria	101	0.05
Delitschia	101	0.05
Humicola	101	0.05
Neotestudina	100	0.05
Chrysosporium	99	0.05
Teichospora	99	0.05
Ramicandelaber	96	0.05
Rhizopus	93	0.04
Exophiala	92	0.04
Lecanicillium	92	0.04
Parasarocladium	91	0.04
Stachybotryaceae unclassified	91	0.04
Trichosporon	90	0.04
Coniothyrium	88	0.04

Genus	Number of DNA sequences	% of Total
Fusariella	86	0.04
Wettsteinina	86	0.04
Corallomycetella	82	0.04
Gaeumannomyces	82	0.04
Minutisphaera	82	0.04
Spegazzinia	82	0.04
Chytridium	80	0.04
Parazalerion	80	0.04
Micropsalliota	79	0.04
Paraconiothyrium	79	0.04
Strophariaceae unclassified	78	0.04
Limnoperdon	78	0.04
Agaricostilbomycetes unclassified	77	0.04
Magnaporthaceae unclassified	77	0.04
Coprinus	77	0.04
Pseudorobillarda	77	0.04
Typhula	77	0.04
Tremellodendropsidales unclassified	77	0.04
Cystoagaricus	75	0.04
Geosmithia	75	0.04
Pezizales unclassified	75	0.04
Tetragoniomyces	74	0.03
Arthrinium	73	0.03
Eocronartium	73	0.03
Laetisaria	73	0.03
Stagonospora	73	0.03
Entoloma	72	0.03
Aseroe	71	0.03
Moesziomyces	71	0.03
Sporocadaceae unclassified	70	0.03
Ustilago	70	0.03
Marasmiellus	69	0.03
Aspergillus	68	0.03
Microascaceae unclassified	67	0.03
Exserohilum	66	0.03
Robillarda	66	0.03
Parasola	65	0.03
Spiromastix	65	0.03

Genus	Number of DNA sequences	% of Total
Orbiliomycetes unclassified	65	0.03
Dendryphiella	63	0.03
Penicillium	63	0.03
Microascus	62	0.03
Kurtzmanomyces	61	0.03
Sparassis	61	0.03
Subramaniula	61	0.03
Claviceps	60	0.03
Sordariales unclassified	60	0.03
Verruconis	59	0.03
Melanommataceae unclassified	58	0.03
Corynespora	57	0.03
Coniochaetales unclassified	57	0.03
Fusicolla	56	0.03
Eurotiales unclassified	56	0.03
Sebacinales unclassified	56	0.03
Itersonilia	54	0.03
Trechispora	53	0.02
Microascales unclassified	53	0.02
Apiosordaria	52	0.02
Meyerozyma	50	0.02
Pseudophialophora	49	0.02
Chytridiomycetes unclassified	49	0.02
Kamienskia	48	0.02
Agrocybe	47	0.02
Pleurotus	47	0.02
Mortierellomycota unclassified	47	0.02
Chlorophyllum	46	0.02
Kazachstania	46	0.02
Schizothecium	46	0.02
Smaragdiniseta	46	0.02
Myriococcum	45	0.02
Pyxidiophora	45	0.02
Boubovia	44	0.02
Xylariales unclassified	44	0.02
Archaeospora	43	0.02
Sporormiella	43	0.02
Trichomerium	43	0.02
Chaetosphaeria	42	0.02
Minimedusa	42	0.02

Genus	Number of DNA sequences	% of Total
Neoidriella	42	0.02
Ophiosphaerella	42	0.02
Rosellinia	42	0.02
Latorua	41	0.02
Lophotrichus	41	0.02
Podoscypha	41	0.02
Trichosphaeriales unclassified	40	0.02
Neohelicomyces	39	0.02
Classiculaceae unclassified	39	0.02
Panaeolus	38	0.02
Xenoacremonium	38	0.02
Entrophospora	37	0.02
Saitozyma	37	0.02
Staphylotrichum	36	0.02
Eurotiomycetes unclassified	35	0.02
Halosphaeriaceae unclassified	35	0.02
Coniophora	35	0.02
Galiicola	35	0.02
Tilletia	35	0.02
Eleutherascus	34	0.02
Nodulisporium	34	0.02
Sagenomella	34	0.02
Septoriella	33	0.02
Taeniolella	33	0.02
Volvariella	33	0.02
Glomeromycota unclassified	33	0.02
Ovatospora	32	0.02
Torulaspora	32	0.02
Roussoella	31	0.01
Pholiotina	30	0.01
Powellomyces	30	0.01
Viridispora	30	0.01
Herpotrichiellaceae unclassified	29	0.01
Arachnomyces	29	0.01
Conlarium	28	0.01
Coronicium	28	0.01
Ophiocordyceps	28	0.01
Pseudaleuria	28	0.01
Pseudocercospora	28	0.01
Stictis	28	0.01

Genus	Number of DNA sequences	% of Total
Athelia	27	0.01
Chalara	27	0.01
Cladorrhinum	27	0.01
Wojnowiciella	27	0.01
Acrogenospora	26	0.01
Auxarthron	26	0.01
Stellatospora	26	0.01
Glomeraceae unclassified	26	0.01
Spizellomycetaceae unclassified	25	0.01
Dactylella	25	0.01
Gymnoascus	25	0.01
Parascedosporium	25	0.01
Scutellinia	25	0.01
Cryptococcus	24	0.01
Myceliophthora	24	0.01
Stemphylium	24	0.01
Diversisporaceae unclassified	24	0.01
Diaporthales unclassified	24	0.01
Onygenales fam Incertae sedis unclassified	23	0.01
Erythrobasidium	23	0.01
Microdiplodia	23	0.01
Occultifur	23	0.01
Phaeoacremonium	23	0.01
Sodiomyces	23	0.01
Malassezia	22	0.01
Pseudocoleophoma	22	0.01
Tulostoma	22	0.01
Pleosporaceae unclassified	21	0.01
Amaurodon	21	0.01
Endophragmiella	21	0.01
Kalmusia	21	0.01
Kiflimonium	21	0.01
Pleurophoma	21	0.01
Hymenochaetales unclassified	21	0.01
Xylariales fam Incertae sedis unclassified	20	0.01
Lacellina	20	0.01
Saccharomycetales unclassified	20	0.01
Wiesneriomyces	20	0.01
Cephalotrichiella	19	0.01
Cutaneotrichosporon	19	0.01

Genus	Number of DNA sequences	% of Total
Loweporus	19	0.01
Simplicillium	19	0.01
Tetraplosphaeria	19	0.01
Thielavia	19	0.01
Cylindrocladium	18	0.01
Degelia	18	0.01
GS14 unclassified	18	0.01
Pleosporales fam Incertae sedis unclassified	17	0.01
Calcarisporiella	17	0.01
Gibberella	17	0.01
Inocybe	17	0.01
Nakataea	17	0.01
Tomentella	17	0.01
*Trematei*a	17	0.01
Tuberculina	17	0.01
Cyphellaceae unclassified	17	0.01
Trichosporonales unclassified	17	0.01
Cantharellales unclassified	17	0.01
Coprotus	16	0.01
Gymnopilus	16	0.01
Idriella	16	0.01
Mucor	16	0.01
Neodeightonia	16	0.01
Phlyctochytrium	16	0.01
Sistotrema	16	0.01
Microthyriales unclassified	16	0.01
Glomeraceae unclassified	15	0.01
Basidiobolus	15	0.01
Hirsutella	15	0.01
Karstenia	15	0.01
Lasiodiplodia	15	0.01
Leucoagaricus	15	0.01
Phlebia	15	0.01
Saksenaea	15	0.01
Sporendonema	15	0.01
Serendipitaceae unclassified	15	0.01
Claroideoglomeraceae unclassified	14	0.01
Stereaceae unclassified	14	0.01
Arthrobotrys	14	0.01
Eucasphaeria	14	0.01

