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# Niche partitioning association of fungal genera correlated with lower *Fusarium* and fumonisin-B1 levels in maize

**Bwalya Katati · Anne D. van Diepeningen · Henry Njapau · Paul W. Kachapulula · Bas J. Zwaan · Sijmen E. Schoustra**

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**Abstract** Through partitioning of ecological niches, several fungi are able to coexist on the same host crop. In (partial) absence of niche partitioning, competitive exclusion among fungi can occur. Competitive exclusion is one of the bases for biocontrol. We investigated fungal correlations, in terms of relative abundance of the fungi, in pre-harvest maize, as a natural ecosystem model. Internal mycobiome fungal relative abundance of maize was used to establish correlations. The maize had been harvested from dry and wet agro-ecological zones of Zambia. The relative abundances of the fungal genera were determined using DNA amplicon sequencing. For this study, positive or absence of correlations between fungal genera signified good niche partitioning (co-existence), whereas negative correlations signified poor niche partitioning and potential for competitive exclusion. When species compete within one niche (competitive exclusion), we may expect to detect higher levels of mycotoxins—since mycotoxins are considered antagonistic agents aimed at defending or invading an ecological niche. To estimate the importance of mycotoxins in competitive exclusion, we measured the influence of the fungal correlations on levels of fumonisin-B1 (FB1) in the maize. FB1 data were derived from a previous study on the maize, determined by HPLC. Results showed that *Sarocladium* and *Stenocarpella* had the strongest significant negative correlation with *Fusarium*, suggesting poor niche partitioning and potential for antagonism of these genera with *Fusarium*. Furthermore, higher levels of *Stenocarpella* resonated with lower levels of FB1 and vice versa. It was also observed that, when *Sarocladium* was in low abundance (< 10%), the frequency of detection of higher levels of FB1 (> 100 μg $kg^{-1}$) in the pre-harvest maize was highest.

**Keywords** Competitive exclusion · Fumonisin-B1 · *Fusarium* · Maize · Niche partitioning · *Sarocladium*

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B. Katati (✉) · B. J. Zwaan · S. E. Schoustra
Laboratory of Genetics, Wageningen University and Research, Droevendaalsesteeg 1, Wageningen 6708 PB, The Netherlands
e-mail: bkatati@nisir.org.zm

B. Katati · H. Njapau
Mycotoxicology Laboratory, National Institute for Scientific and Industrial Research, P.O. Box 310158, Lusaka, Zambia

A. D. van Diepeningen
Business Unit Biointeractions and Plant Health, Wageningen University and Research, Wageningen, The Netherlands

P. W. Kachapulula · S. E. Schoustra
School of Agricultural Sciences, University of Zambia, Great East Road Campus, Lusaka, Zambia

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## Introduction

Fungi are ubiquitous and diverse. In nature, fungi evolve into diverse communities residing in such ecological systems as soil or maize as a host plant. The diversity of the communities of fungal taxa may depend on niche partitioning. Through partitioning of ecological niches, living organisms can coexist in one space without competing for the same resource (Chesson 2000). Several fungal genera can infect the same host crop and coexist through niche partitioning. The niche partitioning in fungi can be driven by various abiotic factors including temperature and rainfall as well as carbon source among others (Arroyo et al. 2008; Giorni et al. 2009; Mohale et al. 2013; Perrone et al. 2020). Where the niche partitioning is 'good,' fungi will likely coexist and on the contrary, the fungi will likely undergo competition (Chesson 2000). The competition may take the form of reduction in abundance of the less competitive genus or species, relative to another. In quantitative terms, the result can be a negative correlation in relative abundance between the two organisms due to change in abundance of one relative to the other. The deterministic ecological mechanisms related to 'good' or 'poor' niche partitioning among microorganisms include neutralism (no effect on abundance of the existing populations), mutualism (co-existing population abundances increasing), predation/parasitism (with part of existing population increasing and other reducing), competition (generally the existing populations reducing), etc. (Abdullah et al. 2017; West et al. 2006) (Fig. 1).

Resource partitioning may make it possible for different fungi to occupy a single host or differentially infect crops in their demand for different carbon sources (Arroyo et al. 2008; Giorni et al. 2009; Mohale et al. 2013), while finite nutrient availability may trigger competition between microorganisms. Resource partitioning, therefore, makes it possible for crops to be contaminated with an array of fungal genera. Furthermore, resource partitioning may lead to differentiation in genera that will infect a specific crop. For example, of the mycotoxigenic fungi, groundnuts in the field are known to be more prone to metabolite aflatoxin (AF) contamination due to its susceptibility to *Aspergillus* infection (Hulikunte et al. 2017; Kachapulula et al. 2017; Ndisio et al. 2017) compared to contamination with the

