

Long-Term Maize-Legume Intercropping Shifts Structure and Composition of Soil Microbiome with Potential Impacts on Ecosystem Services and Food Safety

Aneth Mwakilili (✉ anethdavid367@gmail.com)

University of Dar es Salaam <https://orcid.org/0000-0002-1633-297X>

Kilaza Samson Mwaikono

Dar es Salaam Institute of Technology (DIT)

Sebastian Larsson Herrera

Swedish University of Agricultural Sciences (SLU),

Charles Midega

International Centre for Insect Physiology and Ecology (ICIPE)

Francis Magingo

University of Dar-es-Salaam (UDSM)

Beatrix Alsanius

Swedish University of Agricultural Sciences (SLU)

Teun Dekker

Swedish University of Agricultural Sciences (SLU)

Sylvester Leonard Lyantagaye

University of Dar es Salaam Mbeya College of Health and Allied Sciences (UDSM-MCHAS)

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Abstract

Purpose: Push-pull is an intercropping technology that rapidly spreads among Sub-Saharan smallholder farmers. It intercroops maize with *Desmodium* to fight off stem borers, eliminate parasitic weeds, and improve soil fertility and yields. The above-ground components of push-pull cropping have been well investigated. However, impact on and from the soil microbiome and its role in diverse ecosystem benefits are unknown. Here we describe the soil microbiome associated with push-pull and compare it with maize monoculture.

Methods: Soil samples from long-term maize-desmodium intercropping and maize monoculture plots were analysed using 16S and ITS metagenomics.

Results: Maize-desmodium intercropping caused a strong divergence in fungal microbiome, which was more diverse and species rich than monoculture plots. Zooming into genera revealed that intercropping enhanced fungal genera linked to important ecosystem services. These include mycorrhizal and endophytic groups such as *Edenia*, *Acrocalyma* and *Colletotrichum*, saprophytic and decomposer fungi like *Pithya* and *Cristinia* and fungi with biocontrol properties, for instance, *Talaromyces*, *Penicillin*, *Clonostachys* and *Trichoderma*. Fungal genera enriched in monoculture plots were few, and were functionally linked to plant diseases (for example *Didymella*, *Curvularia* and *Parastagonospora*), and human pathogenic taxa (*Exserohilum*, *Curvularia* and *Aspergillus*). Although separating well, bacterial microbiomes did not, except in a few genera, differ between treatments.

Conclusion: Maize-desmodium intercropping diversifies fungal microbiomes and favors taxa that are associated with important ecosystem services, including plant health, productivity and food safety. Further studies should increase the resolution of shifts noted above, experimentally ascertain the inferred functions, and translate this knowledge to improve cropping systems.

Introduction

In this study, push-pull technology was used as a model for understanding interactions between plants and soil microbes in intercropping farming systems, and the potential impacts of the soil microbiome on plant health and productivity. Push-pull technology is an ecological habitat management strategy for the control of major pests of cereals, particularly maize and sorghum. Since its inception in the 90's, the technology has spread to smallholder farmers across southern and eastern Africa who use the technology to manage stem borers and fall armyworms attack on cereal crops thus increasing yield (Midega et al. 2018). The technology exploits the chemical ecology of leguminous intercrops belonging to the genus *Desmodium* and grass trap crops such as Napier or *Brachiaria spp.* The intercrop "pushes" stem-boring insects from the main crop through release of volatile compounds that signal an unfavourable egg-laying environment. At the same time, the trap crop "pulls" the insects towards itself but does not support their development (Khan et al. 2003; Khan et al. 2010). Both *Desmodium* and trap crops are a reliable source of animal fodder particularly in drought periods as farmers do not uproot them

between farming seasons (Khan et al. 2010). The technology is effective and importantly, affordable for smallholder farmers in Sub-Saharan Africa.

However, over the years several additional benefits of push-pull cropping were uncovered. These indicate diverse soil-based mechanisms and warrant further study. It was found that *Desmodium* employs allelopathic mechanisms that dramatically reduce infestation of the parasitic weed *Striga hermonthica* to cereal crops, further adding to yield increases (Khan et al. 2002). Moreover, push-pull technology provides other ecological benefits contributing to improved cereal crops yield, including nitrogen fixation by *Desmodium*, soil structure improvement and mulching effect in the fields. Finally, push-pull cropping reportedly reduced the incidence of human pathogenic fungal toxins in maize kernels (Njeru et al. 2020; Owuor et al. 2018).

Whereas many components of the push-pull farming technology and their underlying mechanisms have been investigated, one of the areas that has received no attention is the impact of push-pull cropping systems on the soil microbiome and vice-versa. Soil microorganisms are known to promote plant health and productivity through direct and indirect mechanisms mediated through root systems (van der Heijden et al. 2008). Therefore, it is of interest to explore the contribution of soil microorganisms on the effectiveness of the push-pull system.

A better understanding of soil microbial diversity in intercropping agricultural practices is necessary for optimal exploitation of their potential ecosystem benefits in plant health and productivity. Eventually, beneficial plant growth and protection activities of soil microorganisms may be incorporated into sustainable food production, offering perspectives on reducing the reliance on and environmental impacts of agrochemical inputs. The first step towards this understanding is to characterize the microbial diversity and how this has evolved through changing from monoculture to polyculture cropping.

Microbiome studies are increasingly used to discern potential impacts of farming practices such as intercropping on structure and diversity of soil microbiota, in addition to other benefits, such as increase in yield and mineral nutrients availability. A study by Debenport et al. (2015) found an increase in diversity of soil bacterial and fungal communities around the millet rhizosphere when intercropped with woody shrubs compared to monocultures. In another study, an increase in yield as well as overall diversity of soil bacteria was also observed in maize-peanut intercropping systems. In addition, the abundance of beneficial soil bacteria was higher in intercropping systems with complete and semi-below ground interactions compared to monoculture systems (Li et al. 2018). Tang et al. (2020) observed variations in availability of soil minerals and enzymes as a result of intercropping with either peanut or cassava. In the same study, certain bacterial groups including *Nitrospirae* increased in abundance, and others were associated with improved soil quality.

The current study compared the broad diversity of soil microorganisms between long-term maize-desmodium intercropping (push-pull system without the trap crop) and maize monoculture plots. Specifically, 16S and ITS amplicon sequencing were used to investigate the differences in the soil bacterial and fungal population structures between long-term maize-desmodium intercropping and maize

monoculture practices in a context of potential ecological benefits. The mapping of the microbiomes of these maize monoculture and intercropping plots demonstrated that particularly the fungal microbiome was highly diversified in maize-desmodium intercropping plots compared to maize monoculture plots. The results are discussed in the context of reported 'benefits' around maize-desmodium intercropping systems, and known ecological functions of taxa contributing to the observed difference have important ecological functions. Further studies are recommended to discern key determinants of the observed differences, their importance in ecosystem (dis)services. Knowledge and translation of this knowledge into other cropping systems could advance sustainable food production through fostering belowground microbial communities that support plant health and productivity.