Genus	Number of DNA sequences	% of Total
Helicosporium	14	0.01
Hyphoderma	14	0.01
Phanerochaete	14	0.01
Phyllachora	14	0.01
Sulcosporium	14	0.01
Uncinocarpus	14	0.01
Veronaea	14	0.01
Dictyosporella	13	0.01
Fibulochlamys	13	0.01
Kochiomyces	13	0.01
Neurospora	13	0.01
Septoglomus	13	0.01
Stephanonectria	13	0.01
Glomeromycota unclassified	13	0.01
GS16 unclassified	13	0.01
Tulasnellaceae unclassified	13	0.01
Xylaria	13	0.01
Helotiales unclassified	13	0.01
Amesia	12	0.01
Arachniotus	12	0.01
Endogone	12	0.01
Hypoxylon	12	0.01
Leptobacillium	12	0.01
Myxospora	12	0.01
Nanoglomus	12	0.01
Penidiellopsis	12	0.01
Spencerozyma	12	0.01
Stilbella	12	0.01
Thelonectria	12	0.01
Uwebraunia	12	0.01
Wardomycopsis	12	0.01
Dothideales unclassified	12	0.01
Campylocarpon	11	0.01
Clitocybe	11	0.01
Conidiobolus	11	0.01
Cyrenella	11	0.01
Dichotomopilus	11	0.01
Efibulobasidium	11	0.01
Niesslia	11	0.01
Porotheleum	11	0.01

Genus	Number of DNA sequences	% of Total
Stylonectria	11	0.01
Boliniales fam Incertae sedis unclassified	11	0.01
Aschersonia	10	<0.01
Brevicalcar	10	<0.01
Brevistachys	10	<0.01
Chlamydocillium	10	<0.01
Colletotrichum	10	<0.01
Coniocessia	10	<0.01
Cystobasidium	10	<0.01
Emmonsiellopsis	10	<0.01
Monascella	10	<0.01
Paecilomyces	10	<0.01
Yunnania	10	<0.01
Chytridiomycetes unclassified	9	<0.01
Acrodontium	9	<0.01
Aplosporella	9	<0.01
Arthrographis	9	<0.01
Biatriospora	9	<0.01
Immersiella	9	<0.01
Lepidosphaeria	9	<0.01
Monoblepharis	9	<0.01
Montagnula	9	<0.01
Musicillium	9	<0.01
Pluteus	9	<0.01
Trematophoma	9	<0.01
Pleosporales unclassified	9	<0.01
Tetragoniomycetaceae unclassified	9	<0.01
Vaginatispora	9	<0.01
Xenopenidiella	9	<0.01
Niessliaceae unclassified	8	<0.01
Stephanosporaceae unclassified	8	<0.01
Apiotrichum	8	<0.01
Bourdotigloea	8	<0.01
Candida	8	<0.01
Cintractia	8	<0.01
Ctenomyces	8	<0.01
Daldinia	8	<0.01
Funiliomyces	8	<0.01
Gongronella	8	<0.01
Mycoarachis	8	<0.01

Genus	Number of DNA sequences	% of Total
Ramichloridium	8	<0.01
Retroconis	8	<0.01
Scleroderma	8	<0.01
Triparticalcar	8	<0.01
Hypocreales unclassified	8	<0.01
Ustilaginoidea	8	<0.01
Xylodon	8	<0.01
Trichocomaceae unclassified	7	<0.01
Ustilaginaceae unclassified	7	<0.01
Aphanoascus	7	<0.01
Arthopyrenia	7	<0.01
Castanediella	7	<0.01
Codinaea	7	<0.01
Collariella	7	<0.01
Dactylaria	7	<0.01
Devriesia	7	<0.01
Didymocyrtis	7	<0.01
Fonsecaea	7	<0.01
Hongkongmyces	7	<0.01
Meira	7	<0.01
Meripilus	7	<0.01
Neodevriesia	7	<0.01
Ochronectria	7	<0.01
Paraphysoderma	7	<0.01
Perenniporia	7	<0.01
Rasamsonia	7	<0.01
Acrospermum	6	<0.01
Ascotricha	6	<0.01
Astrosphaeriella	6	<0.01
Aureobasidium	6	<0.01
Debaryomyces	6	<0.01
Dicoccum	6	<0.01
Dominikia	6	<0.01
Donkioporiella	6	<0.01
Memnoniella	6	<0.01
Mycophilomyces	6	<0.01
Neolindgomyces	6	<0.01
Phomatospora	6	<0.01
Tilletiopsis	6	<0.01
Spizellomycetaceae unclassified	6	<0.01

Genus	Number of DNA sequences	% of Total
Volutella	6	<0.01
Auriculariales unclassified	6	<0.01
Geminibasidiales unclassified	6	<0.01
Didymosphaeriaceae unclassified	5	<0.01
Ganodermataceae unclassified	5	<0.01
Myrmecridiaceae unclassified	5	<0.01
Polyporaceae unclassified	5	<0.01
Areolospora	5	<0.01
Cephaliophora	5	<0.01
Gliocladiopsis	5	<0.01
Hyweljonesia	5	<0.01
Lindtneria	5	<0.01
Malbranchea	5	<0.01
Melanocarpus	5	<0.01
Melanopsichium	5	<0.01
Minimelanolocus	5	<0.01
Nematoctonus	5	<0.01
Olpidium	5	<0.01
Phialemoniopsis	5	<0.01
Pseudoophiosphaerella	5	<0.01
Pyronema	5	<0.01
Rhodotorula	5	<0.01
Termitomyces	5	<0.01
Clavariaceae unclassified	5	<0.01
Rhizophlyctidales unclassified	5	<0.01
Monoblepharidomycetes unclassified	4	<0.01
Diaporthaceae unclassified	4	<0.01
Phomatosporaceae unclassified	4	<0.01
Pleurotaceae unclassified	4	<0.01
Disciseda	4	<0.01
Hendersonia	4	<0.01
Leucosphaerina	4	<0.01
Limacella	4	<0.01
Limonomyces	4	<0.01
Nigrograna	4	<0.01
Pseudoacremonium	4	<0.01
Pterulicium	4	<0.01
Pyrrhoderma	4	<0.01
Scedosporium	4	<0.01
Septofusidium	4	<0.01

Genus	Number of DNA sequences	% of Total
Agaricales unclassified	4	<0.01
Ceraceosorales unclassified	4	<0.01
Clavulinaceae unclassified	4	<0.01
Coniochaetaceae unclassified	4	<0.01
Herpotrichiellaceae unclassified	4	<0.01
Russulales unclassified	4	<0.01
Xylariaceae unclassified	4	<0.01
Xanthagaricus	4	<0.01
Xeromyces	4	<0.01
Bionectriaceae unclassified	3	<0.01
Inocybaceae unclassified	3	<0.01
Schizoporaceae unclassified	3	<0.01
Tricholomataceae unclassified	3	<0.01
Abrothallus	3	<0.01
Asterostroma	3	<0.01
Bifiguratus	3	<0.01
Calvatia	3	<0.01
Catenulostroma	3	<0.01
Ceratocystis	3	<0.01
Collarina	3	<0.01
Coniella	3	<0.01
Cortinarius	3	<0.01
Crepidotus	3	<0.01
Cystolepiota	3	<0.01
Dactylonectria	3	<0.01
Diaporthe	3	<0.01
Dinemasporium	3	<0.01
Diversispora	3	<0.01
Favolus	3	<0.01
Gyrothrix	3	<0.01
Ijuhya	3	<0.01
Kockovaella	3	<0.01
Laccaria	3	<0.01
Lasiobolidium	3	<0.01
Letendraea	3	<0.01
Leucocoprinus	3	<0.01
Macrophomina	3	<0.01
Micronematobotrys	3	<0.01
Naganishia	3	<0.01
Nectriopsis	3	<0.01