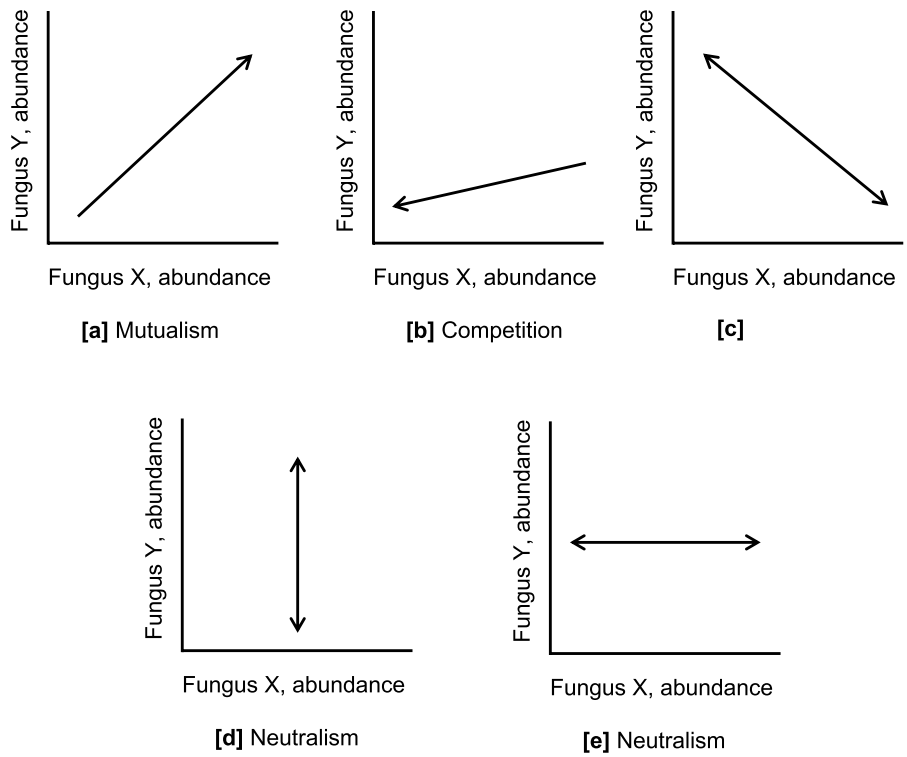


**Fig. 1** Demonstrates idealistic correlations between two fungal genera including mutualism (**a**) (both populations increasing); competition (**b**) (one of the two populations reducing compared to the other); predation (**c**) (one population increasing and the other reducing); and lack of correlation (**d**); **e** (one population changing without any relation to the other). The arrows are not a response to timescale, but rather space

metabolite fumonisin (FB) due to *Fusarium* infection. On the contrary, maize in the field is more prone to the metabolite FB contamination due to *Fusarium* infection, compared to *Aspergillus* infection (Lane et al. 2018; Akello et al. 2021; Katati et al. 2023). However, it should be noted that both *Fusarium* and *Aspergillus* will infect pre-harvest maize besides genera like *Stenocarpella* and *Penicillium* (Lane et al. 2018; Katati et al. 2023).

Lack of resource partitioning will result in niche exclusion due to the competition for specific niche space (Dorner et al. 1999). In competitive exclusion, one fungal genus, or one species, outcompetes another fungus or species effectively reducing the population of the outcompeted fungus. For example, *Fusarium* outcompeting *Ustilago* (Alvarado-Serrano and Knowles 2014) may suggest that under the specific conditions *Fusarium* and *Ustilago* would be occupying the same niche. Competitive exclusion is one of the bases for biocontrol of mycotoxins including important ones such as aflatoxin due to *Aspergilli* (Bock and Cotty 1999; Medina et al. 2017; Raksha et al. 2020). Some important antagonisms include the competition between *Fusarium* and *Trichoderma* for the competitive exclusion of *Fusarium*. *Trichoderma* is a ubiquitous soil dweller (Bacon et al. 2001) and is non-pathogenic to maize (Gromadzka et al. 2019). It is a prospective fungus in the competitive exclusion of wheat and maize pathogenic *Fusarium* (Palazzini et al. 2018; Filizola et al. 2019; Lu et al. 2020).

As part of competition for niche space, fungi can produce biochemicals. For example, it has been demonstrated in vitro that outcompeting of *Ustilago* by *Fusarium* may be through generation of cell wall-degrading secondary metabolites by *Fusarium*, competitively excluding *Ustilago* (Alvarado-Serrano and Knowles 2014). With respect to the production of secondary metabolites by fungi, studies have demonstrated the production of the biochemical aflatoxin in *Aspergillus* as a defence response to invasion of its ecological space (Trienens et al. 2010; Drott et al. 2017). Similar antagonistic mechanisms include the production of antagonistic agents such as bacteriocins in bacteria (Schoustra et al. 2012). In addition to this, the maize pathogen *Stenocarpella* has been demonstrated to produce antifungal metabolite chaetoglobosin K against *Aspergillus flavus* and *Fusarium verticillioides* (Wicklow et al. 2011). Importance of *Stenocarpella* in maize is that, even though *Fusarium* is the most common maize pathogen, it is also an important causative genus for ear and stalk rots (Flett and McLaren 1994; Olatinwo et al. 1999; Rossouw et al. 2009; García-Reyes et al. 2022). Significant infection of maize with *Stenocarpella* leads to yield loss.

