Determining Management-related Factors that Impact the Severity and Incidence of Soybean Taproot Decline (TRD) (Project 15-2021)

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Background

Soybean taproot decline (TRD), an important soilborne root disease, is shaping up to become one of the major diseases facing soybean producers in Mississippi, and beyond. TRD has been increasing in Mississippi since the first reports more than 12 years ago but yield losses and economic damages have yet to be accurately quantified. The causal agent of TRD is identified recently as *Xylaria necrophora*, and vast majority of the *Xylaria* species are saprophytic fungi (decomposers), which is consistent with anecdotal observations linking TRD and prior soybean crop, or other residues and reduced tillage systems. The composition and function of these microbial assemblages can be altered by agronomic practices in association with key soil characteristics. For instance, tillage, residue management, and cover crops have been shown to promote residue decomposition by increasing the population and efficiency of decomposers and improving aeration by promoting soil aggregation. Relatedly, keystone microbial taxa that exploit the same resources as *Xylaria* spp. can potentially exert considerable antagonistic influence on TRD. With an increased understanding of the relationships involved, there is an opportunity for increased field suppression due to more informed selection of management practices and scientific monitoring of the results. Our aim is to understand how management practices in Mississippi soybean production systems (e.g., early-planting, precision seed placement, irrigation systems, residue management) relate to the occurrence of TRD in order to verify the effects of altering management practices, and to determine the potential for enhancing the disease suppressive ability of these endemic soils.

Objectives

1. Survey of fields for TRD incidence and severity as well as distribution within agricultural fields that differ in farming system classification.

2. Evaluation of the ecology of *Xylaria* spp. based on pathogen population in soil and microbiome structure and function.

Report of Progress/Activity

Objective 1. Survey of fields for TRD incidence and severity as well as distribution within agricultural fields that differ in farming system classification.

Field survey and TRD distribution in the Mississippi Delta region

A questionnaire-based field survey of soybean producer farms was conducted and assessed for the intensity of TRD (*Xylaria necrophora)* using a systematic rating system developed based on the percent of infected plants along 10 linear feet of row (0% = asymptomatic, 1-25% = light, 26-50% = moderate, and > 50% = severe). 158 survey transects and a total of 20 soybean fields across the Mississippi delta farms were exploited to study the rate of TRD severity and distribution. Each field was divided into quadrants for assessment (Fig 1). Surveys were completed within two observation areas (10 m radius) per quadrant. This assessment covers an array of factors such as soils, rotation, tillage, irrigation, variety, fertility regime, crop protection practices, etc. The data variables collected was organized within a geodatabase and classification methodologies were applied to generate a range of groupings across farms. Understanding the disease infection (%) and rating is important as these can result in significant economic losses. Based on the field survey and analysis, TRD infection rate ranged from 0-62 percent and there was no trend for the average infection rate across the 20 surveyed farms (Fig.2). Field distribution characteristic of TRD was determined on a 0-5 scale (0 = No disease and 5 = Diseased plants observed in 10+ locations involving multiple rows and variable row length). Characterization of the TRD distribution in the field showed that higher TRD infection rates were associated with distribution patterns where the diseased plants were concentrated in 5-10 areas in the observation area (sub-areas) (Fig. 3a). The distribution of TRD in infected fields exhibits significant complexity with no clear patterns based on the survey instrument. Poor relationship (R^2=0.25, data not shown) was observed between filed distribution rate and TRD infection (%). Overall TRD rating ranged from 0-15, indication of the light incidence of the disease across the survey fields. Surface residue cover (%) was determined as the percent coverage in an area (3 m length x 1 m width) along the same row assessed for infection rate. Surface residue cover (%) ranged from 0-9 (Fig. 3b), and non-significant relationship was observed for TRD infection rate with the percent residue cover. Besides, 97% of TRD infected plants had buried residue present in upper root zone.

Further, based on the first-year survey results, infection period and infection percent of TRD, seven (7) farms were selected for the second-year study. The survey fields included those where no TRD symptoms were previously observed, those with soybean residue remaining on the soil surface, buried residue, and heavy residue loading such as with cover crops. Survey was made at GPS-marked locations which account for soybean row position. From the survey, we observed that TRD symptoms showed in the early reproductive stages (R3-R5) in most of the selected farms. Over 50% of the surveyed fields detect TRD distribution within transect area, maximum of 1-4 infected locations with subsurface and buried residue (Fig. 4). Field symptoms were localized, and symptomatic plants were clustered within the rows.

Objective 2. Evaluation of the ecology of *Xylaria* spp. based on pathogen population in soil and microbiome structure and function.

Confirmation of TRD (*Xylaria necrophora*) pathogen

Symptomatic TRD soybean plants were collected during 2021 growing season, by southern Ag. consultants and transported to the laboratory. The symptoms include interveinal chlorosis, blackened crown, taproot, and lateral root system (Fig.5a and b). The collected roots were disinfected and used for fungal isolation. Plate culturing was done using the PDA and pure culture was identified using the fungal morphology from the previous studies (Garcia- Aroca et al., 2021). TRD pure culture storage method was validated by performing a method comparison study and verified by culturing the 7-d incubation period mycelium from

colonies on the PDA agar plates (Fig.4d and e). Additionally, TRD culture storage method is confirmed by culturing the stored fungal mycelium on the PDA agar plates. Pure culture was stored for the green house experiments and future study. Fungal DNA was extracted from the growing mycelium and confirmation through ITS sequencing is underway.