Genus	Number of DNA sequences	% of Total
Omphalina	3	<0.01
Paradictyoarthrinium	3	<0.01
Paraphaeosphaeria	3	<0.01
Periconiella	3	<0.01
Pestalotiopsis	3	<0.01
Phialemonium	3	<0.01
Poitrasia	3	<0.01
Pringsheimia	3	<0.01
Pseudoseptoria	3	<0.01
Pseudoxylomyces	3	<0.01
Rhodosporidiobolus	3	<0.01
Stypella	3	<0.01
Tetracoccosporium	3	<0.01
Thyridariella	3	<0.01
Vishniacozyma	3	<0.01
Cladochytriales unclassified	3	<0.01
Erythrobasidiales unclassified	3	<0.01
Ascodesmidaceae unclassified	2	<0.01
Beltraniaceae unclassified	2	<0.01
Kondoaceae unclassified	2	<0.01
Absidia	2	<0.01
Amanita	2	<0.01
Angulomyces	2	<0.01
Arcopilus	2	<0.01
Arcuadendron	2	<0.01
Badarisama	2	<0.01
Berkleasmium	2	<0.01
Botryosphaeria	2	<0.01
Calceomyces	2	<0.01
Chaenothecopsis	2	<0.01
Chaetopreussia	2	<0.01
Cordyceps	2	<0.01
Corticium	2	<0.01
Cytospora	2	<0.01
Deconica	2	<0.01
Dichostereum	2	<0.01
Fellomyces	2	<0.01
Gliocephalotrichum	2	<0.01
Glomus	2	<0.01
Hansfordia	2	<0.01

Genus	Number of DNA sequences	% of Total
Hasegawazyma	2	<0.01
Heterospora	2	<0.01
Hyphodontia	2	<0.01
Hypochnicium	2	<0.01
Inonotus	2	<0.01
Knufia	2	<0.01
Koorchaloma	2	<0.01
Lacrymaria	2	<0.01
Lepista	2	<0.01
Liberomyces	2	<0.01
Lyomyces	2	<0.01
Microbotryozyma	2	<0.01
Millerozyma	2	<0.01
Monoblepharella	2	<0.01
Mycena	2	<0.01
Neofusicoccum	2	<0.01
Neophaeococcomyces	2	<0.01
Neoscolecobasidium	2	<0.01
Occultibambusa	2	<0.01
Papulaspora	2	<0.01
Paraphelidium	2	<0.01
Paraphoma	2	<0.01
Phellinus	2	<0.01
Phialocephala	2	<0.01
Phlebiopsis	2	<0.01
Phylloporia	2	<0.01
Polyporus	2	<0.01
Protomyces	2	<0.01
Pseudodactylaria	2	<0.01
Ruinenia	2	<0.01
Saccharomycopsis	2	<0.01
Sebacina	2	<0.01
Sesquicillium	2	<0.01
Setosphaeria	2	<0.01
Smardaea	2	<0.01
Sporisorium	2	<0.01
Sporothrix	2	<0.01
Stilbocrea	2	<0.01
Subplenodomus	2	<0.01
Talbotiomyces	2	<0.01

Genus	Number of DNA sequences	% of Total
Trimorphomyces	2	<0.01
Tylopilus	2	<0.01
Cephalothecaceae unclassified	2	<0.01
Cystobasidiales unclassified	2	<0.01
Endogonales unclassified	2	<0.01
Exobasidiales unclassified	2	<0.01
GS06 unclassified	2	<0.01
Sebacinales unclassified	2	<0.01
Stephanosporaceae unclassified	2	<0.01
Trichomeriaceae unclassified	2	<0.01
Urocystis	2	<0.01
Valsonectria	2	<0.01
Vermispora	2	<0.01
Zygosporium	2	<0.01
Capnodiales unclassified	2	<0.01
Chytridiales unclassified	2	<0.01
Kickxellales unclassified	2	<0.01
Microthyriales unclassified	2	<0.01
Blastobotrys	1	<0.01
Chlamydosporiella	1	<0.01
Dissoconium	1	<0.01
Hydropus	1	<0.01
Neoascochyta	1	<0.01
Rachicladosporium	1	<0.01
Verrucocladosporium	1	<0.01
Tremellales unclassified	1	<0.01

Supplementary Table A.2 Relative abundance of all genera detected in maize ears from both district.

Genus	**Number of DNA sequences**	**% of Total**
Sarocladium	59444	32.53
Fusarium	33849	18.52
Ceratobasidium	18091	9.90
Neodeightonia	12030	6.58
Waitea	9547	5.22
Trichoderma	8751	4.79
Talaromyces	7401	4.05
Lasiodiplodia	7254	3.97
Bipolaris	6384	3.49
Aspergillus	6176	3.38
Wallemia	3107	1.70
Cyphellaceae unclassified	1652	0.90
Curvularia	1373	0.75
Nigrospora	1228	0.67
Meyerozyma	990	0.54
Penicillium	921	0.50
Trichosphaeriales unclassified	670	0.37
Capillosclerotium	362	0.20
Blastobotrys	357	0.20
Papiliotrema	302	0.17
Exophiala	222	0.12
Pseudofusicoccum	190	0.10
Debaryomyces	124	0.07

Genus	**Number of DNA sequences**	**% of Total**
Wallemiales unclassified	122	0.07
Exserohilum	121	0.07
Acremonium	112	0.06
Marasmius	80	0.04
Typhula	74	0.04
Neocosmospora	69	0.04
Malassezia	68	0.04
Kazachstania	65	0.04
Paecilomyces	64	0.04
Trichomonascus	63	0.03
Cladosporium	62	0.03
Exobasidiales unclassified	62	0.03
Rhizoctonia	60	0.03
Pyxidiophora	57	0.03
Saccharomycetales unclassified	51	0.03
Alternaria	48	0.03
Botryosphaeria	44	0.02
Trichothecium	40	0.02
Ascomycota unclassified	40	0.02
Wickerhamomyces	38	0.02
k__Fungi unclassified	37	0.02
Sordariaceae unclassified	35	0.02
Hypocreales unclassified	35	0.02
Ustilago	33	0.02
Niesslia	30	0.02
Anthracocystis	27	0.01
Periconia	22	0.01

Genus	**Number of DNA sequences**	**% of Total**
Eurotiomycetes unclassified	21	0.01
Eremothecium	21	0.01
Candida	20	0.01
Engyodontium	20	0.01
Diaporthaceae unclassified	18	0.01
Arthrinium	18	0.01
Colletotrichum	18	0.01
Thanatephorus	18	0.01
Neofusicoccum	17	0.01
Geosmithia	16	0.01
Gibellulopsis	16	0.01
Rhizopus	15	0.01
Moesziomyces	14	0.01
Neodevriesia	14	0.01
Arachnomyces	12	0.01
Atractiella	12	0.01
Dissoconium	12	0.01
Filobasidium	12	0.01
Microascus	12	0.01
Spissiomyces	12	0.01
Eurotiales unclassified	12	0.01
Coprinopsis	11	0.01
Mortierella	11	0.01
Aureobasidium	10	0.01
Cyphellophora	10	0.01
Neoascochyta	10	0.01