More generally, mycotoxins could be important compounds when species compete within an ecological niche. In mixed fungal communities, it is plausible that the levels of mycotoxins such as FB or AF in the fungal environment could be linked to the way fungal genera in an ecological niche correlate either negatively or positively by relative abundance. In this regard, where poor niche partitioning occurs, and thus high levels of competitive exclusion is expected, it would be anticipated that certain genera may negatively correlate with respect to relative abundance with each other. In addition, it would also be expected that competing fungi would secrete anti-competitor toxins under a negative correlation. Specifically, negative correlation in relative abundance between *Fusarium* and another genus would be expected to lead to higher levels FB in maize. Importance of FB is its carcinogenicity (IARC 2012; Yu et al. 2021). Of the FB variants (FB1, FB2, FB3, FB4), FB1 is known to form the major compound (70–80%) on average of the total unmasked fumonisin in maize grain (Gil-Serna et al. 2014) and has also been found to be the most toxic one (Wentzel et al. 1992; Yu et al. 2020). The identification of fungal genera with negative correlation in relative abundance to *Fusarium* would provide prospects for combat of FBs, where the *Fusarium* is able to be competitively excluded. Unlike the biocontrol of *Aspergillus* to control aflatoxin, the field biological control of *Fusarium* to control FBs in maize has been elusive, with success limited to laboratory experimentation (Kagot et al. 2019). Hence, this calls for the need to identify additional biological control agents (BCA) naturally adapted to the same natural environment in which *Fusarium* thrives on maize.

Fungi will exist on crop through niche partitioning. The aim of this study was to elucidate the existence of evidence that niche partitioning has an effect on competitive exclusion in fungi. We investigated negative and positive correlations in relative abundance of fungal genera present on maize kernels in order to make reliable predictions for competitive exclusion of specific genera of interest. Once consistent

correlations would be observed, direct experimental studies could be designed to test if antagonism between strains is indeed a mechanism by which competitive exclusion takes place. The study had two specific objectives: (1) To determine correlations in relative abundance among fungal genera present in maize kernels in order to identify potential mutualistic and antagonistic relations between the fungi. We suggest that absence of negative correlation in relative abundance between fungal genera demonstrates 'good' niche partitioning in the maize grain whereas negative correlation may indicate 'poor' niche partitioning, signifying potential for competition between such negatively correlated genera. We specifically predict that fungal genera with negative correlation with *Fusarium* exist. Such genera can potentially be used as BCA to competitively exclude *Fusarium* from a crop. (2) To evaluate the influence of fungi that are negatively correlated with *Fusarium* on the levels of the mycotoxin FB1. Our specific hypothesis was that the presence of fungal genera negatively correlated with *Fusarium* in relative abundance will lead to a decrease in FB in maize, owing to the resulting low abundance of *Fusarium,* which may consequently lead to its reduced competitive edge to produce FB. Conversely, we hypothesize that a high relative abundance of *Fusarium* in presence of another negatively correlated fungus will still lead to higher level of FB in the maize, because *Fusarium* in competition with other fungi will secrete anti-competitor toxins (including FB) in order to outcompete the other fungi.

## Materials and methods

### Determination of fungal relative abundance correlations

#### *Sample collection and preparation*

We investigated fungal relative abundance correlations by analysing the internal mycobiome of previously dried maize kernels (Katati et al. 2023) stored at −35 °C. The maize samples ($n=40$) were collected during the 2018/2019 season from four northerly ($n=20$ samples) and four southerly ($n=20$ samples) districts of Zambia. The northerly districts are located in a high-rainfall agro-ecological zone (AEZ), while the southerly districts lie in a low rainfall AEZ (Bunyolo et al. 1995; Phiri et al. 2013). For each sample, a 150 g homogenised portion was sterilised by dipping the kernels in 10% (v/v) sodium hypochlorite solution (household grade Jik®) for 3 min to remove unwanted surface DNA (Kampmann et al. 2017, 2022; Nilsson et al. 2022) and microbes. The kernels were then rinsed three times in sterile water and dried inside sterilised cotton bags placed in a Forced Draft Oven (Heraeus, model D-6450, Hanau, Germany) for 24 h, set at 42 ± 2 °C to rapidly drive out the moisture. The dried kernels were then milled using an Ultra Centrifugal Mill (model ZM200, Retsch, Haan, Germany) fitted with a 1.0 mm ring sieve and then stored in sterile polyethylene bags. The mill's pot and ring were cleaned between samples.

#### *DNA extraction*

Extraction of internal mycobiome DNA from the milled maize was carried out according to the Qiagen PowerSoil Quick-start Spin Protocol (detailed at https://www.qiagen.com/nl, product catalogue number HB-2495 of 2019) as follows: small portions of homogenised milled maize were carefully scooped from different positions of the sample, collecting about 200 mg into a bead beating tube for DNA extraction. Next, 800 µl of CD1 lysis buffer was added to the tube, then the prescribed PowerSoil Quick-start Spin Protocol was followed. To adapt to the protocol, tube beating was carried out for 12 min continuously using a sideways homogeniser (Model MM400 Retch, Haan, Germany) set at 25 beats per second. Tube centrifuge was done at room temperature in a microcentrifuge (model 5424, Eppendorf, Hamburg, Germany). The concentration and quality of the isolated DNA were read on a spectrometer (Nanodrop model 2000, Thermo Fischer Scientific, Wilmington, DE, USA).