Methodology

Sampling site

To compare soil microbial profiles between maize-desmodium intercropping and monoculture maize farming, we obtained soil samples from long-term experimental plots at the International Centre for Insect Physiology and Ecology (ICIPE), Mbita campus, Kenya (0°25.877 S 34°12.425 E).

Soil samples

Soil samples were collected from three long-term experimental plots that have been continuously maintained as pairs of maize-desmodium intercropping and maize monoculture each since 1998, 1999 or 2003. We collected three samples from maize-desmodium intercropping plots established each year and four samples from each monoculture plot.

For each individual sample, sampling was done by randomly taking three 15–18 cm deep cores across the plot between rows of maize as close to *Desmodium spp.* root system as possible. Afterwards, each soil sample was homogenized and sieved through a 4 mm wire mesh. About 200 g soil sub-sample was collected and stored at -20 °C until further processing.

DNA extraction and sequencing

DNeasy Powersoil kit (Qiagen, Manchester, UK) was used for total DNA extraction from the soil samples following the manufacturer's protocol. Briefly, 0.25 g soil was added to PowerBead Tubes containing a lysis buffer and vortexed for a few seconds. The resulting mixture was centrifuged at 10,000 g for 30 s before discarding the pellet and centrifugation of the supernatant in spin columns. Tris-HCl solution was used to wash off DNA from the spin column. Nanodrop spectrophotometer and gel electrophoresis were used to assess the quality of the extracted DNA. The DNA samples were stored at -20 °C until further processing.

DNA sequencing was done at Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa). The primers targeting V1-V3 of the 16S rDNA gene of the bacteria (27F and 518R primer pairs) were used to amplify DNA under the following PCR conditions: initial denaturation at 95 °C for 2 minutes, followed by

30 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final elongation at 72 °C for 5 minutes. For fungi, ITS1F and ITS2 primer pairs targeting the ITS1 region was used for PCR amplification under the following conditions: 95 °C for 2 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 50 °C for 30 seconds, and extension at 72 °C for 1 minute. Final elongation was held at 72 °C for 5 minutes.

Resulting amplicons were gel purified, end repaired and illumina specific adapter sequence were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). Following quantification, the samples were individually indexed (NEBNext Multiplex Oligos for illumina Dual Index Primers Set 1), and another AMPure XP bead based purification step was performed. Amplicons were then sequenced on Illumina's MiSeq platform, using a MiSeq v3 kit with 600 cycles (300 cycles for each paired read and 12 cycles for the barcode sequence) according to the manufacturer's instructions. Demultiplexed 300 bp paired-end reads were obtained.

Bioinformatics and statistical analysis

FASTQC (Wingett and Andrews 2018) was used to assess the quality of raw sequence reads. Then, QIIME2 v2020.8 suit of packages was used for quality control, construction of a feature table, taxonomic classification and diversity analyses (Bolyen et al. 2019). Briefly, the dada2 plugin (Callahan et al. 2016) was used to trim and truncate poor regions of both the 16S and ITS raw reads. The truncation and trimming was set to `-p-trim-left-f 8, -p-trim-left-r 8`; and `-p-trunc-len -f 290, -p-trunc-len-r 260`, for the 16S; while for the ITS, parameters used were `p-trim-left 22, -p-trunc-len 299`. Bacterial taxonomic assignment was done using feature-classifier `classify-sklearn` (Bokulich et al. 2018; Pedregosa et al. 2011), only including reference genes that was classified to at least genus level, by using SILVA v.138 99% database (Quast et al. 2013) pre-trained to V1-V3 region of the 16S. For the ITS, we used UNITE v8.2 reference database (Nilsson et al. 2019) pre-trained to ITS1.

Further, the feature table was converted into biom format (using qiime 2 export tool), and then imported into R (R Core Team 2020) using "qiime2R" (Bisanz 2018). For visualising the number of ASV, genera, families and orders present in the dataset we filtered out everything that was present only once at each level and then Venn diagrams were produced using function `vennCounts` from package "limma" (Ritchie et al. 2015). Then, everything that was unassigned at family level was filtered out. Genera that were present in either all samples, in one treatment from one area or were present in more than 60% in the samples from either maize or maize-desmodium intercropping were kept.

For constructing dendrograms, primary component analysis (PCA) and heatmaps data was transformed using CSS (cumulative sum scaling) by using a package "metagenomeseq" (Paulson et al. 2013). To perform a principal component analysis (PCA), we used package "recipes" (Kuhn and Wickham 2020), and annotated ellipses using a Khachiyan algorithm from package "ggforce" (Pedersen 2020). Dendrograms were constructed using a jaccard index from package "vegan" (Oksanen et al. 2020) with a presence absence standardization and plotted using "ggtree" (Guangchuang et al. 2017).

Species diversity (Shannon) and richness (chao1) were calculated on untransformed and unfiltered data using “vegan” through the package “phyloseq” (McMurdie and Holmes 2013), while evenness was calculated as the Shannon index divided by the natural logarithm of the total number of species. All indices were tested for significance using a Student’s t-test.

Differential expression analysis was done on untransformed but filtered data based on a negative binomial distribution through “DESeq2” (Love et al. 2014). The resulting log₂fold changes were shrunken using the adaptive shrinkage estimator from package “ashr” (Stephens et al. 2020). Genera were deemed to significantly impact treatments if they had an adjusted p-value smaller than 0.05 (Wald test), and an absolute log₂fold change of over one, which was then visualised on a volcano plot modified from package “EnhancedVolcano” (Blighe et al. 2020). The result from the differential expression analysis also was used to group data in the heatmap and label significant genera in the PCA. All data was manipulated using “tidyverse” (Wickham et al. 2019) and visualised using “ggplot2” (Wickham 2016).

Results

Below we describe some of the major differences found between plots that have been under long-term maize-desmodium intercropping or monoculture management. The results outline the differences in composition and abundance of bacterial and fungal taxa, measures the diversity indices within and between plots and treatments, assesses the degree of separation, and highlights differences in taxa with known significance in plant and human health. In addition to the data presented here, more detailed information is deposited online.

Composition and abundance of soil microbiome in maize-desmodium intercropping and monoculture plots

A total of 1085 fungal ASVs distributed across 225 fungal genera were identified from mono-culture plots, whereas 1262 ASVs from 284 genera were identified in maize-desmodium intercropping plots. For bacteria, a total of 1934 ASVs were identified from monoculture plots and 1333 ASVs from maize-desmodium intercropping plots, distributed across 195 and 162 genera, respectively. Figure 1 shows the Venn diagrams of unique and common taxonomic units in maize-desmodium intercropping and monoculture plots. ASVs and genera that were present only in single samples were excluded from the Venn diagram. Maize-desmodium intercropping plots had a more diverse representation of fungal genera compared to maize monoculture plots (see also diversity indices, below).