Genus	**Number of DNA sequences**	**% of Total**
Udeniomyces	10	0.01
Didymellaceae unclassified	8	<0.01
Cystofilobasidium	8	<0.01
Diaporthe	8	<0.01
Gibberella	8	<0.01
Hydropus	8	<0.01
Plectosphaerella	8	<0.01
Scutellinia	8	<0.01
Stilbocrea	7	<0.01
Tilletia	7	<0.01
Trematosphaeria	7	<0.01
Fomes	6	<0.01
Microdochium	6	<0.01
Paraconiothyrium	6	<0.01
Phaeosphaeria	6	<0.01
Ramichloridium	6	<0.01
Schizophyllum	6	<0.01
Yamadazyma	6	<0.01
Cystobasidium	5	<0.01
Edenia	5	<0.01
Meira	5	<0.01
Ochronectria	5	<0.01
Pseudothielavia	5	<0.01
Symmetrospora	5	<0.01
Xeromyces	5	<0.01
Basidiomycota unclassified	5	<0.01

Genus	**Number of DNA sequences**	**% of Total**
Chytridiomycota unclassified	5	<0.01
Rhizophlyctidaceae unclassified	4	<0.01
Saccharomycetales fam Incertae sedis unclassified	4	<0.01
Agrocybe	4	<0.01
Anthostomella	4	<0.01
Basidiobolus	4	<0.01
Dendryphiella	4	<0.01
Lactocollybia	4	<0.01
Phoma	4	<0.01
Rhodotorula	4	<0.01
Sterigmatomyces	4	<0.01
Verticillium	4	<0.01
Magnaporthaceae unclassified	3	<0.01
Ustilaginaceae unclassified	3	<0.01
Ascobolus	3	<0.01
Chaetomium	3	<0.01
Chordomyces	3	<0.01
Clonostachys	3	<0.01
Daedaleopsis	3	<0.01
Dirinaria	3	<0.01
Glomus	3	<0.01
Hirsutella	3	<0.01
Hypsizygus	3	<0.01
Lectera	3	<0.01
Pseudohyphozyma	3	<0.01
Rhizophlyctis	3	<0.01

Genus	**Number of DNA sequences**	**% of Total**
Sarcodon	3	<0.01
Spegazzinia	3	<0.01
Stachylidium	3	<0.01
Sympodiomyces	3	<0.01
Toxicocladosporium	3	<0.01
Filobasidiales unclassified	3	<0.01
Pleosporales unclassified	3	<0.01
Tremellales unclassified	3	<0.01
Agaricomycetes unclassified	2	<0.01
Dothideomycetes unclassified	2	<0.01
Botryosphaeriaceae unclassified	2	<0.01
Nectriaceae unclassified	2	<0.01
Trichomeriaceae unclassified	2	<0.01
Acrocalymma	2	<0.01
Albonectria	2	<0.01
Chlamydosporiella	2	<0.01
Coprinus	2	<0.01
Corynespora	2	<0.01
Cumuliphoma	2	<0.01
Eutypella	2	<0.01
Flavodon	2	<0.01
Metarhizium	2	<0.01
Occultifur	2	<0.01
Paradictyoarthrinium	2	<0.01
Parasarocladium	2	<0.01
Poaceascoma	2	<0.01

Genus	**Number of DNA sequences**	**% of Total**
Rachicladosporium	2	<0.01
Stemphylium	2	<0.01
Teratoramularia	2	<0.01
Trichosporon	2	<0.01
Onygenales unclassified	2	<0.01
Ceratobasidiaceae unclassified	1	<0.01
Chaetomiaceae unclassified	1	<0.01
Alfaria	1	<0.01
Arnium	1	<0.01
Beauveria	1	<0.01
Bovista	1	<0.01
Bullera	1	<0.01
Clitopilus	1	<0.01
Cordana	1	<0.01
Cristinia	1	<0.01
Cryptococcus	1	<0.01
Cyathus	1	<0.01
Cytospora	1	<0.01
Didymocrea	1	<0.01
Dimorphiseta	1	<0.01
Emericellopsis	1	<0.01
Ganoderma	1	<0.01
Geastrum	1	<0.01
Macrophomina	1	<0.01
Malbranchea	1	<0.01
Metacordyceps	1	<0.01

Genus	**Number of DNA sequences**	**% of Total**
Micropsalliota	1	<0.01
Minutisphaera	1	<0.01
Mucor	1	<0.01
Ochroconis	1	<0.01
Oliveonia	1	<0.01
Ophiosphaerella	1	<0.01
Phaeosphaeriopsis	1	<0.01
Phialophora	1	<0.01
Podoscypha	1	<0.01
Preussia	1	<0.01
Pterula	1	<0.01
Ramicandelaber	1	<0.01
Remotididymella	1	<0.01
Rinaldiella	1	<0.01
Robillarda	1	<0.01
Scolecobasidium	1	<0.01
Setophoma	1	<0.01
Subramaniula	1	<0.01
Torula	1	<0.01
Trematophoma	1	<0.01
Diversisporaceae unclassified	1	<0.01
Mortierellomycota unclassified	1	<0.01
Verrucocladosporium	1	<0.01
Westerdykella	1	<0.01
Dothideales unclassified	1	<0.01
Russulales unclassified	1	<0.01

Supplementary Table A.3. Alpha Diversity metrics calculation

Sample	Observed number of unique OTUs (Sobs)	Chao1*	Shannon Diversity Index**	Evenness***
DM1	74	85.7	2.70	0.63
DM2	44	68.0	2.17	0.57
DM3	52	78.3	1.79	0.45
DM4	65	77.7	2.48	0.59
DM5	48	78.3	2.42	0.62
DM6	61	64.0	2.11	0.51
DS1	415	462	4.92	0.82
DS2	442	536	4.74	0.78
DS3	377	449	4.61	0.78
DS4	417	560	4.53	0.75
DS5	419	536	4.37	0.72
DS6	372	479	4.33	0.73
KM1	77	92.4	1.95	0.45
KM2	52	73.0	1.24	0.31
KM3	80	90.9	2.18	0.50
KM4	44	49.5	0.76	0.20
KM5	43	49.1	0.91	0.24
KM6	54	60.0	1.41	0.35
KS1	434	587.2	4.46	0.74
KS2	430	512	4.68	0.77
KS3	503	611	4.59	0.74
KS4	477	563	4.96	0.80
KS5	510	659	5.10	0.82
KS6	599	737	5.13	0.80

* **Chao1:** Extrapolated species richness = Sobs $+ \frac{f_1(f_1-1)}{2(f_2+1)}$

Sobs: number of unique OTUs observed in sample

f_1: number of singletons

f_2: number of doubletons.

****Shannon Diversity Index : $-\sum pi*ln(pi)$**

$\mathbf{p_i}$: relative abundance of ith taxon, calculated internally as $\mathbf{p_i} = \mathbf{n_i / N}$

$\mathbf{n_i}$: raw read count of taxon i

N : Total sum of all reads in sample.