#### *DNA amplicon sequencing and bioinformatic analysis*

For amplicon sequencing and bioinformatic analysis, the purified DNA was normalised to 10 ng $\mu l^{-1}$. The DNA was sequenced at LGC Genomics (Biosearch Technologies, Berlin, Germany) on Illumina platform, Miseq V3, by paired-end amplicon sequencing

(2 × 300 bp). The ITS1 (nuclear ribosomal internal transcribed spacer 1) region of the fungal genome was sequenced, as described in a previous study (Katati et al. 2023). The following primer pairs were used to amplify and sequence the region, partly overlapping into the 5.8S region: ITS1F_Kyo2 (forward) TAGAGGAAGTAAAAGTCGTAA and ITS86R (reverse) TTCAAAGATTCGATGATTCAC. The sequencing output data were received in the form of adaptor- and primer-clipped demultiplexed samples with a sequencing depth of about 105,000 to 158,000 total reads per sample, with reads less than 100 bp discarded. The bioinformatic analysis of the obtained raw amplicon sequence data was processed using the Divisive Amplicon Denoising Algorithm version 2 (DADA2) pipeline (Callahan et al. 2016) as described in a previous study (Katati et al. 2023). The Unite Fungal Database (UNITE Community 2019) was used to assign taxa to the DADA2-generated amplicon sequence variants (ASVs). The assigned ASVs were the measure for genera abundance. Fungal taxon resolution was done up to genus level as this was sufficient for the niche mapping.

*Determination of fungal relative abundance correlations*

A Spearman correlation matrix (SCM) was used to assess fungal relative abundance correlations. The top 30 genera out of the full internal mycobiome ($n \sim 40$ genera) were used, given that these were the more abundant members (≥ 12% frequency of field appearance) likely to drive significant correlations. In the SCM, relative abundance correlations between −1.0 and −0.3 were considered (strongly) negatively correlated (potential for antagonism). Correlations between +1.0 and +0.3 were considered (strongly) positively correlated (potential for mutualism). Values close to or equal to zero (−0.30 to +0.30, inclusive) denoted a weak or no correlation.

*Influence of fungal abundance correlations on fumonisin-B1 (FB1)*

Data for FB1 were retrieved from a previous study (Katati et al. 2023) in order to associate fungal genera abundances with FB1 in current study.

Data analysis

All statistical computations were conducted in software R (R Core Team 2023) version 4.3.2 (for the R code see Data Availability) using the following packages and their functions, partly described in a previous investigation (Katati et al. 2023): "phyloseq" (McMurdie and Holmes 2013) for internal mycobiome census; "reshape2" (Wickham 2007) for generation of Spearman correlation matrix (SCM) of fungal relative abundance correlations; "Hmisc" (Harrell and Dupont 2019) for determination of significance of fungal relative abundance correlations; 'vegan' (Oksanen et al. 2010) and 'dplyr' (Wickham et al. 2021) for MDS (Multi-Dimensional Scaling) data decomposition of agronomic factors; "ggplot2" (Wickham 2016) for visualisation of data as figures (SCM and MDS).

*Influence of fungal correlations on FB1*

The internal mycobiome was studied for this purpose. This is presuming that this would provide a more discrete relation between the mycobiome colonising the grain and mycotoxin, as the FB is harboured in the grain, even though the phyllosphere would still be expected give a good relation. To associate fungal genera relative abundance with FB1 levels, Spearman rank correlation (rho) was used such that FB1 data and the corresponding fungal relative abundances are ranked in the algorithm, due to the heterogenous distribution of the FB1 datum.

*Agronomic (confounding) factors with potential to influence Fusarium and fumonisin-B1 levels*

Additional agronomic factors, as confounding elements, with potential to influence *Fusarium* proliferation (Roucou et al. 2021) as well as FB1 levels in the maize, were appraised. The factors were obtained through a questionnaire (Supplemental Questionnaire S1). The questionnaire was administered with the help of agricultural field extension officers who routinely work with the farmers. To assess the link between the agronomic factors and FB1 levels as a result of *Fusarium* proliferation, each factor per field was assigned the corresponding levels of *Fusarium* relative abundance (if response to question was affirmative) or '0' (if response to question

was negative). The factors, included seed type (early, medium or late maturing variety), cropping type, and presence or absence of pests. Responses were computed into a dataframe (see Data Availability).

Multi-Dimensional Scaling (MDS) was used to determine if the agronomic factors did not differentially influence *Fusarium* proliferation as well as FB1 levels in the maize. The measure used in the MDS was *Fusarium* relative abundance. The FB1 data were derived from a previous study (Katati et al. 2023), whose maize concentration we ordinated in the current study as low ($<50$ µg kg$^{-1}$, $n=17$), medium (50–300 µg kg$^{-1}$, $n=13$) and high ($>300$ µg kg$^{-1}$, $n=10$) across the 40 sampled fields. *Fusarium* relative abundance was similarly ordinated into arbitrary levels as high ($>65\%$, $n=14$), medium (21–65%, $n=13$) and low ($\leq 20\%$, $n=13$) relative abundance. Cutoff point for agronomic factors with influence on *Fusarium*/FB1 levels was set at a $P$ value of 0.05.