In Fig. 2, the relative abundances of soil bacterial and fungal genera in both maize-desmodium intercropping and monoculture plots were plotted across the thirty most abundant genera. Not all bacteria were fully classified to genus level (Fig. 2A), whereas almost all fungi were classified to genus (Fig. 2B). Noteworthy is that several fungal genera, even though abundant to both cropping practices, significantly differed between the maize monoculture and push pull systems. These include *Aspergillus* (known human pathogens overrepresented in maize monoculture), *Didymella* (plant pathogens), *Edenia* (known

beneficial genera overrepresented in maize-desmodium intercropping), *Curvularia* (plant pathogens), *Albifimbria* (saprophytic), *Glomus* (mycorrhizal), *Parastagonospora* (genus of many plant pathogens), and *Achroiostachys*.

The most abundant bacterial phyla were Acidobacteri, Actinobacteria, Chloroflexi, Proteobacteria, and Planctomycetes, whereas Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Mortierellomycota were the most abundant fungal phyla.

Diversity of soil microbiome between maize-desmodium intercropping and monoculture plots (alpha diversity)

No significant difference in diversity of soil bacteria genera was observed between monoculture and maize-desmodium intercropping plots ($p = 0.246$). In contrast, fungal genera were more diverse in maize-desmodium intercropping plots compared to monoculture ($p = 0.047$). Similarly, bacterial genera did not differ in richness ($p = 0.238$) and evenness ($p = 0.206$) between monoculture and maize-desmodium intercropping plots, whereas fungal general had significantly higher richness in maize-desmodium intercropping plots ($p = 0.012$). The evenness of fungal genera in maize-desmodium intercropping plots was not significantly different from that of monoculture plots ($p = 0.386$).

Diversity of soil microbiome between maize-desmodium intercropping and monoculture plots (beta diversity)

Figure 4 shows the impact of long-term maize-desmodium intercropping farming practice on composition of soil microbes at the genus level, which shows separation of bacterial and fungal profiles based on treatment (maize monoculture and maize-desmodium intercropping). Generally, bacterial communities did not separate the treatments strongly (Fig. 4A). In contrast, the effect of the cropping system on fungal microbial communities more strongly separated maize-desmodium intercropping and monoculture plots along the first principal component (Fig. 4B). It is notable that in addition to separation by treatment, plots from the same treatment separated out, particularly on PC2, albeit explaining roughly 10 % of the variation.

Differential abundance of soil microorganisms across maize-desmodium intercropping and monoculture plots

Several fungal and bacterial genera showed varying abundances between maize-desmodium intercropping and monoculture plots and some occurred in one farming practice only. Figure 5 shows a heatmap plot of the abundance of bacterial (A) and fungal (B) genera both common and unique to each farming practice. The figure shows that fungal genera were relatively richly represented than fungal genera and were significantly higher in maize-desmodium intercropping plots than in maize monoculture plots (Fig. 5B), whereas there was a little differential expression levels of bacterial genera between the

two farming practises (Fig. 5A). The plots separated largely by treatment (dendrogram, left hand panel of each heatmap plot).

More detailed analysis shows the differences in abundance at genus level (Fig. 6, supplementary tables 1 and 2). As can be inferred from the heatmap plot (Fig. 5A) and further reinforced here, fewer bacterial genera exhibited a differential abundance between maize-desmodium intercropping (push-pull) (1 genus) or monoculture plots (4 genera, Fig. 6A), whereas a larger number of fungal genera (52 genera) were significantly more abundant in maize-desmodium intercropping (push-pull) plots compared to monoculture plots (16 genera, Fig. 6B).

In maize-desmodium intercropping plots, fungal genera that were significantly more abundant represent a diversity of functional groups of which species have been described as saprophytic (most notably *Pithya*, *Albifimbria*, *Acremonium*, *Pseudorobillarda* and many less common taxa e.g. *Cristinia*), mycorrhizal and endophytic (most notably *Edenia*, *Acrocalyma* and *Colletotrichum*), and having biocontrol functions such as antifungal, antibacterial and/or nematicidal effects (most abundantly *Edenia*, *Talaromyces*, *Penicillin*, *Clonostachys* and *Trichoderma*). A few pathogenic genera were also enriched (most notably *Mycocleptodiscus*, a pathogen of legumes).

In contrast, maize monoculture plots were enriched in many genera with described plant pathogenic (*Didymella*, *Curvularia*, *Parastagonospora*, *Tetracladium*) or human pathogenic effects (*Aspergillus*, *Curvularia*, *Exserohyllum*), and only a few with known beneficial effects (most abundantly mycorrhizal genus *Glomus*, and endophytic *Laetisaria*).

Discussion

Intercropping is increasingly studied to provide sustainable alternatives to monoculture production systems. Intercropping provides diverse ecosystem services, some of which are immediate and pronounced (such as productivity, pollinator support, pest and disease reduction, nitrogen fixation (Bybee-Finley and Ryan 2018; Nourbakhsh et al. 2019), whereas others are acquired over a longer time. Effects on and impact of the soil microbiome fall in the latter category and have been, in part for that reason, little studied. This particularly holds true for cereal crops, as they are frequently cropped annually and part of a rotational cropping scheme, which hampers long-term studies.

The maize-desmodium push-pull intercropping system, originally developed to reduce stem borer impacts (Khan et al. 2006), has evidently very pronounced below-ground benefits, including control of the parasitic weed striga, increase in soil carbon and fertility through perenniation and nitrogen fixation (Khan et al. 2016; Midega et al. 2018), and a lower incidence of mycotoxin-producing fungal infection (Owuor et al. 2018; Njeru et al. 2020). By virtue of the long-term cropping plots managed by ICIPE, long-term push-pull and monoculture plots (more than 14 years old) are exquisitely suited for studies on long-term effects of legume intercropping on soil microbiomes.

In the current study, we demonstrate substantial qualitative and quantitative effects of long-term maize monoculture and maize-desmodium intercropping on structure and diversity of soil microbial communities. Below, we elaborate on the differences in bacterial and fungal microbiomes associated with these plots, and elaborate on differences of selected taxa with a known functional significance as plant symbionts or pathogens, or human pathogens.