Soil disease suppression-microbial ecology- latent period study (2020): To study the microbial ecology of soil samples from the selected seven farms, soil sampling was done at GPS-marked locations which account for soybean row position. Within a single soil type (soil map unit) in each field, a known volume of soil and residue was collected to a depth of 7.5 cm (approx. 3 inches). Three of these samples were collected close to one another at each of two separate locations within the soil unit. Sampling scheme in the selected farms included three crop rotations, corn-soybean, cotton-soybean and soybean-soybean with a tillage/residue management combinations, minimum till-surface residue, conventional till-buried residue and cover crop-high residue. Sampling of bulk and residue (detritusphere) soil was done in November 2020 and April 2021 of the latent period the year from the selected TRD infected farms. Further, in the succeeding soybean crop season, infectious year 2 (2021) rhizosphere samples were collected. The collected soil samples were placed and transported on the ice and stored in the -80 freezer in the lab until the analysis. To determine the diversity of the microbial network as well as estimates of the microbial taxa associated with functions such as, decomposition, nutrient processes and disease suppression, soil genomic DNA was extracted and amplicon sequencing of bacterial and fungal (16S and ITS fragments, respectively) genes were carried out for both year soil samples at the MSU Institute for Genomics, Biocomputing & Biotechnology using Illumina MiSeq sequencing. Data processing and statistical analysis was done using the QIIME 2 (version 2021.11) (Quantitative Insights Into Microbial Ecology) software. Amplicon Sequence Variants (ASV's) were generated using the DADA2 version. Statistical analysis was performed using the MicrobiomeAnalyst (https://www.microbiomeanalyst.ca/) pipeline. Analysis of the first-year bulk and detritusphere soil samples for bacterial rRNA gene showed significance ($p<0.001$) difference for the bacterial community compositions, which accounts for 10.8% of the variation. However, no difference was observed for bacterial diversity index (Alpha diversity, Chao1 and Shannon diversity index). The bacterial community species, *moabensis* and *japonicum* showed higher abundance in the detritusphere soil and significantly ($p<0.05$) different for the soil types. Species, *moabensis* generally present in the top centimeters of the soil, creating a crust of soil particles bound together by organic materials. The soil bacteria, *Bradyrhizobium japonicum* develops a symbiosis with the soybean plant and is the N_2-fixing partner of soybean. This supported the higher abundance of forementioned species in the detritusphere soil and as some of the farms were following the residue cover, soybean, corn and cotton residue remaining on the soil surface.

Fungal beta diversity analysis showed that fungal communities differ significantly ($p<0.001$) between the bulk and the detritusphere soil, with 32.5% of total variation (Fig. 6). Microbial relative abundance analyses are effective in revealing taxonomic changes under the differential sample environment. Analysis of the taxonomic composition at the phylum level revealed that both bulk and detritusphere soil samples were dominated by members of phylum *Ascomycota* (85-90%) and followed by *Basidiomycota*. A combination of pattern correlation and heat map analysis showed positive correlation between the phylum *Ascomycota* and the detritusphere soil. Similar observation was seen in the heat map (Fig. 7). Metagenomic sequencing analysis displayed higher abundance of *Fusarium solani*, *Trichoderma asperellum*, *Humicola repens* and *Pseudeurotium ovale* in bulk soil (Fig. 8 a and b). *Fusarium solani* is known phytopathogenic soil borne fungus which causes the root rot. Despite of pathogenicity, interestingly this taxon has reports for the disease suppression of the pathogen FocTR4 (Fusarium wilt) in combination with *Aspergillus fumigatus,* which was obtained from the plant residue manipulation (Yuan et al., 2021). This indicates that residue cover practice would help in tackling the important soil-borne diseases. In Detritusphere soil samples, fungal alpha diversity was significantly ($p< 0.01$) higher in the asymptomatic farms, and lower in the TRD symptomatic farms (soybean-soybean) (Fig. 9a). In bulk and detritusphere soil, significant ($p<0.001$) differences were observed for the fungal community compositions in TRD asymptomatic and symptomatic

farms (Fig. 9b and c). Thus, TRD asymptomatic farms possess differential fungal communities. Likewise, in the study differential fungal communities were observed for the buried, high and surface residue cover.

Soil properties, pH, total C, N and Easily Extractable Glomalin-Related Soil Protein (EE-GRSP) were estimated in the study. The bulk soil samples were air-dried overnight, grounded, passed through a 2.0mm sieve and stored at room temperature for measurement of all the above parameters. In the study, neutral soil pH was recorded in both year soil samples. Further, Relationship between soil physiochemical properties and microbial community structure was estimated using the Mantle test. Total C and N content were significantly correlated with bacterial communities and total C content was correlated with fungal communities (Table 1).

Soil disease suppression-microbial ecology infectious period study (2021): Bulk (latent period) and rhizosphere (infectious period) soil samples were used to study the soil microbial diversity and community composition in the TRD symptomatic and asymptomatic farms. Rhizosphere samples were collected (May 25, 2021) by uprooting the plant by making wedge using sharpshooter and shift the wedge to the labelled ziplock bag, delivered to the lab for rhizosphere collection. In the lab loose soil attached to roots was collected and roots were placed in the phosphate buffer (with Silwet L-77) for the extraction of rhizosphere soil. The obtained soil was used to extract the total genomic DNA for amplicon sequencing. Amplicon libraries were prepared using two-step PCR targeting the V3-V4 region of the 16S rRNA gene for bacterial community and ITS2 for fungal community. Analysis of fungal community data revealed that, alpha diversity indices (Chao1 and Shannon diversity index) were significantly ($p > 0.05$) different for bulk and rhizosphere soil samples (Fig.10a and b). Soil fungal communities showed distinct clusters for the soil types (Fig. 10c). Positive correlation was observed for the phyla *Mortierellomycota* and *Basidomycota* for the rhizosphere samples. Additionally, significant ($p > 0.01$) differences in the abundance of phylum *Ascomycota* and *Mortierellomycota* were noticed across the bulk and rhizosphere soil samples. There was no significant relationship between soil physiochemical properties and fungal community structure was observed.

Further, to study the abundance of *Xylaria* spp. in latent and infectious period soil samples, abundance level was analyzed at different taxonomic level. Taxonomic classification at the order level revealed the *Xylariales*, which belongs to the phylum *Ascomycota*. In the study, dominance of phylum Ascomycota was observed. Bulk and detritusphere soil samples for the latent period of the year, 2020 showed the presence of the order *Xylariales*. Positive correlation of the order *Xylariales* was observed for the TRD symptomatic farm (Soybean-Soybean). Whereas asymptomatic farms showed the negative correlation for both bulk and detritusphere soil samples (Fig. 11 a and b). In addition, higher abundance of the *Xylariales* were detected in continuous soybean- soybean farms compared to cotton-soybean rotation farms (Fig. 11c and d). However, in the second year (2021), only rhizosphere soil samples exhibited the order *Xylariales*. Positive correlation was found for the TRD symptomatic farms and negative correlation was observed for the asymptomatic farms (Fig. 12a). Univariate analysis of abundance of *Xylariales* showed significant difference across the TRD symptomatic and asymptomatic farms (Fig. 12b).