## Results

### Fungal relative abundance correlations

On average, *Sarocladium* and *Fusarium* had the highest internal mycobiome relative amplicon sequence variant (ASV) abundance of 47% and 26%, respectively. *Stenocarpella* was third highest at 13%. The full table of the ASV relative abundance representation of the internal mycobiome genera is available on a public repository as (https://github.com/bkatati/nichemap/blob/main/InternoBiome.csv). Of the mycotoxin- important genera, and the high relative abundance genera, *Fusarium-Sarocladium* and *Fusarium-Stenocarpella* had the strongest negative significant correlations by relative abundance (Fig. 2; see Supplemental Table S1). There was no clear correlation between *Sarocladium* and *Stenocarpella* relative abundances (Fig. 2; rho $=-0.15$, $P=0.011$). Similarly, the correlation between *Ustilago* and *Fusarium* was very weak (Fig. 2) although the dry weather pattern showed an inclination for a strong negative correlation between the two genera (rho $=-0.650$, $P<0.001$) compared to the wet weather pattern with a non-significant correlation (rho $=0.205$, $P=0.058$).

### Influence of fungal genus abundance on FB1 levels

From the internal mycobiome, there was a negative correlation between relative abundance of *Stenocarpella* and levels of FB1 (rho $=-0.33$, $P=0.04$). The observed negative correlation between the levels of *Sarocladium* and FB1 was not significant (rho $=-0.10$, $P=0.52$). However, fields with lower relative abundance of *Sarocladium* ($<10\%$) had a higher frequency of higher FB1 levels when compared to fields with medium or higher levels of *Sarocladium* (Table 1). Similarly, there was no correlation between *Ustilago* relative abundance and levels of FB1 (rho $=-0.21$, $P=0.20$). *Fusarium* relative abundance positively correlated with levels of FB1 (rho $=0.39$, $P=0.01$).

### *Assessment of influence of confounding (agronomic) factors on Fusarium and fumonisin-B1 levels*

The additional agronomic factors, as confounding elements, did not differentially influence *Fusarium* and FB1 levels (Fig. 3). The factors with significance to be able to influence FB1/*Fusarium* proliferation included seed type by maturity (early: $P=0.001$, $R=0.37$; medium: $P=0.001$, $R=0.46$; late maturing: $P=0.001$, R $=0.39$), and pest incidence during cropping ($P=0.006$, $R=0.27$). Factors with no significance to influence FB1/*Fusarium* proliferation were field burning ($P=0.166$) and monocropping ($P=0.937$).

## Discussion

### Fungal relative abundance correlations defined by niche partitioning

In this study, we associate the fungal genera relative abundance correlations with niche partitioning. *Sarocladium* and *Fusarium* had the highest relative abundance by amplicon sequence variant representation in the maize internal mycobiome (47% and 26%, respectively) followed by *Stenocarpella* (13%). The strong negative relative abundance correlation between *Fusarium* and *Sarocladium* suggests poor niche partitioning between the two genera. The poor niche partitioning is characteristic of competition, a likely consequence of similarity in niche utilisation.

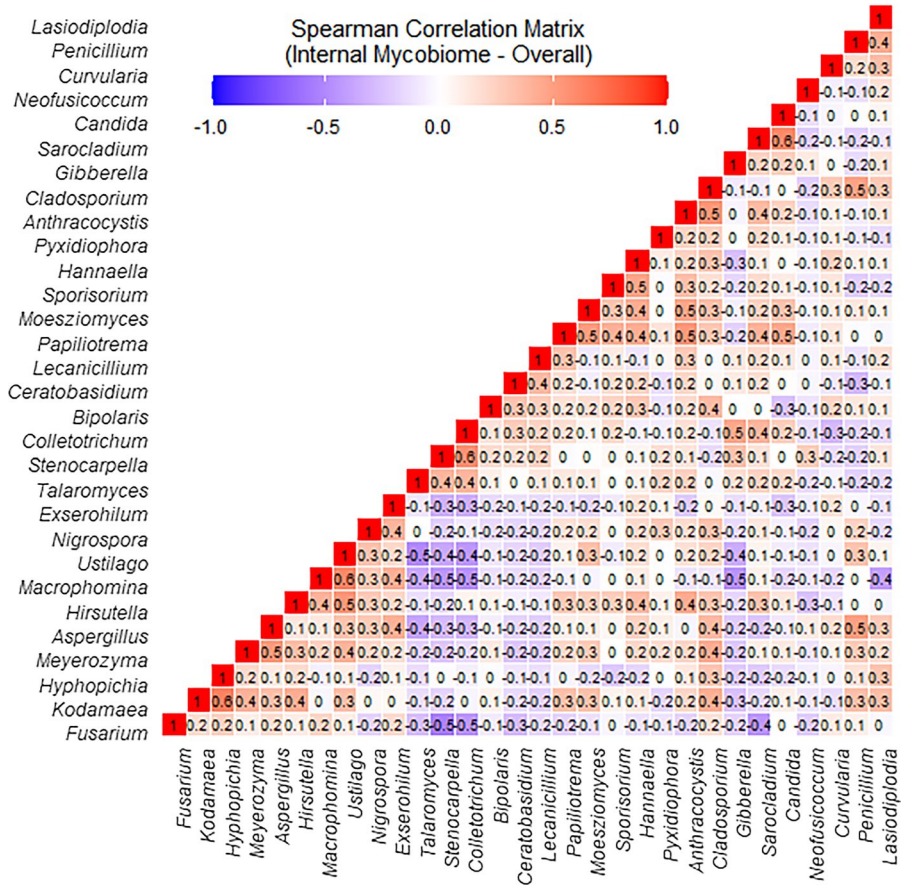


**Fig. 2** Spearman correlation matrix, showing top 30 genera in descending abundance. The correlations are the overall combining internal mycobiome fungal relative abundances for both dry (southerly) and wet (northerly) agro-ecological zones of Zambia. Negative values (blue) are negative correlations and positive (red) are positive correlations in fungal relative abundances. Individual correlations of the genera per district are presented in Supplemental Table S1. Of the total number of correlations appraised ($n=435$ pairs out of 30 genera), most correlations were either strongly positive (13.6%) or were weak in either direction (82.7%). Strong negative correlations were 3.7%. Note: *Fusarium* (*equiseti*) is detected as a distinct anamorph of the former *Gibberella* (*intricans*) based on the UNITE fungal database (UNITE Community 2019). *Talaromyces* is assessed as a distinct clade from its asexual anamorph *Penicillium*