Diversity of cropping systems links to diversity in soil microbiome in maize-desmodium intercropping

Diversifying cropping systems, often using legumes as intercrop, may have been devised originally for purposes other than increasing biodiversity, such as food security, pest control (push-pull), green manure, or to avoid negative plant-soil feedback and soil legacy (Stagnari et al. 2017). However, ripple effects on biodiversity and other ecosystem services are apparent. Maize-desmodium push-pull intercropping is a good example of this. Initially designed to combat stemborers, it gradually revealed a range of additional ecosystem services, including combating parasitic weeds of cereals, increase soil nitrogen and carbon, and reducing incidence of mycotoxins in maize (Balaso et al. 2019; Cook et al. 2007; Xu et al. 2018). Here we add to these benefits a diversification of the soil microbial communities, with a particularly strong shift in the composition of fungal taxa, whose diversity was significantly higher in maize-desmodium intercropping than monoculture plots. By itself diversity in ecosystems is generally regarded as increasing stability, resilience and productivity (Prieto et al. 2015), mostly as a consequence of resource complementarity and functional redundancy (Cleland 2011; Rosenfeld 2002).

Whereas a perennial crop, such as *Desmodium*, could have shaped the rhizosphere and reduced biodiversity, intercropping may also lead to niche diversification in the rhizosphere and thereby increase biodiversity. Generally, research supports the later, i.e., an increased diversity of soil microorganisms in intercropping systems, particularly fungal microbes. For example, in wheat-soybean intercropping (Bargaz et al. 2017), maize/wheat-faba bean intercropping (Wang et al. 2020), millet-mung bean intercropping (Dang et al. 2020), and cucumber-cereal intercropping (Li and Wu 2018). Tang et al. (2020), observed a higher diversity of both bacterial and fungal species in cassava-peanut intercropping, but also peanut monoculture, suggesting that the leguminous peanut intercrop was responsible for the observed higher soil microbial diversity, a factor we could not correct for in our study. Similarly, Gao et al. (2019) observed that peanut monoculture plots had the highest richness and abundance of soil bacteria compared to intercropping tobacco after two and four years. These observations, including the ones we made in the current study, indicate that the impact of legume intercropping on soil microbial communities is complex and system dependent. Regardless of the biodiversity, more critical is the functional biodiversity in terms of plant health and food safety, details of which we discuss below.

Differential abundance of taxa and their potential functional significance

Whereas in general many fungal taxa abundant in maize-desmodium intercropping plots appear to fulfill a saprophytic role (for example *Arthrographis*, *Malbranchea*, *Lepiota*, *Cristinia* and *Pleurophragmium* (supplementary Table II)), and thus may indicate higher carbon sequestration associated with the perenniation in these plots, other enriched fungal genera likely confer more distinct ecosystem services, such as *Edenia*, *Talaromyces*, *Clonostachys*, *Trichoderma*, *Penicillium* and *Colletotrichum* (supplementary Table II). Species belonging to these genera can form mycorrhizal and endophytic associations with plant roots that promote plant growth activities through increased nutrient supply, and/or directly or indirectly stimulate production of beneficial secondary metabolites and protection against pathogens and insect pests (Díaz-González et al. 2020; Hiruma et al. 2016; Macías-Rubalcava et al. 2008; Munasinghe et al. 2017; Ravnskov et al. 2006; Zin and Badaluddin 2020). Indeed, effects can be indirect and intricate: *T. atroviride* promoted growth and herbivory resistance of maize against *Spodoptera frugiperda*, possibly linked to induction of the jasmonic acid pathway leading to heightened induced defence (Contreras-Cornejo et al. 2018). Similarly indirect, soil microbe composition and diversity was positively correlated to diversity and productivity of plants above ground in most systems (Schnitzer et al. 2011), which in turn would favor above ground biodiversity and biological control (Winqvist et al. 2011).

Although we do not know to what extent these direct and indirect interactions are functionally relevant in the push-pull systems, it is reasonable to assume that below ground intricacies do contribute to ecosystem services observed in push-pull and maize-desmodium intercropping systems, including above ground tritrophic interactions. For instance, in a recent study it was shown that maize grown on soil from push-pull plots showed a higher induced-defence response, including higher release of induced volatiles and lower herbivore damage (Mutymbai et al. 2019). Of particular notice in this context is the higher abundance of *Edenia* and *Clonostachys* species, which are associated with increased plant health, biocontrol and increased resistance against herbivore damage (Iqbal et al. 2018; Macías-Rubalcava et al. 2008; Poveda et al. 2020).

Recent papers reported lower incidences of maize ear rot and associated mycotoxins (aflatoxins and fumonisins) (Owuor et al. 2018) as well as lower rate of infection of maize kernels with *Fusarium verticillioides* and *Aspergillus flavus* (Njeru et al. 2020) in smallholder farmers' push-pull plots compared to monoculture. Push-pull thus appears to promote food safety by reducing the risk of mycotoxins entering the human food chain, although the mechanisms remained unclear. We therefore were particularly keen on assessing the abundance of these fungal genera in soil samples of monoculture and maize-desmodium intercropping plots. Interestingly, a lower relative abundance of *A. flavus* was indeed associated with maize-desmodium intercropping cropping, whereas no association was found for *F. verticillioides*. The lower incidence of these ear rot infections thus may partially be explained by the abundance of the species in the soil. Additional factors, for instance related to the observed high fungal biodiversity, may further contribute to the reported lower mycotoxin incidence in push-pull plots. These may include fungal competition or biocontrol such as has been reported for *Edenia*, *Myriococcum*, *Humicola*, *Penicillium*, *Clonostachys*, and *Trichoderma*, which are enriched in maize-desmodium intercropping plots. Other studies have reported on the role of both bacteria (Venkatesh and Keller 2019) and fungal competition (Sarocco et al. 2019; Wang et al. 2020) in reducing mycotoxins production.

Further research on how mycotoxin incidence in maize kernels can be reduced by interactions between mycotoxin producing fungi and other soil microbes in maize-desmodium intercropping, would help in devising strategies to increase food safety through more healthy plant production systems.

Although the overall diversity of bacterial genera was not significantly different between the two farming practices, several genera were significantly abundant in maize-desmodium intercropping plots compared to monoculture. Most of them were unclassified, however, among those classified is a nitrogen-fixing soil bacteria genus *Nitrospira spp* (supplementary table I) (Koch et al. 2015). Enriched presence of *Nitrospira spp*. in maize-desmodium intercropping plots suggests involvement in nitrogen fixation, potentially through symbiosis with *Desmodium spp*, and thus an increased supply in Nitrogen that benefits maize plants in maize-desmodium intercropping plots.

Concluding remarks

This study evaluated the microbiome associated with maize-desmodium intercropping systems in order to understand its contribution to the well-described ecosystem services of these cropping systems. Understanding the complex interactions between plants, soil, microbiome and cropping systems is key in harnessing the benefits that are rendered to plant health and productivity. Whereas in some instances single genera and species appear to play key roles in protecting plants or promoting plant growth (Reva et al. 2019), many functions may arise through complex interactions within very diverse soil microbial communities, which give rise to emergent ecosystem properties that may not be readily assigned to functional roles of single taxa.