Quantitative PCR for estimating gene copy numbers of *Xylaria* in soil

Based on pathogen tree of homology, we designed a primer set to target RNA polymerase II (RPB1) and BTUB region sequences of both *Xyleria arbuscula* and *Xylaria striata* species. PCR was performed to quantify the TRD pathogen load from the soil samples. We worked on the standardization of the protocol (annealing temperature) for both *Xylaria* species. We were not successful in quantifying the pathogen load.

Conclusions:

- The distribution of TRD in infected farms exhibits significant complexity with no clear patterns based on our survey technique.
- Based on the survey, the incidence of TRD was observed in the vegetative and early reproductive (R3-R5) stages of soybean.
- TRD distribution within the transect area were concentrated in 5-10 areas in the observation field for the first survey period. And for the second survey period, TRD distribution was observed in 1-4 infected locations within the transect area, with subsurface residue.
- Fungal community diversity was significantly different in asymptomatic farms compared to the TRD symptomatic farms.
- Bacterial communities in the latent period samples, *Bradyrhizobium japonicum* were identified as N_2-fixing partner of soybean, thus improving the soybean plant growth and development.
- Soil management practices influenced the soil bacterial and fungal communities differentially in both latent period and infectious period. And a strong correlation of total soil C and N between the bacterial/ fungal communities was observed for the bulk soil samples (latent period, 2020).
- The continuous soybean system (TRD symptomatic soil samples) resulted in higher abundance of order *Xylariales* and lower abundance was detected in the asymptomatic soil samples.

Impacts and Benefits to Mississippi Soybean Producers

Soybean taproot decline has been increased steadily in the southern US, invade plants through roots, reach above-ground plant organs and can affect at any time during the growing season. Thus, leads to significant decrease in soybean production, approaching 25 percent. Field survey results of the Mississippi Delta region showed significant increase in the disease severity from first year to second year. Substantial progress has been made from researchers in the characterization of soybean TRD pathogen, mainly identification of causal agent, *Xylaria necrophora* from the onset of this study. At present, fungicides are not labeled for management of TRD, however it has been observed that certain fungicides applied in-furrow observed as advantageous.

Little is known about impacts of soil management and plant residues trigger the development of protective microbiomes. Development of disease suppressive soils is important strategy to shield plants for soil borne pathogens. Incorporation of plant residues and crop rotation strategies improve disease resistant properties, by the changes in the soil physiological properties. The results of fungal amplicon sequencing indicated that the differential *Ascomycota* abundance in asymptomatic farms compared to TRD symptomatic farms. Soybean-Soybean crop rotation sequence possessed the higher abundance of order *Xylariale*. However, cropping sequence of cotton-soybean showed lesser abundance of the *Xylariale*. As, *Xylaria* spp. are saprophytes and soil borne fungi, soybean rotation with other crops, like cotton would benefit in reducing the TRD pathogen load in the soybean fields.

These data illustrate that disease was observed in the patches and TRD pathogen can be observed in both vegetative (V12-V14) and reproductive (R2-R5) stages. The survey and classification of farming systems in relation to TRD occurrence and severity is anticipated to provide direct benefits to soybean growers by identifying practical management decisions that may reduce the impact of TRD. Outcomes and information

from this project will help soybean producers in both understanding and managing taproot decline disease and to develop effective management strategies for stakeholders.

End Products–Completed or Forthcoming

- Poster presentation at the APS Southern Division Meeting (Virtual), held on February 15-19, 2021. William Kingery, Dan Prevost, Shankar Ganapathi Shanmugam, *In-season Survey of Soybean Taproot Decline Incidence and Severity on Selected Mississippi Delta Farms.*

- Poster presentation at the ASA Southern Branch Meeting, held on February 12-14, 2022. Nisarga Kodadinne Narayana, Daniel Prevost, William Kingery and Shankar Ganapathi Shanmugam, *Understanding and determining the impacts of soybean Taproot decline (TRD) disease severity in Mississippi Delta farms.*

- Results will be written as manuscript for publication in peer-reviewed journal. The manuscript will describe function and composition of surrounding soil microbial communities which exploit the same resources as TRD can potentially exert an antagonistic influence on TRD, thus potentially enhancing disease suppression.

- Progress gained by the project will be used to write project proposal to accomplish the research gap and study further.

- Results will be presented in regional and national professional meetings.

Graphics/Tables

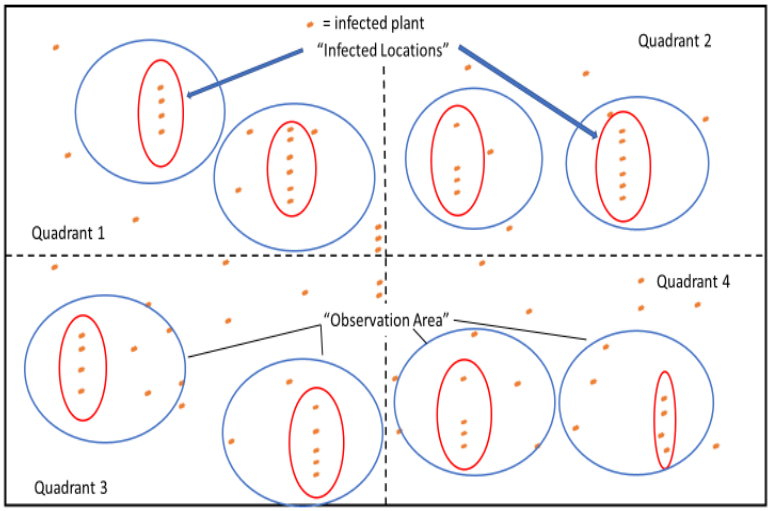

Figure 1: TRD field survey method. Field was divided to into quadrants for the survey/observations.