**Table 1** Frequency of FB1 (μg kg$^{-1}$) levels in maize fields in relation with *Sarocladium* levels

| *Sarocladium* level | *Sarocladium* relative abundance (& number of fields) | FB1 Geometric Mean$^{\chi}$ | gSE$^{\#}$ | > 100 μg kg$^{-1}$ FB1 ($n$) | > 500 μg kg$^{-1}$ FB1 ($n$) | > 1000 μg kg$^{-1}$ FB1 ($n$) |
|---|---|---|---|---|---|---|
| Low | < 10% ($n=22$) | 116.5 (±6.3) | 1.5 | 13 | 6 | 2 |
| Med | 10–50 ($n=9$) | 44.7 (±3.1) | 1.8 | 2 | 0 | 0 |
| High | > 50% ($n=9$) | 79.6 (±4.1) | 1.6 | 6 | 0 | 0 |

In this table, fields with lower levels of *Sarocladium* (< 10%) had more than twice the frequency ($n=13$) of detection of higher FB1 levels (geometric mean > 100 μg kg$^{-1}$) when compared to fields with medium or higher levels of *Sarocladium*. However, the negative correlation between the levels of *Sarocladium* and FB1 was not significant (rho = −0.10, $P=0.52$). $^{\chi}$Figures in parenthesis are geometric SD. $^{\#}$The gSE is exponentiated SE of the logarithmic data of samples within the categorical variable '*Sarocladium* level.'

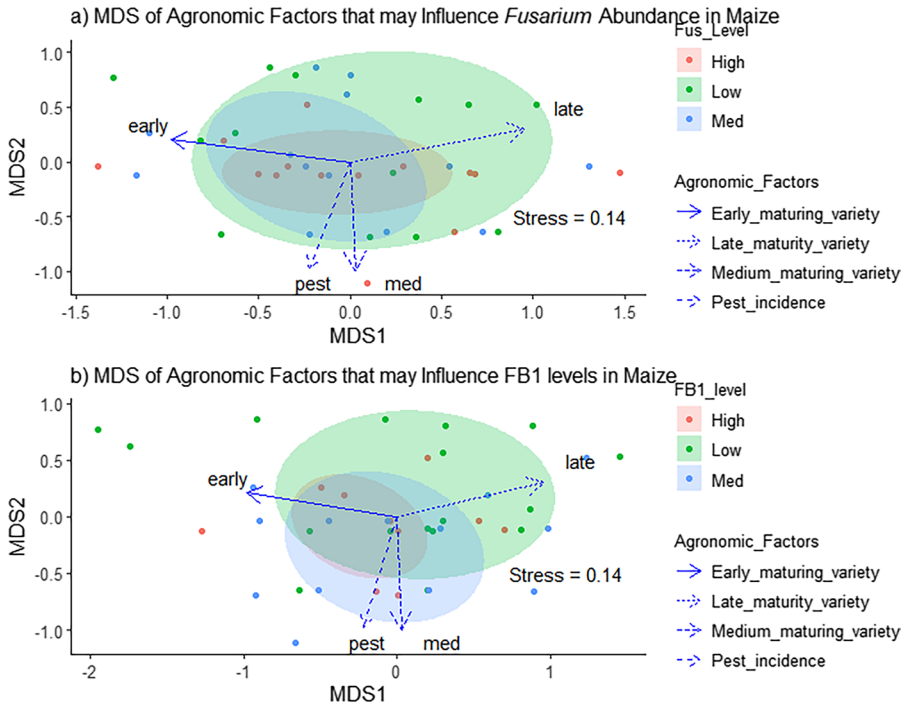


**Fig. 3** In the MDS, arrows are orientation of agronomic factors based on relative abundance of *Fusarium*. For example, the arrow 'med' refers to a medium maturing seed variety. Dots are fields ($n=40$), and each field has a value of FB1 level (μg $kg^{-1}$) and *Fusarium* relative abundance (%). Ellipses link fields of similar arbitrary levels of *Fusarium* (**a**) or FB1 (**b**). The dots have been scattered by MDS based on relative abundance of *Fusarium*. Levels of *Fusarium* are ordinated as 'High' (>65%, $n=14$), 'Med' (21–65%, $n=13$) and 'Low' (≤20%, $n=13$). Similarly, FB1 values are ordinated as 'High' (FB1 > 300 μg $kg^{-1}$, $n=10$), and 'Med' (FB1 = 50–300 μg $kg^{-1}$, $n=13$) and 'Low' (FB1 < 50 μg $kg^{-1}$, $n=17$). Only arrows of the significantly ($P<0.05$) important agronomic factors on *Fusarium* proliferation are shown ($n=4$ out of six), namely seed type by maturity (early: $P=0.001$, R = 0.37; medium: $P=0.001$, R = 0.46; late maturing: $P=0.001$, R = 0.39) and pest incidence during cropping ($P=0.006$, R = 0.27). Thus, monocropping ($P=0.93$) and burning ($P=0.18$) are excluded. The arrows (agronomic factors) subsequently orient towards ellipses for *Fusarium*/FB1 levels. The ellipse overlaps in the MDS for both *Fusarium* and FB1 and the absence of clear orientation of the arrows to a particular ellipse, even at reduced confidence level (67%), indicate a lack of correlation of the appraised agronomic factors with *Fusarium* proliferation or FB1 levels in the pre-harvest maize