Intercropping systems, particularly those involving leguminous perennial components, promote complementarity in farming systems that facilitate effective land use in agriculture (Duchene et al. 2017). The various knock-on ecosystem effects of these polyculture systems have perhaps most clearly been described for maize-desmodium intercropping (Midega et al. 2010). Hitherto, the impacts on and contributions of the microbiome have, however, received little attention. We show significant interactions between maize-desmodium intercropping and the soil microbiome, with apparent ecosystem services in terms of diversity, production, plant health and food safety. Given the increasing availability of sequencing tools, we suggest that research on intercropping studies for improvement of crop health and productivity more integrally include metagenomics to assess the impact on and effects of the microbiome on plant productivity, plant health, and more broadly ecosystem health.

This study contributes to the extensive literature around push-pull technology through describing the bacterial and fungal microbiome associated with long-term monoculture and maize-desmodium intercropping cropping, and linking above ground benefits to below-ground microbial communities. It is a first step that should be further complemented with higher resolution analyses such as whole genome sequencing (WGS), to provide a finer-grained overview of functional groups of soil microorganisms. We further hope that future studies will experimentally dissect the contribution of the components of the microbiome identified here, in providing the diverse benefits reported (Midega et al. 2018), such that push-pull cropping techniques can be further improved, and translated to other cropping systems.

Declarations

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Consent for publication: NA

Availability of data and material: The datasets generated during and analysed during the current study are available in the Genbank Sequence Read Archive (SRA) repository under project number PRJNA667690 linked here <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA667690/>.

Authors' contributions

Conceptualization and advice: TD, SL, ADM; Methodology and advice: TD, SL, ADM, BA, CM, FM; Formal analysis and investigation: TD, SL, ADM, KSM, SLH; Writing - original draft preparation: ADM, KSM, TD Writing - review and editing: all authors. Funding acquisition: TD, SL, FM Supervision: TD, SL, FM, CM, BA. All authors approved the submitted version for publication.

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Figures

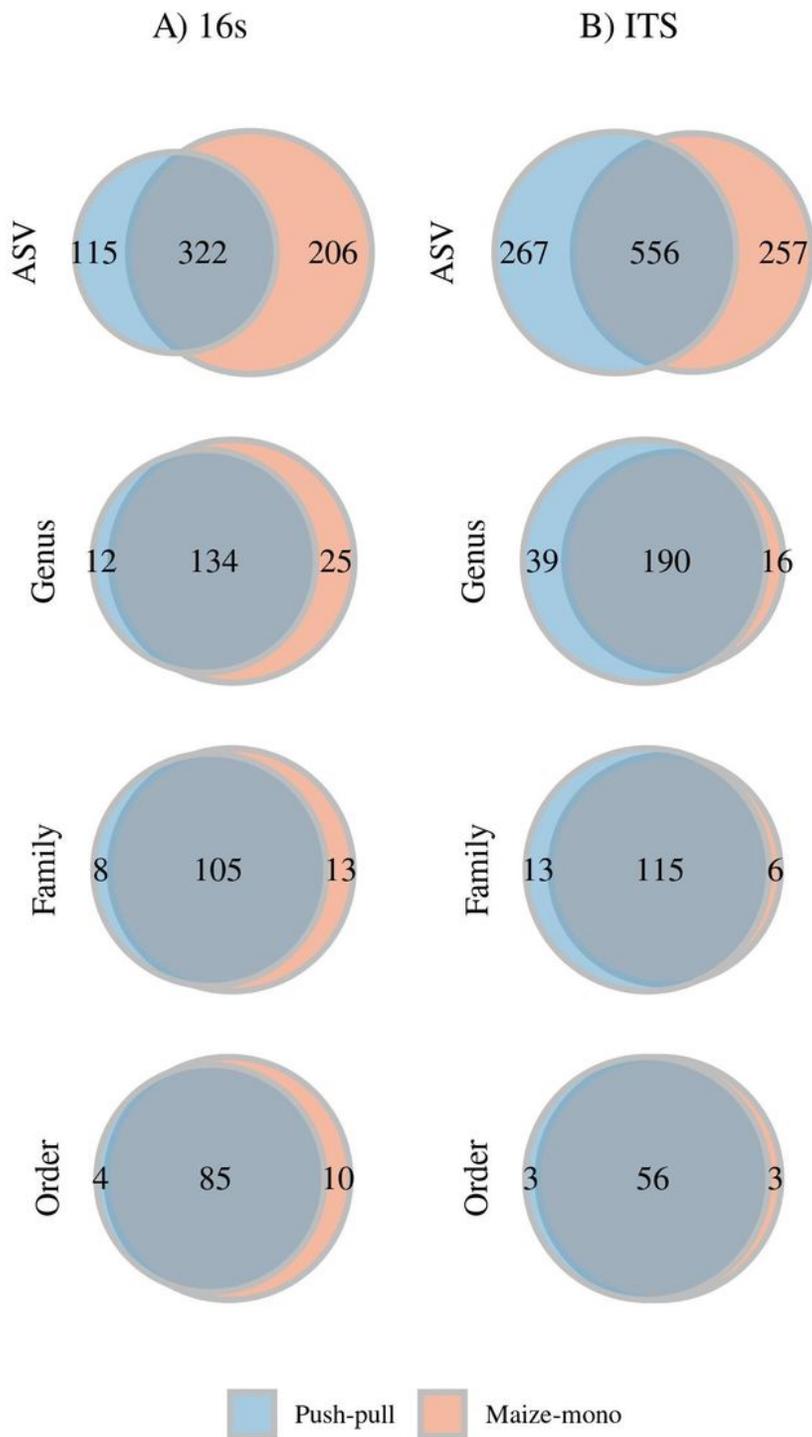


Figure 1

Venn diagrams showing unique and common taxonomic units (ASVs, genera, families and order) for A) bacteria and B) fungi in maize-desmodium intercropping (push-pull) and maize monoculture plots. Only groups that were found more than once in the dataset were included