*****Evenness:** Shannon Diversity Index/ ln (observed num of unique OTUs)

Supplementary Table A.4 Maize ear Indicator Taxon Analysis

OTU	Genus	Indicator Value	P value	Significance	P value_FDR	Significance
0002	*Sarocladium*	0.83	0.09	.	0.54	ns
0006	*Ceratobasidium*	0.93	0.01	**	0.16	ns
0008	*Neodeightonia*	0.91	0.03	*	0.29	ns
0009	*Waitea*	1.00	0.01	*	0.20	ns
0012	*Bipolaris*	1.00	0.00	**	0.13	ns
0017	*Aspergillus*	0.91	0.08	.	0.53	ns
0022	*Ceratobasidium*	0.94	0.02	*	0.24	ns
0027	*Trichoderma*	0.91	0.09	.	0.54	ns
0029	*Talaromyces*	0.92	0.03	*	0.29	ns
0031	*Curvularia*	0.95	0.04	*	0.36	ns
0035	*Bipolaris*	0.98	0.01	**	0.16	ns
0040	*Cyphellaceae* unclassified	0.99	0.00	**	0.13	ns
0065	*Nigrospora*	0.99	0.00	**	0.13	ns
0083	*Trichosphaeriales* unclassified	0.89	0.03	*	0.29	ns
0091	*Talaromyces*	0.88	0.06	.	0.42	ns
0156	*Ceratobasidium*	0.91	0.02	*	0.24	ns
0162	*Waitea*	0.82	0.06	.	0.46	ns

Supplementary Table A.5. Soil Indicator Taxon Analysis

OTU	Genus	Indicator value	P value before FDR	Significance	P value_FDR
0007	*Fusarium*	0.89	0.01	**	0.02
0010	*Pseudothielavia*	0.88	0.02	*	0.05
0014	*Cyphellophora*	0.94	0.01	**	0.02
0016	*Curvularia*	0.88	0.03	*	0.06
0024	*Lectera*	0.92	0.05	.	0.09
0025	*Mortierella*	1.00	0.01	**	0.02
0028	*Periconia*	0.97	0.01	**	0.02
0029	*Talaromyces*	0.92	0.02	*	0.04
0031	*Curvularia*	0.89	0.01	*	0.03
0032	*Acremonium*	0.88	0.03	*	0.07
0033	*Sordariacea* unclassified	0.99	0.01	*	0.03
0034	*Plectosphaerella*	0.90	0.02	*	0.04
0036	*Setophoma*	0.96	0.01	**	0.02
0037	*Phoma*	0.98	0.01	**	0.02
0039	*Gibellulopsis*	0.98	0.02	*	0.04
0041	*Chaetothyriaceae* unclassified	1.00	0.01	**	0.02
0042	*Phaeosphaeria*	0.83	0.01	*	0.04
0043	*Nigrospora*	0.94	0.03	*	0.06
0044	*Alternaria*	0.84	0.07	.	0.11
0045	Fungi unclassified	1.00	0.01	**	0.02
0048	*Pyrenochaetopsis*	1.00	0.01	**	0.02
0049	*Rhizoctonia*	0.92	0.08	.	0.12
0051	*Torula*	1.00	0.01	**	0.02

OTU	Genus	Indicator value	P value before FDR	Significance	P value_FDR
0052	*Edenia*	0.94	0.01	**	0.02
0053	*Clitopilus*	0.82	0.06	.	0.09
0054	*Acrocalymma*	0.97	0.01	**	0.02
0060	*Rhizophlyctidaceae* unclassified	1.00	0.01	**	0.02
0062	*Westerdykella*	0.96	0.03	*	0.05
0063	*Mortierella*	0.96	0.02	*	0.04
0064	*Verticillium*	0.82	0.06	.	0.10
0067	*Kernia*	0.87	0.05	*	0.08
0072	*Thanatephorus*	0.88	0.07	.	0.11
0073	*Didymocrea*	0.91	0.02	*	0.04
0075	*Pleosporales* unclassified	1.00	0.01	**	0.02
0076	*Didymocrea*	0.99	0.01	**	0.02
0077	*Cyathus*	0.82	0.06	.	0.10
0078	*Mortierella*	0.91	0.02	*	0.04
0079	*Ascobolus*	0.87	0.07	.	0.11
0080	*Arrhenia*	1.00	0.01	**	0.02
0081	*Fusarium*	0.99	0.01	**	0.02
0082	*Stachybotryaceae* unclassified	0.91	0.02	*	0.05
0084	*Atractiella*	0.99	0.01	**	0.02
0087	*Pyrenochaetopsis*	0.98	0.01	**	0.02
0090	*Talaromyces*	0.94	0.04	*	0.08
0092	*Chaetomiaceae* unclassified	0.82	0.05	*	0.08

OTU	Genus	Indicator value	P value before FDR	Significance	P value_FDR
0093	*Clonostachys*	0.98	0.01	**	0.02
0094	*Chaetothyriales* unclassified	1.00	0.01	**	0.02
0095	*Rhizophlyctis*	1.00	0.01	**	0.02
0096	*Mortierellomycota* unclassified	1.00	0.01	**	0.02
0097	*Mycothermus*	0.95	0.01	**	0.02
0099	*Preussia*	1.00	0.01	**	0.02
0100	*Fusarium*	0.87	0.09	.	0.13
0104	*Fusidium*	1.00	0.01	**	0.02
0107	*Chytridiomycota* unclassified	1.00	0.01	**	0.02
0108	*Phaeosphaeriopsis*	0.88	0.02	*	0.04
0109	*Stachybotryaceae* unclassified	0.92	0.01	*	0.03
0112	*Chaetothyriales* unclassified	0.98	0.01	**	0.02
0113	*Phialophora*	1.00	0.01	**	0.02
0114	*Mortierellomycota* unclassified	0.93	0.01	**	0.02
0115	*Ochroconis*	0.89	0.04	*	0.07
0116	*Scolecobasidium*	1.00	0.01	**	0.02
0118	*Polyschema*	0.92	0.01	*	0.04
0120	*Dothideomycetes* unclassified	1.00	0.01	**	0.02
0121	*Spizellomyces*	0.90	0.03	*	0.06

0126	*Powellomycetaceae* unclassified	1.00	0.01	**	0.02

Supplementary Table A.6 List of reference standards used in LC/MS-MS

I would like to thank Dr. Michael Sulyok for the LC/MS/MS data in this table.

Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology, IFA-Tulln, University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Str. 20, 3430 Tulln, Austria.