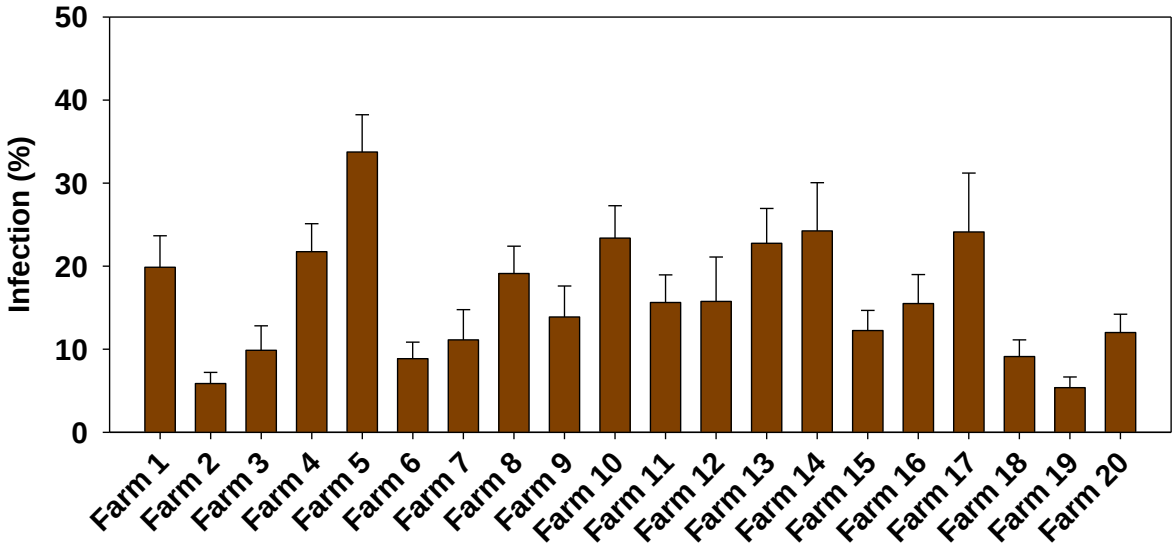

Figure 2: Average TRD infection rate for the 20 Mississippi Delta farms surveyed during latent period of the year (2020).

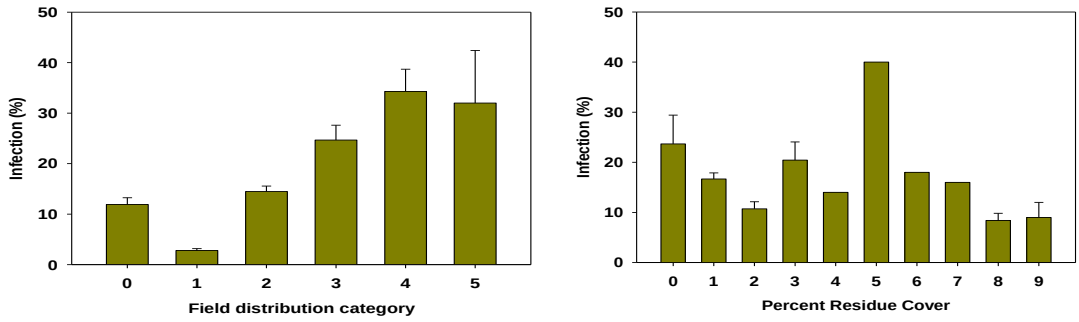

Figure 3: Average TRD infection rate for the (a) field distribution categories describing TRD distribution and (b) different levels of percent surface residue cover within observation areas of the 20 surveyed farms during latent period of the year (2020).

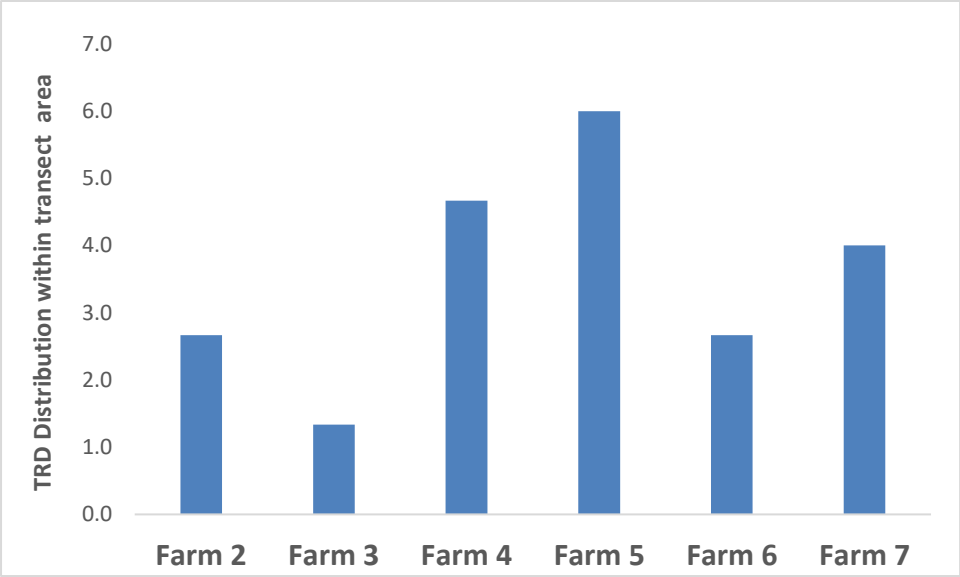

Figure 4: Average TRD distribution within transect area across the selected farms, in the infectious year (2021)