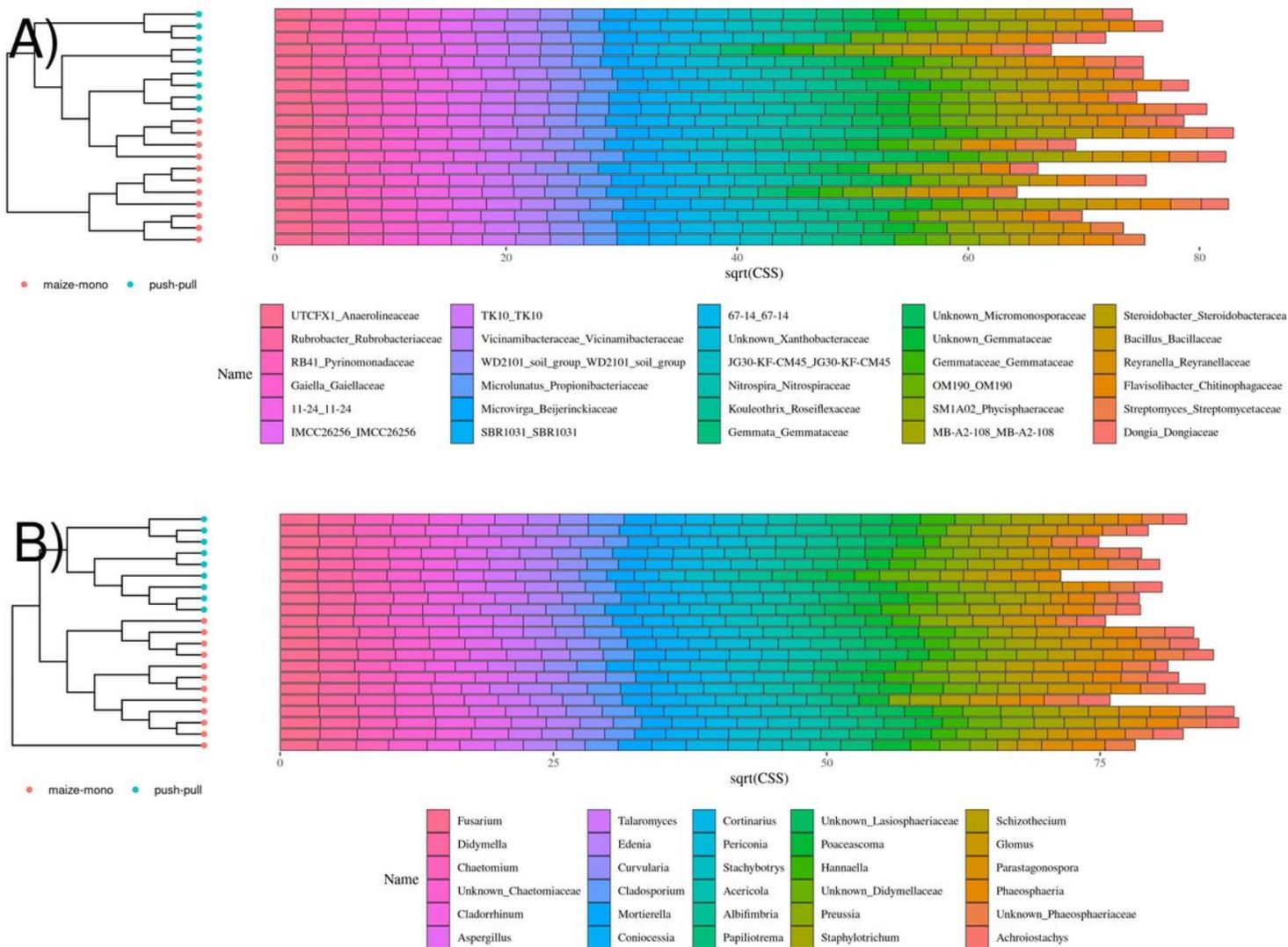
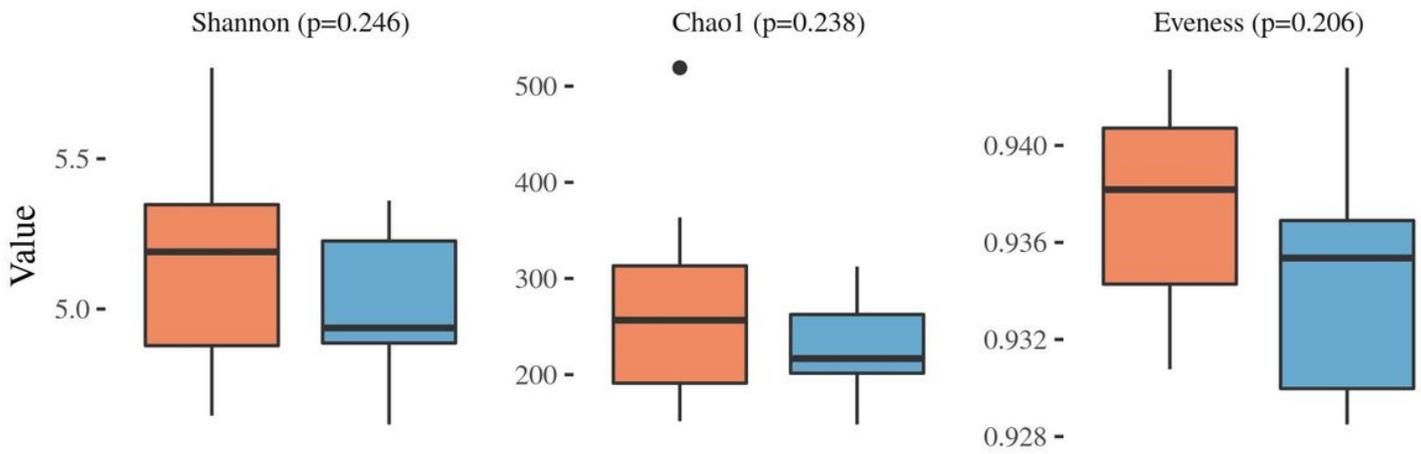


Figure 2

Bar plots showing relative abundance of soil bacterial (A) and fungal (B) genera in maize-desmodium intercropping (push-pull) and maize monoculture plots. Presented on the left are distances between samples calculated using a Jaccard dissimilarity index and presented as a dendrogram