15-Acetyldeoxynivalenol	Aflatoxicol
15-Hydroxyculmorin	Aflatoxin B_1
15-Hydroxyculmoron	Aflatoxin B_2
15-Methyl-epi-Fumiquinazolin A	Aflatoxin G_1
16-Ketoaspergillimide	Aflatoxin G_2
2-Chlorunguinol	Aflatoxin M_1
2-Methylmitorubrin	Aflatoxin M_2
3-Acetyldeoxynivalenol	Aflatoxin P_1
3-Nitropropionic acid	Aflatoxin Q_1
4-Hydroxyalternariol	Aflatrem
4-Monoacetoxyscirpenol	Aflavarin
5-Hydroxyculmorin	Agistatin B
5-Methylmellein	Agistatin D
7-Hydroxykaurenolide	Agistatin E
7-Hydroxypestalotin	Agroclavine
A 23187	Aigualomycin D
AAL TA-Toxin	AJ 296

Abscisic acid

Acetylchaetoglobosin D

acetyldeoxyPHS

acetylPHS

Acuminatum B

Acuminatum C

Alternarian acid

Alternariol

Alternariol-3-Glucoside

Alternariol-9-Glucoside

Alternariolmethylether

Alternariolmethylether-Glucoside

Altersetin

Altersolanol

Altertoxin II

Altertoxin-I

Amidepsin B

Aminodimethyloctadecanol

Amoxycillin

Amphotericin B

Anacin

Andrastin A

Andrastin B

Andrastin C

Andrastin D

Alamethicin

α-Zearalenol

α-Zearalenol Glucoside

Alteichin

Altenuene

Altenusin

Ascofuranone

Ascomycin

Asparason A

Aspercolorin

Asperfuran

Aspergamid A

Aspergillicin Derivative

Aspergillimide

Asperglaucide

Asperlactone

Asperloxine A

Asperphenamate

Asperthecin

Aspinolid B

Aspinonene

Aspochalasin D

Aspochalasin I

Aspochalasin J

Aspochlasin C

Anisomycin
Anomalin A
Antbiotic L 696474
Antibiotic F 1839 A
Antibiotic PF 1052
Antibiotic Y
Apicidin
Ascochlorin

Aspterric acid
Aspulvinone E
Aspyrone
Asteltoxin
Asterric acid
Atlantinon A
Atpenin A5
Atroventinmethylether

Aurantiamin A
Auranticin A
Aurantine
Aurantioclavin
Aurasperon B
Aurasperon C
Aurasperon G
Aureobasidin A
Aurofusarin
Austalide A

Bassianolide
Beauvericin
Benzomalvin A
Benzomalvin C
Berkedrimane B
Berkeleyacetal B
β-Zearalenol
β-Zearalenol-Glucoside
Bikaverin
Bis(methylthio)gliotoxin

Austalide B
Austalide F
Austamide
Austdiol
Austinol
Austocystin A
Austocystin D
Austocystin I

Brasiliamide A
Brefeldin A
Brevianamid F
Brevicompanine B
Butenolid
Butyrolacton III
Butyrolactone I
Butyrolactone II

Australide D

Averantin

Averantinmethylether

Averufanin

Averufin

Averufin Derivative

Bacitracin

Bafilomycin A1

Barceloneic acid A

Cereulide

Cerulenin

Chaetocin

Chaetoglobosin A

Chaetoglobosin D

Chaetoviridin A

Chanoclavin

Chetomin

Chetoseminudin A

Chevalone C

Chlamydospordiol

Chlamydosporol

Chloramphenicol

Chlorocitreorosein

Chloronectrin

Chlortetracyclin

Calonectrin

Calphostin

Calyxanthone

Carnequinazolin A

Carviolin

Cephalochromin

Cephalosporin C

Cercosporamide

Cercosporin

Citrinin

Citromycetin

Cladosporin

Clonostachydiol

CNM 115443

Cochliodinol

Cochlioquinone A

Colchicin

Communesin B

Cordycepin

CPA hydroly

Culmorin

Curvularin

Curvulin

cyclo(L-Leu-L-Pro)

cyclo(L-Pro-L-Tyr)

Chrodrimanin

Chromomycin A3

Chrysogin

Chrysophanol

Cinereain

Citreohybriddione

Citreohybridonol

Citreorosein

Citreoviridin

Citreoviridin C

Citreoviridinol

Cyclosporin D

Cyclosporin H

Cylindrocarpon A4

Cylindrol B

Cytochalasin A

Cytochalasin B

Cytochalasin C

Cytochalasin D

Cytochalasin E

Cytochalasin H

Cytochalasin J

Daunorubicin

Deacetylneosolaniol

Decalonectrin

Decarestrictin

cyclo(L-Pro-L-Val)

Cycloaspeptide A

Cycloechinulin

Cycloheximide

Cyclopenin

Cyclopenol

Cyclopeptine

Cyclopiazonsäure

Cyclosporin A

Cyclosporin B

Cyclosporin C

Deoxyfusapyron

Deoxynivalenol

Deoxynortryptoquivalin

deoxyPS

Deoxytryptoquialanine

Deoxytryptoquivaline A

Desoxypaxillin

Desoxyverrucosidin

Destruxin A

Destruxin B

Destruxin CHL

Destruxin D

Destruxin-Ed Derivative

Dethiosecoemestrin

Diacetoxyscirpenol

Dechlorogriseofulvin	Dichlordiaportin
Dechloroisochromophilon IV	Dichlormethylasterric acid
Dechloronornidulin	Dihydrochlamydocin
Deepoxy-deoxynivalenol	Dihydrocitrinone
Dehydroaustinol	Dihydroergosine
Dehydrocurvularin	Dihydroergotamine
Dehydrocyclopeptine	Dihydrogriseofulvin
Dehydrogriseofulvin	Dihydroinfectopyron
Demethylasteltoxin	Dihydrolysergol
Demethylsulochrin	Dihydrosterigmatocystin
DeoxyAltersolanol	Dihydrotrichotetronine
Deoxybrevianamid E	Dihydroxycalonectrin
Dihydroxymellein	Epiequisetin
DihydroxyZONMethylether	Epoxyagroclavin
Dimethoxymethylgrisantrion	Epoxycytochalasin C
Dinactin	Equisetin
Diplodiatoxin	Eremofortin A
Deoxynivalenol-15-Sulfate	Eremofortin B
Deoxynivalenol -3-glucoside	Ergine
Deoxynivalenol -3-Sulfate	Ergocornine
Deoxynivalenol -Glutathione	Ergocorninin
Doxorubicine	Ergocristam
Doxycycline	Ergocristinam
Drimane 6	Ergocristine
Drimane 8	Ergocristinine