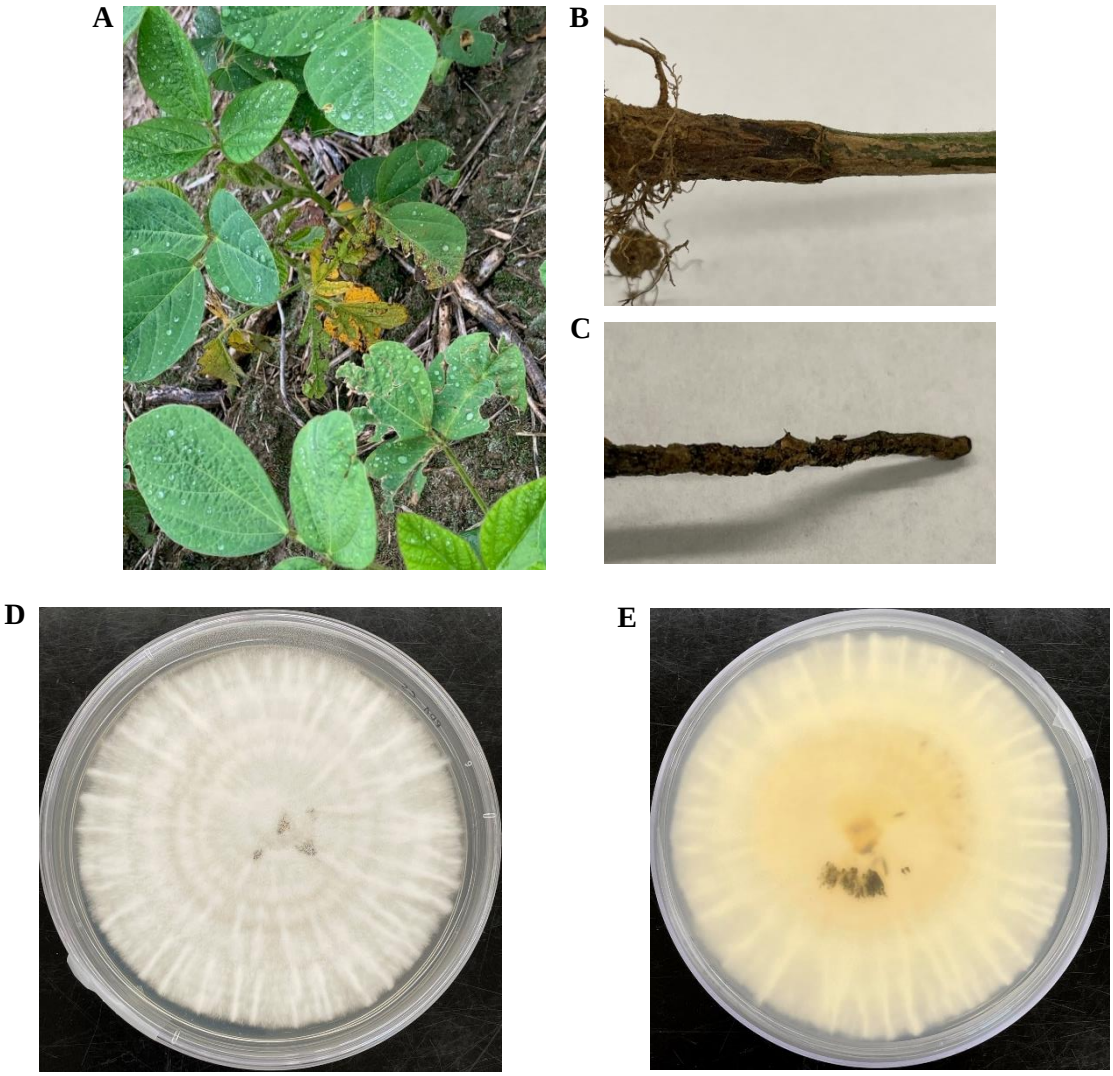

Figure 5. TRD symptoms and colonies. TRD infected (A) soybean plant; (B) Crown; (C) Black stromata on the lateral root and *Xyleria necrophora* colonies, 14 days-old culture (D) Surface view; (E) Reverse view. Plant samples were collected during the infectious period, 2021.

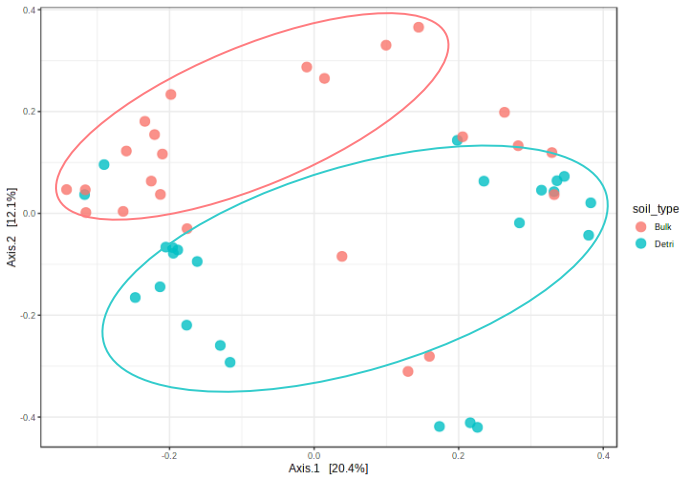

Figure 6. Beta diversity index: Fungal community variation in the bulk and detritusphere soil samples (latent period, 2020). Derived using Principal coordinate analysis (PCoA)-Bray Curtis dissimilarity matrix.

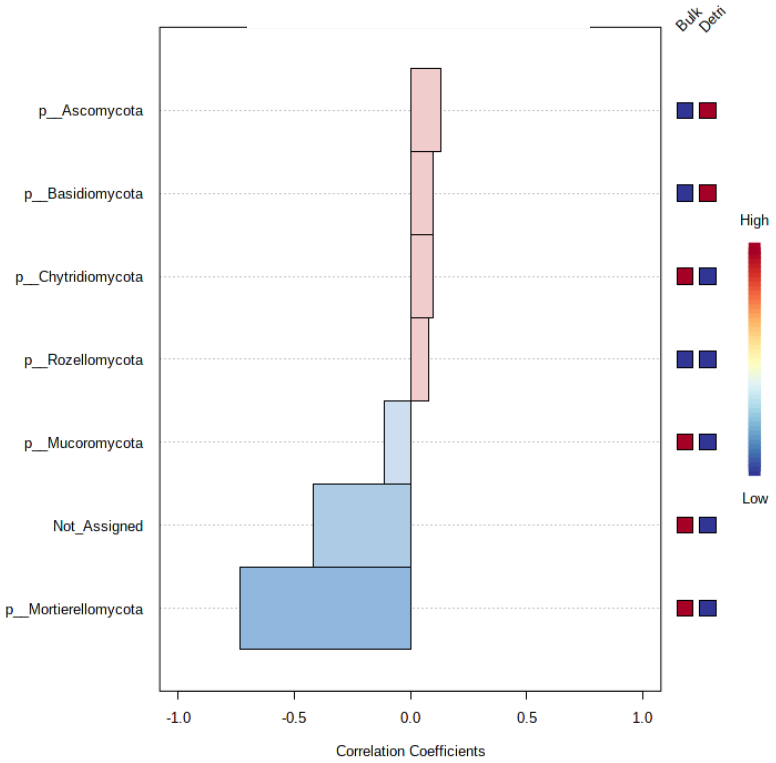

Figure 7. Differential fungal community profiles at phylum level and correlation analysis for bulk and detritusphere soil.