Similar niche antagonism between *Fusarium* and *Sarocladium* has been demonstrated by previous solid substrate plating in vitro studies (Comby et al. 2017; Kemp et al. 2020). Similarly, our simulation data of 2018/2019 cropping season between *Sarocladium* and *Fusarium* external mycobiome relative abundances from a previous study (Katati et al. 2023) reveals a consistent negative correlation (rho = −0.70, $P<0.001$) (Supplemental Data S1 available as https://github.com/bkatati/nichemap/blob/main/S1_ExternoBiome.csv).

A similar negative correlation in relative abundance was observed between *Stenocarpella* and the mycotoxin-important genera *Fusarium* and *Aspergillus* (Fig. 2; see Supplemental Table S1). These observations are consistent with a past study done by solid substrate plating that demonstrated a negative correlation between *Stenocarpella* and two species, *Fusarium verticillioides* and *Aspergillus flavus* (Wicklow et al. 2011). Our findings suggest that even in natural ecosystems, such negative or positive correlations in abundance between certain specific genera are likely to exist, even at higher taxonomic ranking (genus level in the current study).

There was no demonstrable overall correlation in relative abundance between *Fusarium* and *Ustilago* (rho = 0.08, $P<0.001$). This is contrary to findings by Alvarado-Serrano and Knowles (2014) who demonstrated growth ability competition between the two genera in vitro. The variance between our findings and

those by Alvarado-Serrano and Knowles (2014) may be attributed to our in situ abiotic conditions. For example, a strong negative correlation, similar to the report by Alvarado-Serrano and Knowles (2014), is observed in the current study between the *Fusarium* and *Ustilago* under the drier conditions (rho $< -0.60$, $P < 0.001$), whereas under the wetter conditions the positive correlation was not significant ($P = 0.058$; see Supplemental Table S1).

While other studies have demonstrated that *Trichoderma* is a ubiquitous dweller of soil (Bacon et al. 2001; Egidi et al. 2019), which is the natural reservoir for fungi translocating to crop, we did not detect this fungus in the maize internal mycobiome. Similarly, in previous Zambian studies, *Trichoderma* was only detected on maize in one of 11 districts (Mukanga et al. 2010) and not detected at all on maize grain phyllosphere (Katati et al. 2023). We may attribute the absence of *Trichoderma* on maize to its poor colonisation of this crop. It would be worth testing for *Trichoderma* presence or absence in the soil mycobiome from the sampled areas of the current study to better understand if the fungus' poor or non-colonisation of the maize mycobiome was due to its exclusion in the environment (soil) or on the maize. Importance of *Trichoderma* to the maize mycobiome would be its non-pathogenicity to maize (Gromadzka et al. 2019), which would make it the desirable endophyte on maize compared to maize-pathogenic *Fusarium*.

## Influence of genera abundance correlations on FB1 levels

With respect to antagonism that may be mediated by presence of mycotoxins, it would have been expected that higher levels of FB1 would have correlated with higher levels of *Stenocarpella.* This is taking into account that production of secondary metabolites in fungi can be triggered as a response by a fungus to defend its ecological niche due to competition with other fungi. For instance, *Fusarium* is reported to produce cell-degrading metabolites when in competition with *Ustilago* (Alvarado-Serrano and Knowles 2014). Similarly, *Stenocarpella* produces the metabolite chaetoglobosin K, which inhibits the proliferation of *Fusarium verticillioides* and *Aspergillus flavus* (Wicklow et al. 2011). In the current study, a negative correlation was detected between *Stenocarpella* and FB1 besides its negative correlation with *Fusarium* relative abundance. We attribute this to the reduced growth competitiveness of *Fusarium* when the relative abundance of *Stenocarpella* is high, as seen from the negative correlation between the two genera (Fig. 2; see Supplemental Table S1), leading to a diminutive effect on the FB-producing ability of *Fusarium*. The observed relationship between *Stenocarpella* and levels of FB demonstrates for the first time the (indirect) negative influence of *Stenocarpella* on levels of FB1 in pre-harvest maize.