Elymoclavine

Elymoclavine-Fructoside

Emericellamide A

Emericellamide C

Emericellamide E

Emindole SA

Emodin

Endocrocin

Enniatin A

Enniatin A_1

Enniatin B

Enniatin B_1

Enniatin B_2

Enniatin B_3

Fellutannine B

Festuclavine

FK 506

FK 9775 A

FK 9775 B

Flavipucin

Flavoglaucin

Folipastatin

Fonsecin

FS-4

Fulvic acid

Fumagillin

Ergocryptine

Ergocryptinine

Ergometrine

Ergometrinine

Ergosine

Ergosinine

Ergotamine

Ergotaminine

Ergovalin

Erythromycin

Expansolid

F01 1358-A

Fallacinol

Fellutanine A

Fumonisin A_1 precursor

Fumonisin A_2

Fumonisin B_1

Fumonisin B_2

Fumonisin B_3

Fumonisin B_4

Fumonisin B_6

Fungerin

Fusaproliferin

Fusapyron

Fusarenone-X

Fusaric acid

Fumagillol

Fumifungin

Fumigaclavine

Fumigaclavine C

Fumiquinazoline A

Fumiquinazoline C

Fumiquinazoline D

Fumiquinazoline Derivative

Fumiquinazoline E

Fumiquinazoline F

Fumiquinazoline I

Fumitremorgine A

Fumitremorgine B

Fumitremorgine C

Fumonisin A_1

Griseophenone B

Griseophenone C

Harzianopyridine

Harzianum A

HC Toxin

Helvolic acid

Helvolinic acid

Heptaibin

Heptelidic acid

Herquline A

Fusarieline A

Fusarin C

Fusarinolic acid

Fuscofusarin

Fusidic acid

Geldanamycin

Geodin

Geodin hydrate

Gibberellic acid

Gibepyron D

Gliocladic acid

Gliotoxin

Glyantrypine

Gregatin B

Griseofulvin

Irgasan

Isocereulide A

Isochromophilon III

Isochromophilon IV

Isochromophilon IX

Isochromophilone VI

Isofusidienol

Isokotanin B

Iso-Rhodoptilometrin

Josamycin

HT-2 Glucoside

HT-2 toxin

hydrolysed Fumonisin B_1

hydrolysed Nidulin

Hydroxycarnequinazolin A

Hydroxycurvularin

Hydroxyroquefortine C

Hydroxysydonic acid

Hypothemycin

Ilicicolin A

Ilicicolin B

Ilicicolin C

Ilicicolin E

Infectopyron

Integracin A

Integracin B

Ionomycin

Lysergol

Macrosphelide A

Macrosporin

Malformin A

Malformin A_2

Malformin C

Marcfortine A

Marcfortine B

Marcfortine C

K252a

K252b

K-76 Derivative 4

Kipukasin B

Kipukasin D

KO 143

Kojic acid

Koninginin D

Koninginin E

Linamarin

Lincomycin

LL-Z 1272e

Lolitrem B

Lolitrem N

Lotaustralin

Luteoskyrin

Luteusin A

Mycophenolic acid

Mycophenolic acid IV

Myriocin

Mytoxin C

N-benzoyl-phenylalanine

Neoechinulin A

Neosolaniol

Neoxaline

NG 012

Meleagrin
Meleagrin Derivative
Methoxycurvularin
Methoxysterigmatocystin
Methylasterric acid
Methylequisetin
Methylfunicone
Methylsulochrin
Methysergide
Mevastatin
Mevinolin
Mithramycine A
Mitomycine C
Monactine
Moniliformin
Monoacetoxyscirpenol
Monocerin
Monomethylcurvulin
NT-2 Toxin
NX-1
NX-2
NX-3
Ochratoxin A
Ochratoxin α
Ochratoxin B
Nidulin
Nidurufin
Nigericin
Nigragillin
Nigrosporoate A
Nivalenol
Nivalenol glucoside
Nonactin
Norlichexanthone
Nornidulin
Norsolorinic acid
Nortryptoquialanine
Norverrucosidin
Notoamide C
Notoamide E
NP 12318
NP 19199
NP-8140
Paraherquamide A
Paraherquamide E
Paspalic acid
Paspalin
Paspalinin
Paspalitrem A
Paspalitrem B

Ochratoxin C

Ochrephilone

Okaramine B

Okaramine D

Oligomycin A

Oligomycin B

O-Methylsterigmatocystin

O-Methylviridicatin

Ophiobolin A

Ophiobolin B

Orsellinic acid

Oxalicine B

Oxaline

Oxaspirodion

Oxidized elymoclavine

Oxidized Luol

Oxytetracyclin

Papyracillic acid A

Paracelsin A

Paracelsin B

Pinselin

Piscarinin A

Porritoxinol

Prehelminthosporol

Prehelminthosporollacton

Patulin

Paxillin

Pencillazaphilone B

Penicillic acid

Penicillide

Penicillin G

Penicillin V

Penigequinolone A

Penitrem A

Pennigritrem A

Pentahydroxyscirpenol

Pentoxyfylline

Pestalotin

Phenopyrrozin

Phomalactone

Phomalone

Phomopsin A

Phomopsin B

Phomopsolide B

Physcion

Radiclonic acid

Rapamycin

Rasfonin

Roquefortine C

Roquefortine D

Prelaptin

PR-Toxin

Pseurotin A

Pseurotin D

Puberulin A

Puromycin

Purpactin A

Purpuride

Pyranonigrin

Pyrenocin A

Pyrenophorol

Pyripyropene A

Pyripyropene B

Pyripyropene O

Pyrophen

Quadrone

Questiomycin A

Quinadoline A

Quinadoline B

Quinolactacin A

Quinolone A

Radicicol

semi Xanthomegnin

semi-Vioxanthin

Setusosin

Siccanin

Roquefortine E

Roridin A

Roridin L_2

Roritoxin A

Rubellin D

Rubratoxin A

Rubrofusarin

Rugulosin

Rugulotrosin

Rugulovasine A

Rugulusovin

Sambucinol

Satratoxin F

Satratoxin G

Satratoxin H

Scalusamid A

Sch 725680

Sclerotioramin

Sclerotiorin

Secalonic acid D

Secoemestrin C

seco-Sterigmatocystin

Trichothecin

Trichothecolone

Trypacidin

Tryprostatin A

Siccanol
Skyrin
S-Methyl Deoxynivalenol
Sorbicillacton A

Sphingofungin B
Sphingofungin D
Spiculisporic acid
Spiramycin
Spirodihydrobenzofuranlactam IV
Sporogen AOI
Stachybotryamide
Stachybotrylactam
Staurosporin
Stemphylperylenol

Sterigmatocystin
Sulochrin
Sydonic acid
Sydowinin A
Synerazol
T-2 glucoside
T-2 toxin
T2-tetraol
T2-triol
Taxol

Tenellin
Tryprostatin B
Tryptophol
Tryptoquialanine
Tryptoquialanine Derivative

Tryptoquialanone
Terrecyclic acid
Terrein
Terretonin
Terretonin F
Territrem B
Tetracycline
Tetrahydrobostrycin
TR-2 Toxin
Triacetyl-deoxynivalenol

Trichalasin B
Trichodermamide C
Trichodermin
Trichodimerol
Trichostatin A
Trichotetronine
Trichothecin
Trichothecolone
Trypacidin
Tryprostatin A