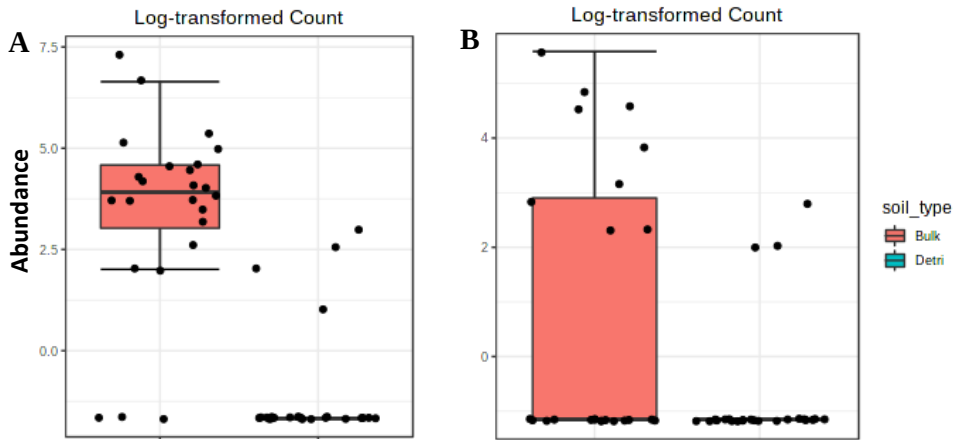

Figure 8. *Fusarium solani* (A) and *Trichoderma asperellum* (B) abundance in bulk and detritusphere soil. Soil samples were collected during the latent period of the year, November 2020.

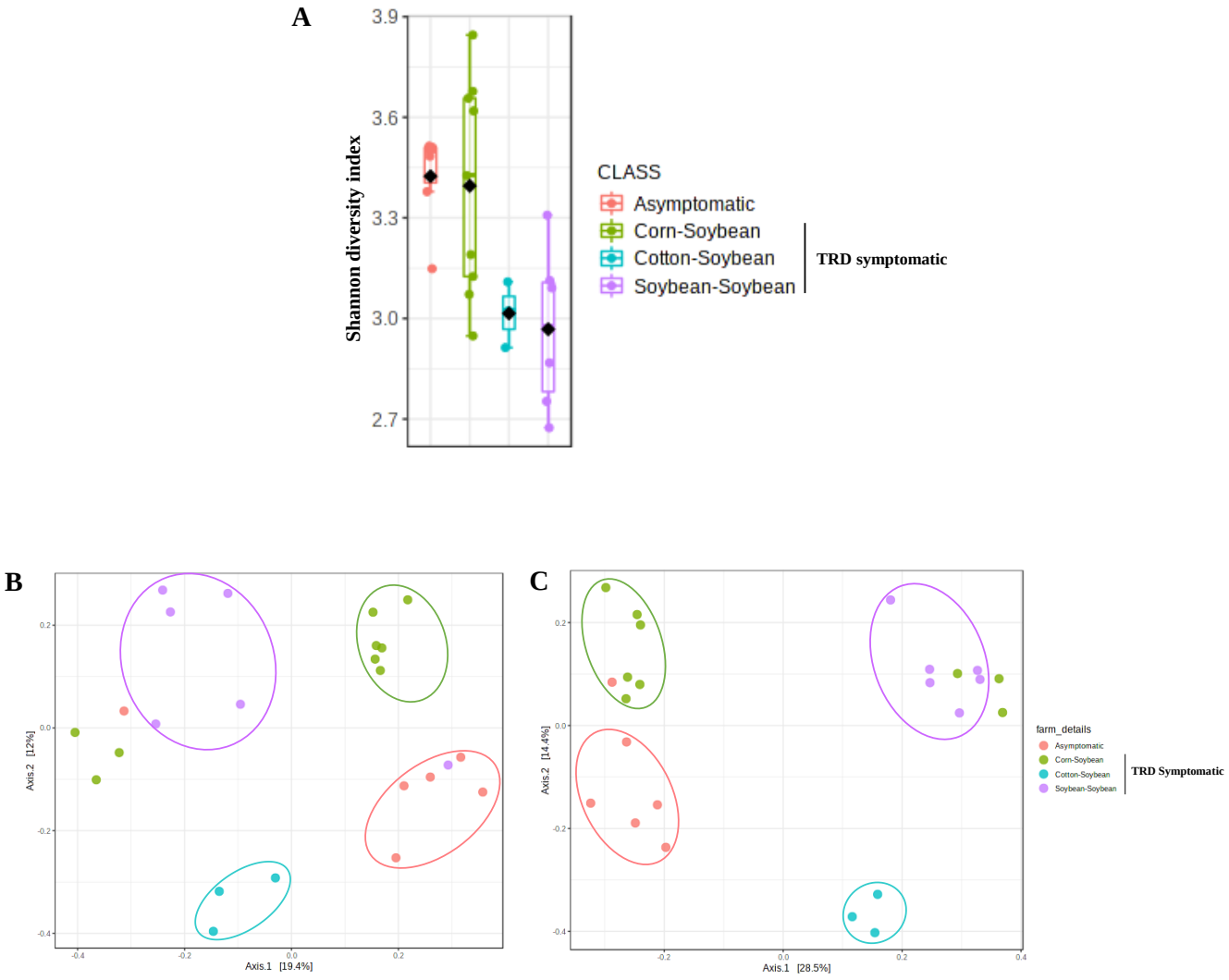

Figure 9. Fungal alpha and beta diversity indices. Shannon diversity index (species richness and evenness) (A) and the Bray Curtis dissimilarity matrix -PCoA of fungal community structures in (B) bulk and (C) detritusphere (Detri) soil samples across TRD symptomatic and asymptomatic farms. Soil samples were collected during the latent period of the year, November 2020.