With respect to *Sarocladium*, another high relative abundance genus in this study, we did not detect a significant correlation between levels of FB1 and *Sarocladium* abundance. However, it was observed from ordinal data that, when *Sarocladium* was in very low abundance (< 10%), the frequency of detection of high FB1 levels (> 100 μg $kg^{-1}$) increased (Table 1). Similarly, we did not detect any correlation between *Ustilago* abundance and FB1 levels in maize (rho $= -0.21$, $P = 0.20$), which resonates with absence of an overall correlation in relative abundance between *Ustilago* and *Fusarium*. We note, however, that, where confrontation between *Fusarium* and *Ustilago* is demonstrated, the upregulation of the excretion of secondary metabolites and cell wall-degrading proteins by *Fusarium* may occur (Alvarado-Serrano and Knowles 2014). Although *Sarocladium* is a reported rice pathogen (Sakthivel et al. 2002), our current findings and those from a previous study (Katati et al. 2023) indicate its capability to naturally and extensively infect maize, satisfying preliminary conditions as a genus with prospects for the competitive exclusion of *Fusarium* and subsequently FB in maize.

## Prospects in biocontrol of fumonisin (FB)

Considering that the biocontrol of mycotoxins such as AF, FB and deoxynivalenol is premised on competitive exclusion of one fungus by another (Bandyopadhyay et al. 2016; Tian et al. 2016; Błaszczyk et al. 2017; Filizola et al. 2019), the observed negative correlations in fungal relative abundances are a useful attribute in prospecting for fungal genera that could be used for the competitive exclusion of *Fusarium* and its mycotoxin FB. In this regard, the observed negative correlation between *Fusarium* and *Sarocladium* relative abundances helps to define prospects for the utilisation of such a genus as

biological control agent (BCA) for the biocontrol of FB. It should also be noted that one of the attributes for the success of biocontrol is that the BCA should be able to effectively and widely contaminate the crop and thrive in the natural ecosystem in which the target pathogen thrives. In this investigation, this attribute has been demonstrated by *Sarocladium* (Fig. 2; see Supplemental Table S1). This then suggests the identification of strains of *Sarocladium* that are non-pathogenic to humans and maize for the prospective competitive exclusion of *Fusarium*. For example, non-pathogenic *Sarocladium zeae* is prospective candidate for the biocontrol of *Fusarium* in wheat (Kemp et al. 2020). Although *Stenocarpella* showed a negative correlation with *Fusarium* relative abundance and FB1 levels, the fungus is largely a pathogen to maize and produces harmful metabolites that can induce diplodiosis in livestock (Snyman et al. 2011; Masango et al. 2015). Hence, unlike genera such as *Stenocarpella* and *Ustilago*, non-pathogenic strains of *Sarocladium* may be good candidates for the control of FB1 contamination of pre-harvest maize.

A critical next step in this work is to design directed experiments to assess if these relative abundance correlations are indeed caused by niche partitioning and that mycotoxins play a critical mechanistic role. Experiments using pairs of strains grown under various levels of niche partitioning (e.g., different types of nutrients) could be used to test direct and specific predictions on what strains may outcompete others under what levels of niche partitioning, and whether or not mycotoxins such as FBs would be important antagonistic agents.

In conclusion, we studied correlations between fungal genera relative abundances, under the assumption that these correlations may be driven by niche partitioning. We found evidence for these correlations using internal mycobiome fungal populations on sampled maize, as the natural ecosystem model. We have demonstrated that niche partitioning may affect the potential for competitive exclusion of fungi on maize. In this regard, the overall hypothesis that lower levels of *Fusarium* and FB1 would ensue due to fungus with a negative correlation in abundance with *Fusarium*, and vice versa, was subsequently not rejected.

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**Data availability** Code for the mycobiome census as well as characteristics of the sampled maize and its full internal mycobiome composition is available on a public repository at https://github.com/bkatati/nichemap. Additional information is available on request from the corresponding author.

**Declarations**

**Conflict of interest** Authors duly declare no conflicts of interest.

**Ethical approval** No human or animal subjects were studied in this investigation.



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**Bwalya Katati** is a scientist at National Institute for Scientific and Industrial Research (NISIR), Lusaka, Zambia, with research interest in the ecology of food fungi, with particular interest in *Aspergillus* and *Fusarium* and their mycotoxins, from a food safety perspective. The work presented here was part of his PhD project on the fungal contamination dynamics in maize and the prospects for competitive exclusion. The work was executed at the Wageningen University and Research, The Netherlands and partly at NISIR.

**Anne D. van Diepeningen** is senior researcher in genetics and phytopathology at Wageningen University and Research, Wageningen, the Netherlands. Her research focus is on the diversity of mycotoxigenic fungi *Fusarium* and *Aspergillus* and their population genetics and evolution.

**Henry Njapau** is executive director of the Zambia Academy of Sciences, Lusaka, Zambia. At time of this investigation he was director for National Institute for Scientific and Industrial

Research, Lusaka, Zambia, and a principal scientific officer focused on food safety research, in particular the mycotoxins.

**Paul W. Kachapulula** is lecturer and head of the Department of Plant Science at University of Zambia, in Lusaka, Zambia. His research interests are in the etiology and management of mycotoxin contamination and in food safety.

**Bas J. Zwaan** is professor and chairholder of Laboratory of Genetics at Wageningen University and Research, Wageningen, the Netherlands. He specialises in quantitative and population genetics, evolution and adaptation in living organisms.

**Sijmen E. Schoustra** is associate professor in evolutionary genetics at Wageningen University and Research, Wageningen, the Netherlands. His work focuses on ecology and evolution of microorganisms, particularly bacteria and fungi. He is also a visiting professor at the Department of Food Science of the University of Zambia, in Lusaka, Zambia.