Tryprostatin B

Tensidol B

Tentoxin

Tenuazonic acid

Ternatin

Terpendole C

Terpendole E

Terphenyllin

Unguinol

Unugisin E

Usnic acid

Ustiloxin A

Ustiloxin B

Ustiloxin D

Ustusol A

Valinomycin

Vancomycin

Vermistatin

Verrucarin A

Verrucarin J

Verrucarol

Verrucofortine

Verrucosidin

Verruculogen

Verruculotoxin

Violaceic acid

Tryptophol

Tryptoquialanine

Tryptoquialanine derivative

Tryptoquialanone

Tryptoquivaline A

Tryptoquivaline F

Tryptoquivaline G

Tylosine

Viridicatin

Viridicatol

Viridicatum toxin

Viridol

WIN 68577

WIN-64821

Wortmannin

Xanthomegnin

Xanthotoxin

Xantocillin X_1

Xantocillin X_2

Yaequinolone J_2

Zaragozic acid A

Zearalenone

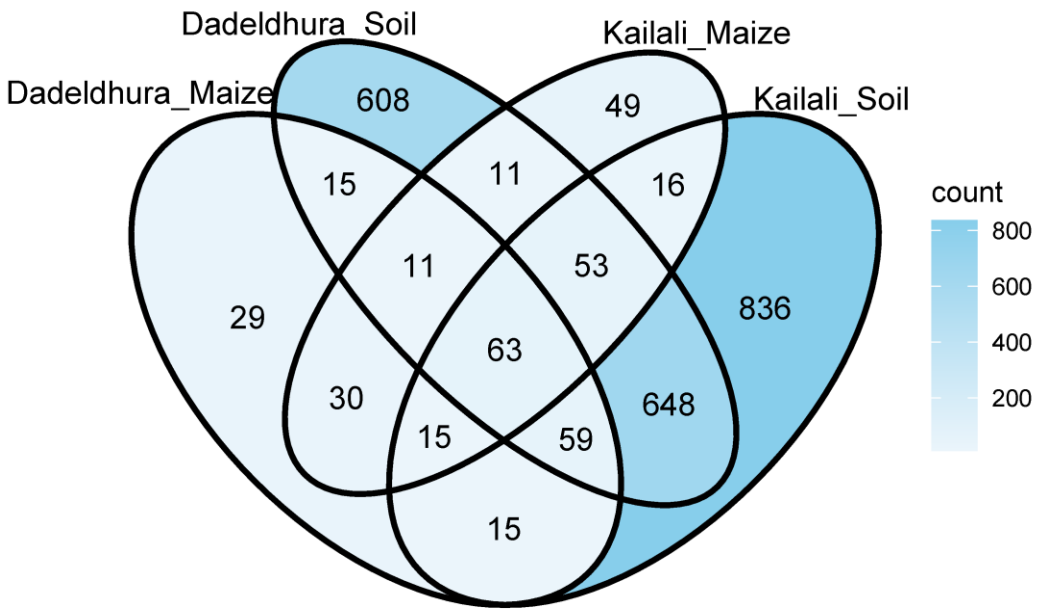
Zearalenone-14-glucoside

Zearalenone-14-sulfate

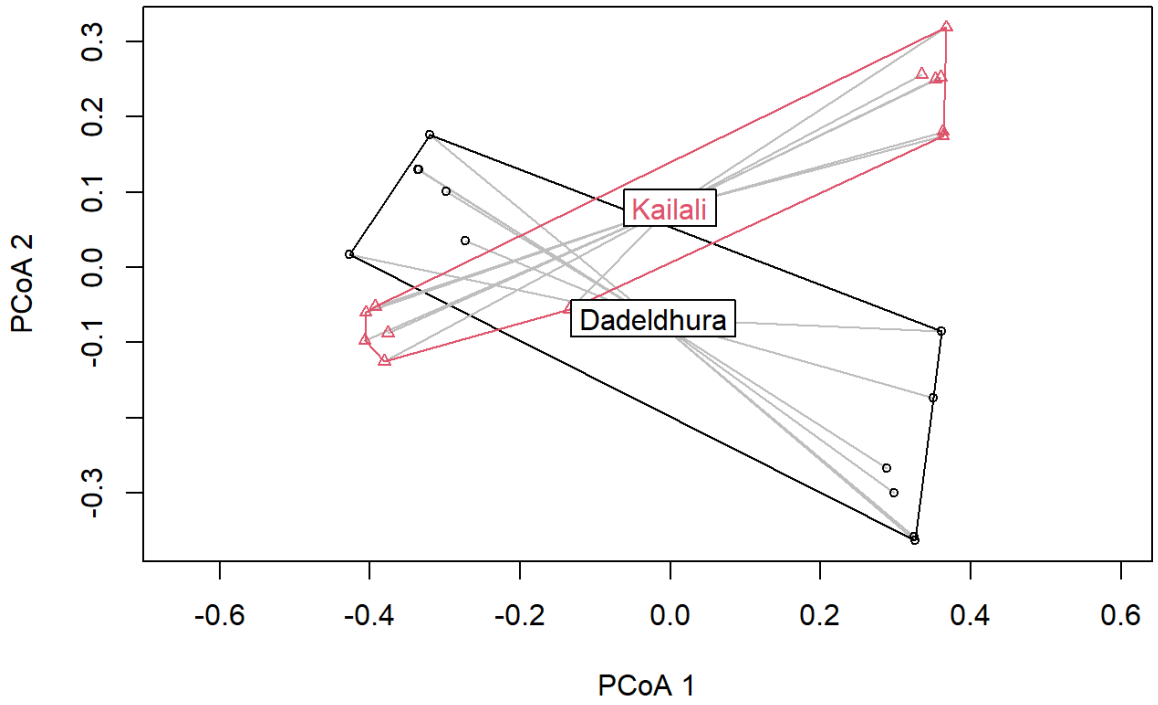
Zeralenone-16-glucoside

Violaceol I	Zinn diol
Violaceol II	Zinniamide
Viomellein	Zinniol
Vioxanthin	

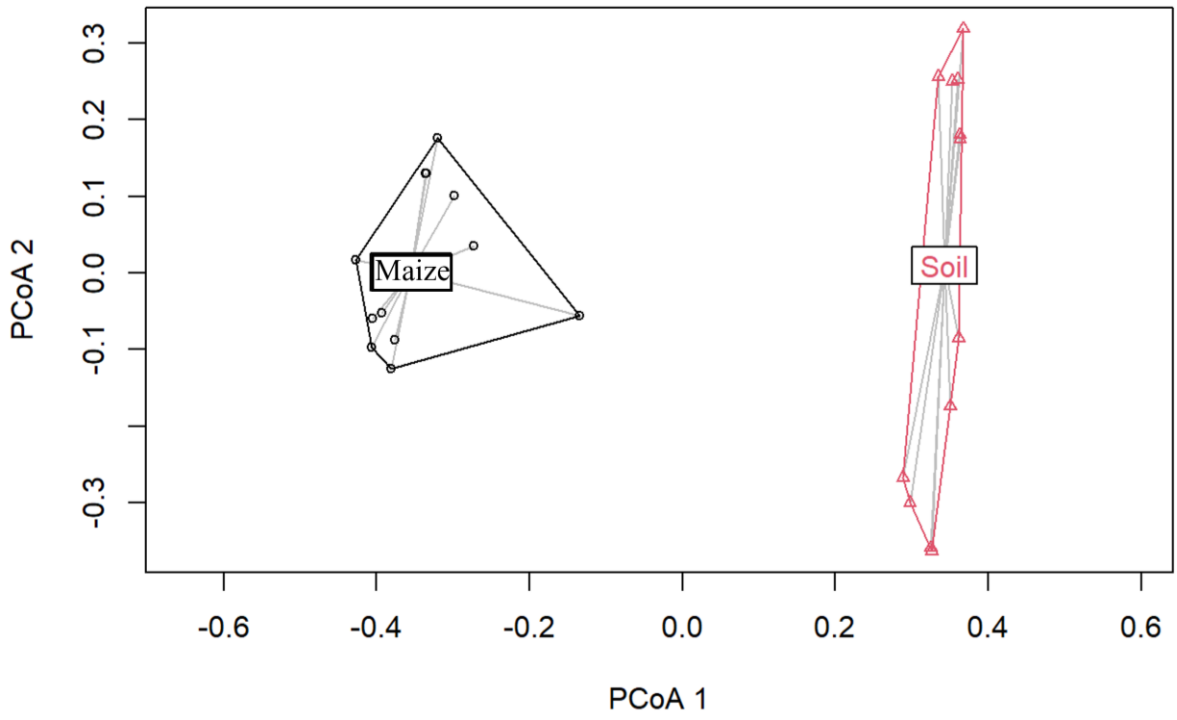
Supplementary Figures



Appendix Figure A.1. Four set Venn Diagram of OTUs distributed in maize and soil in Dadeldhura and Kailali



Appendix Figure A.2. Beta-dispersion analysis of samples was assessed based on the distance of individual samples from the respective group centroids for the sites. Open black circle represents samples (maize ears and soil samples from Dadeldhura). Open red triangle represents samples (maize ears and soil samples from Kailali).



Appendix Figure A.3. Beta dispersion analysis of samples was assessed based on the distance of individual samples from the respective group centroids for the sample types(maize ear and soil). Open black circle represents maize ears from both the districts: Dadeldhura and Kailali. Open red triangle represents soil samples from both the districts