Table 1. Correlation analysis between soil characteristics and bacterial/ fungal communities (significance level at p=0.05) using Mantle test in latent period bulk soil samples, 2020.

Mantle Test	Bacterial communities				Fungal communities			
	pH	C	N	EEGSP	pH	C	N	EEGSP
R	0.253	0.261	0.289	0.164	-0.030	0.249	0.317	0.166
p value	0.057	0.018*	0.010*	0.172	0.773	0.006*	0.343	0.086

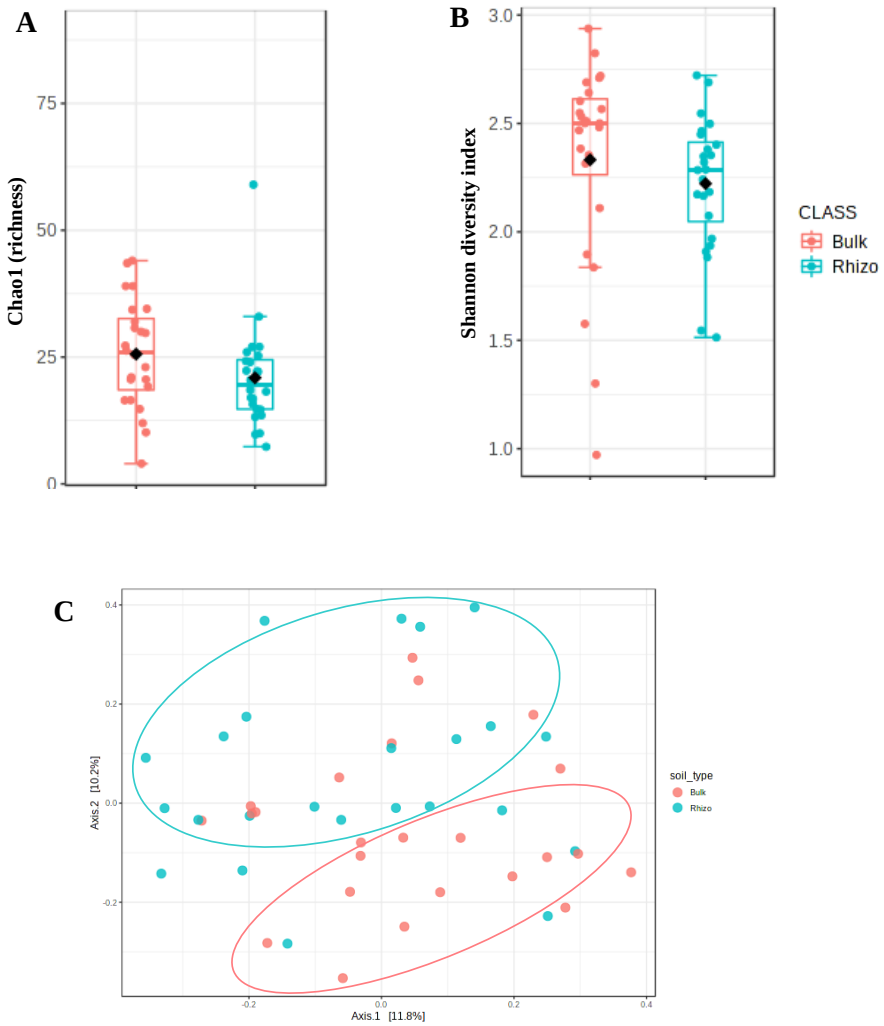

Figure 10. Fungal alpha and beta diversity indices: Species richness (A), fungal diversity index (B) and (C) estimation of differences in the fungal communities as a measure of beta diversity analysis in bulk and rhizosphere soil samples, sampled in the year 2021.