A) 16s



B) ITS

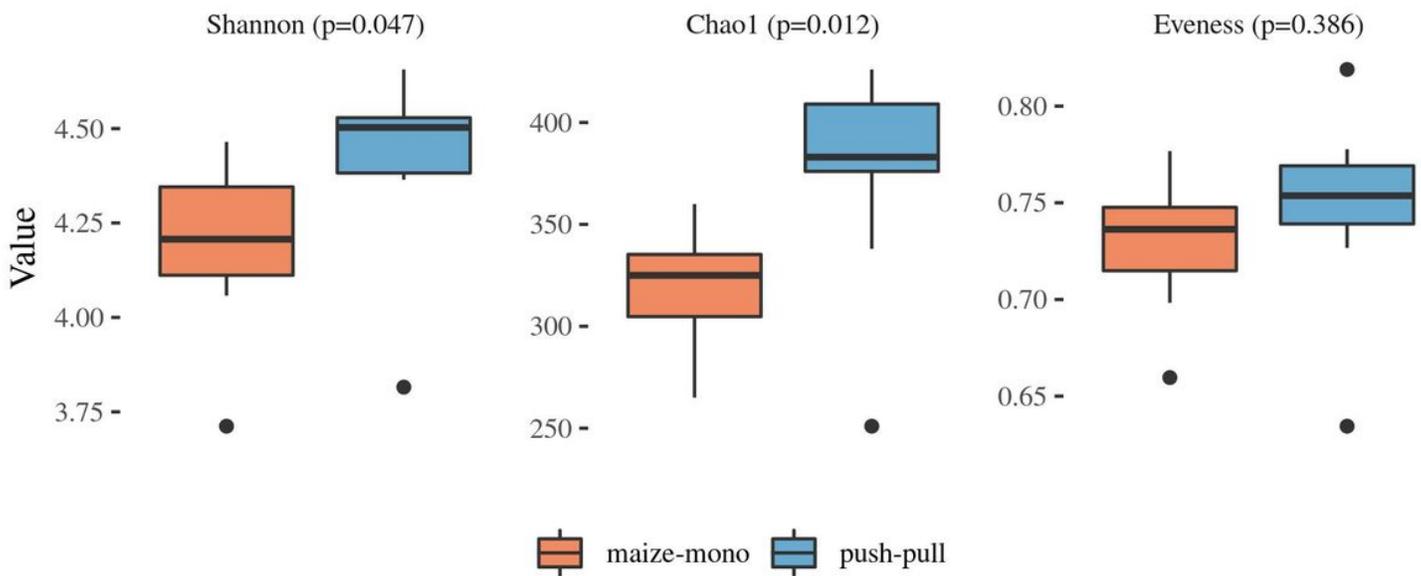


Figure 3

The diversity index (shannon), richness (chao1) and evenness (shannon/ \ln [number of species]) of bacterial (A) and fungal (B) genera across monoculture and maize-desmodium intercropping (push-pull) plots and p-values (Student's t-test)

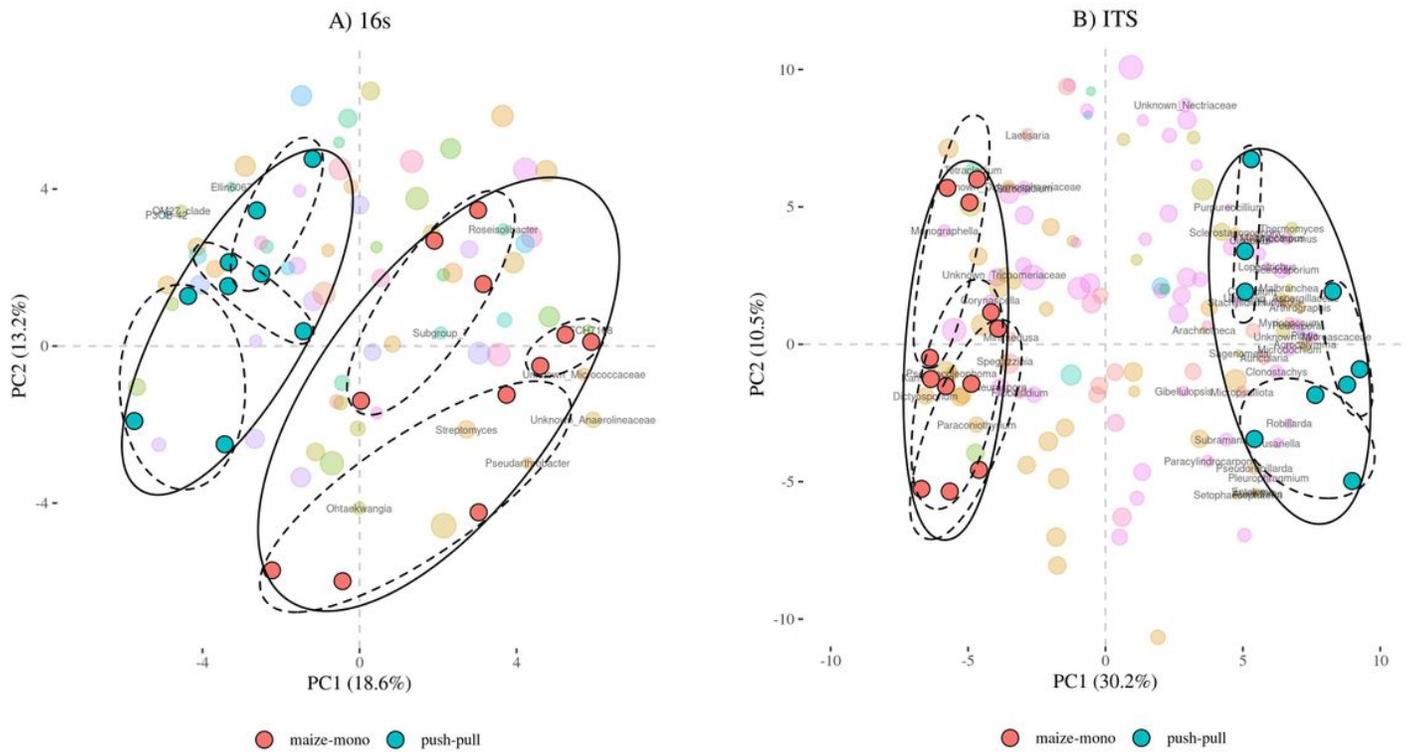


Figure 4

Principal coordinate analysis of bacterial (A) and fungal genera (B) across maize monoculture compared to maize-desmodium intercropping (push-pull). Solid ellipses around each treatment were drawn using the Khachiyan algorithm. Dotted ellipses represent samples from the same area. In the background are the eigenvectors scaled up and colored according to family

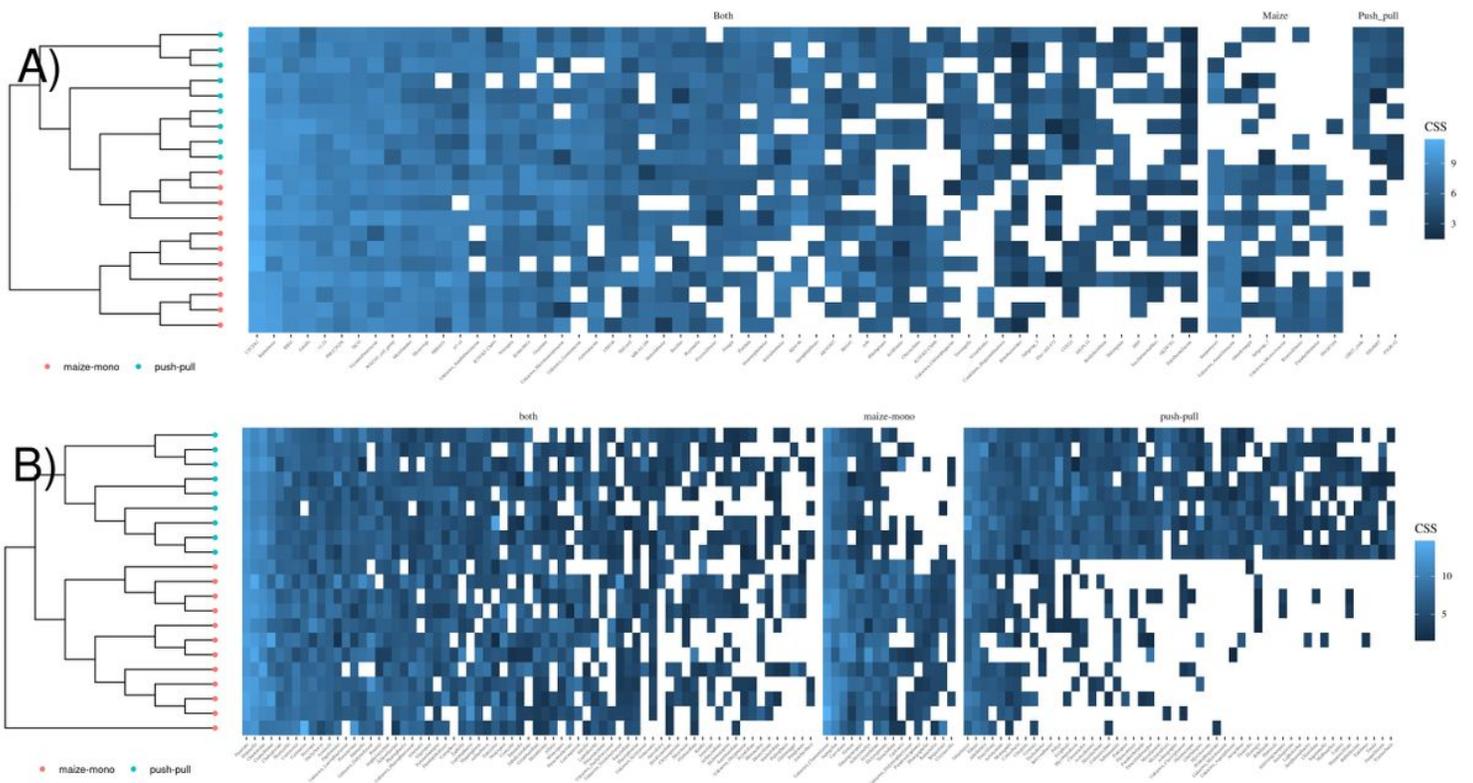


Figure 5

Heatmap showing normalized CSS (cumulative sum scaling) abundance across genera of bacteria 16s (top) and fungal ITS (bottom) in monoculture and maize-desmodium intercropping (push-pull) plots. Each horizontal line represents a different plot. The data are horizontally grouped by 'shared', 'maize' (monoculture) and 'push-pull' and within these from left-to-right by decreasing summed abundance. The dendrogram on the left is produced using a Jaccard dissimilarity index, with a presence-absence standardization. Colors indicate treatments with red representing monoculture and blue push-pull (maize-desmodium intercropping)

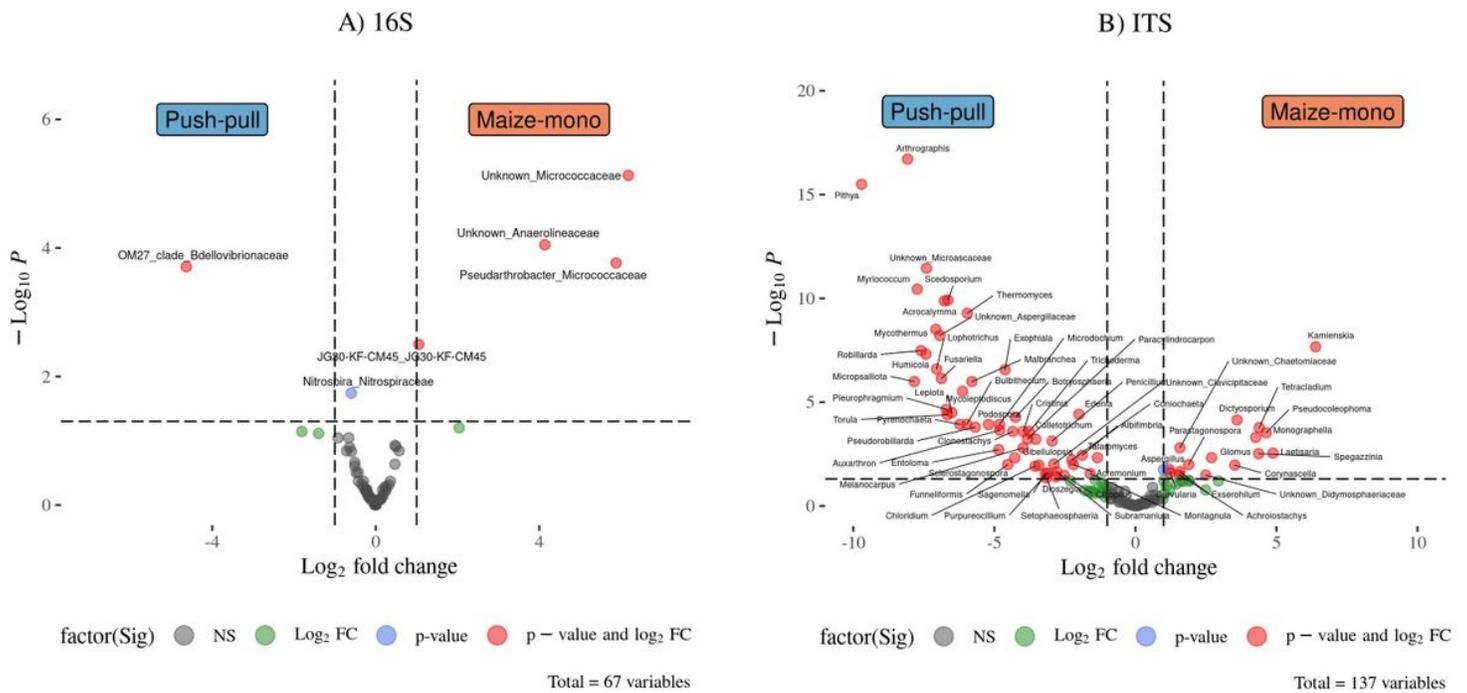


Figure 6

Volcano plots showing bacterial (left) and fungal (right) genus level features that are differentially abundant in maize-desmodium intercropping (push-pull) and monoculture plots. Genus fold changes are shown on X-axis and the negative logarithm (base 10) of the p-value resulting from a Wald test on a generalized linear model using a differential negative binomial analysis on the Y-axis. Red dots represent genus entities that are significantly abundant in each group and with an absolute log₂ fold change over 1, with genera on the negative side of X-axis being significantly abundant in maize-desmodium intercropping (push-pull) plots. The grey and green dots represent the genus features whose abundance is similar between the two farming systems and the blue dots represents values where the p-value is significant between the treatments but where the log₂ fold change is smaller than one

Supplementary Files

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- [Supplementarymaterials.docx](#)