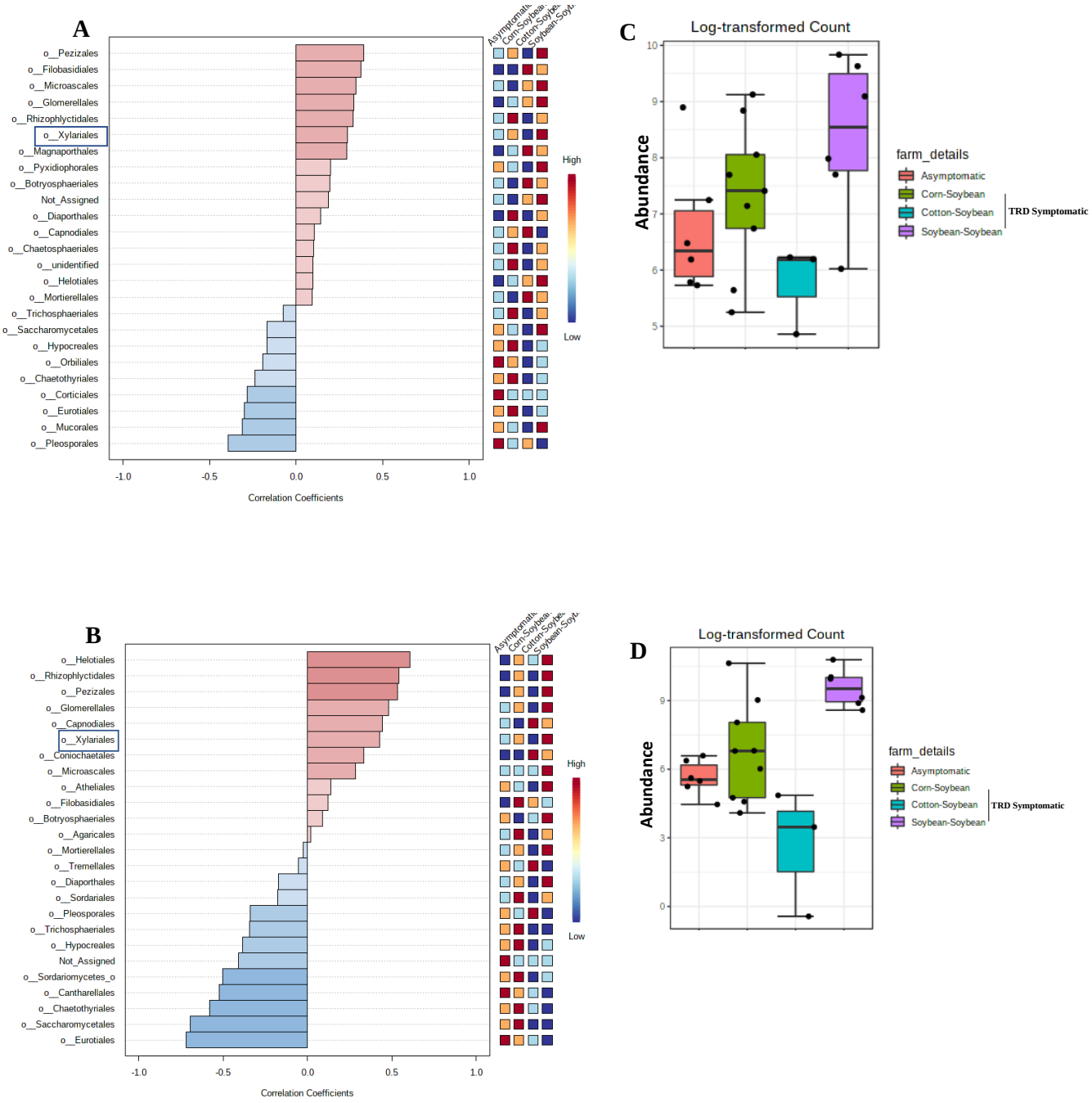

Figure 11. Correlation analysis of top 25 fungal order in the bulk (A) and detritusphere (B) soil. Relative abundance of order *Xylariale* in the asymptomatic and symptomatic farms for the latent period soil samples (2020), bulk (C) and detritusphere (D) soil.

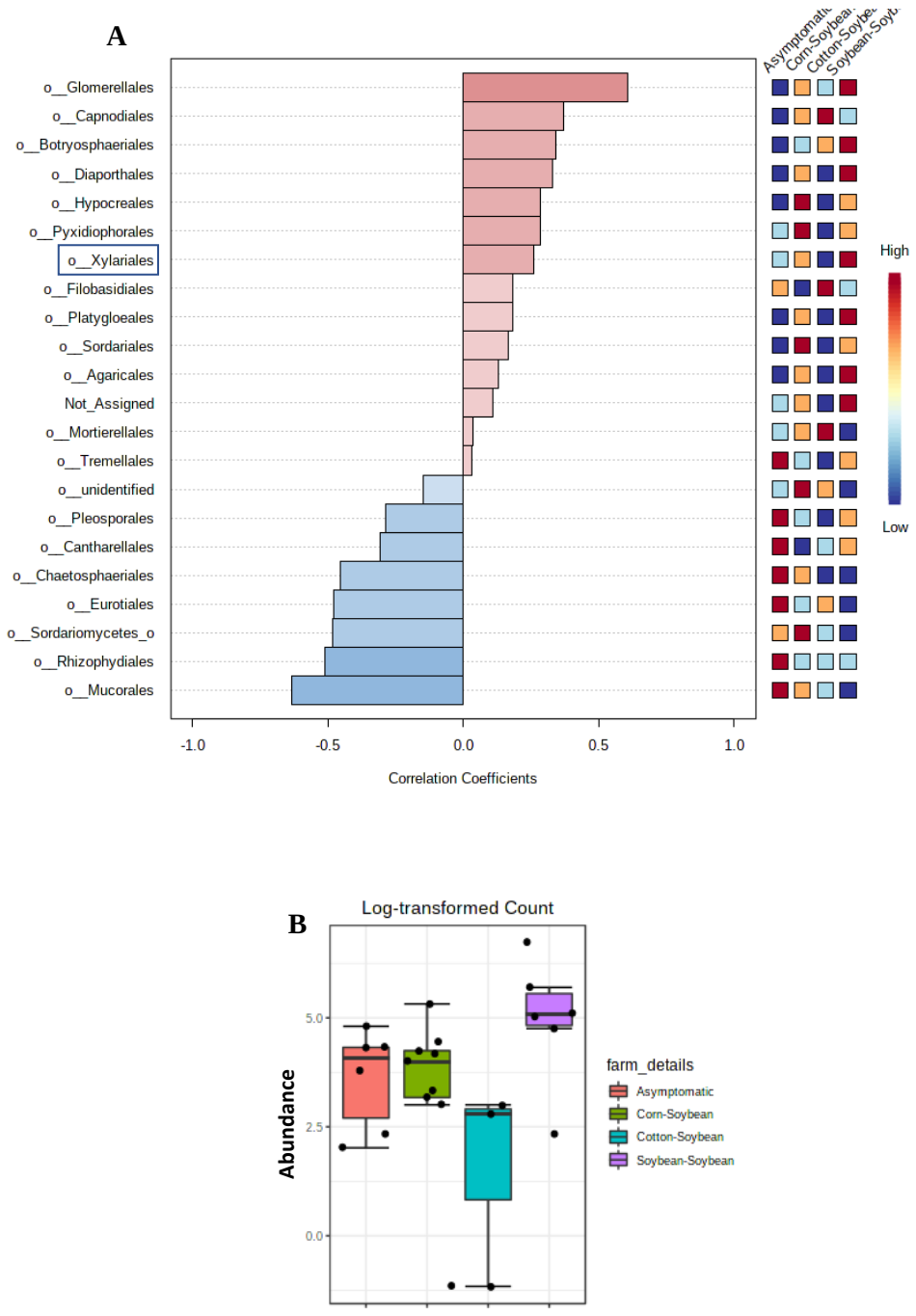

Figure 12. Correlation analysis of top 25 fungal order in the rhizosphere soil (A). Relative abundance of order *Xylariales* in the asymptomatic and TRD symptomatic farms for the infectious period soil samples.