CHARACTERIZE ROOT MICROBIAL COMMUNITIES WITH GROWTH-PROMOTING AND ANTI-FUNGAL ACTIVITIES IN SOYBEAN (53-2020)

REPORT FOR THE NON-COST EXTENSION PERIOD 01 APRIL 2021 – 30 JULY 2021

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RATIONALE/JUSTIFICATION FOR RESEARCH

Plant roots associate with a microbial community that is distinct from the microbes present in surrounding soil. The microbes colonize the rhizosphere (immediately surrounding the root) and the superficial root tissues (endophytic compartment). The root microbiota comprises a wide diversity of microorganisms, and it can benefit plant health or have detrimental effects; shifting this balance towards beneficial plant-microbiota interactions is of high agronomic interest. Microbes may provide the plant with nutrients and hormones that accelerate plant growth and development, may suppress soil pathogens, or may help plants withstand environmental stress. The advantages in the exploitation of microbiota are evident and include the safety of consumers and farmers, and the use of sustainable practices that preserve the environment and protect biodiversity.

Taproot decline (TRD) caused by *Xylaria sp*. is recognized as an important soil-borne disease of soybean, causing significant rot of the taproot. Previous MSPB studies by Dr. Maria Tomaso-Peterson demonstrated TRD is widespread throughout MS soybean production, and affected soybeans were identified in 73 counties. Through the support of MSPB, we are developing a better understanding of TRD and the causal agent *Xylaria sp*. This pathogen overwinters as stroma on crop residue across successive growing seasons and colonizes the roots of corn, cotton, rice, sorghum, and wheat in greenhouse studies. We demonstrated variable degrees of virulence among *Xylaria sp*. isolates, which may be due to the influence of microbes within the rhizosphere of the soybean plants. The optimal temperature of *Xylaria sp*. is similar to that of soybean seed germination, suggesting *Xylaria sp*. may be colonizing the root at the time of germination and continue throughout taproot development. Field observations show dead soybean seedlings resulting from TRD at the V4–V5 growth stage; however, most symptomatic soybeans observed in production fields are in growth stages greater than R3.

Soil microbiome investigations have been strongly promoted over the past ten years. In suppressive soils, crops are protected from soil-borne fungal or bacterial root pathogens. Microbiome studies have provided insights into the composition of disease-suppressing soils. For example, soils suppressing wilt disease caused by *Ralstonia solanacearum* were reported to contain a higher abundance of Proteobacteria and

Acidobacteria compared to soils with disease symptoms. Species with anti-fungal activity include non-pathogenic strains of *Fusarium oxysporum* and bacteria in the genera *Pseudomonas* and *Bacillus*. Thus, the evaluation of root microbiomes for crop growth promotion and disease resistance presents unique opportunities. We aim to understand the diversity of rhizobiome microorganisms that cause specific phenotypes in soybeans, test rhizobiome isolates for beneficial activities, and utilize this knowledge to establish methods for improved growth and biological control of soil pathogens.

Primary goals of the project: (1) Characterize soybean root microbial communities; (2) Identify and test growth-promoting and biocontrol activities of root-associated microorganisms in soybean.

Specifically, during the No-Cost Extension Period we focused primarily on bioinformatics analysis of the metagenome data sets obtained from soybean roots

Activities and significant results:

Several major activities were undertaken during this period to finalize the objectives of the project:

1. We completed the computational analysis of metagenome datasets obtained by sequencing the microbial and fungi collected from healthy and soybean roots (in the summer of 2019). The analysis was performed using the software MicrobeAnalyst (https://www.microbiomeanalyst.ca/). Using this program, we were able to obtain a comprehensive statistical. Visual and meta-analysis of the microbiome and mycobiome datasets resulted from 16SrRNA and ITS Illumina sequencing.
2. In preparation for the first manuscript resulting from our work on this project, we assembled a set of figures (**see Appendix I**) and supplementary figures. This manuscript will describe the microbiota and mycobiota associated with soybean roots from plants at three stages of increasing severity of taproot decline disease. WE estimate that the manuscript will be completed and ready for peer review in approximately one month.
3. A higher-level comparative analysis of healthy and diseased microbiomes and mycobiomes of soybean (collected in the summer of 2020) is in process. For now, we finalized uploading all metagenomic data into a public repository. The analysis is ongoing; we are utilizing the MGnify platform available through EMBL-EBI (https://www.ebi.ac.uk/metagenomics). MGnify is an automated pipeline for determining the taxonomic diversity and functional and metabolic potential of microbiome and mycobiome samples.
4. Our group has been writing and recently finalized a project proposal to be submitted on July 15th, 2021 to NIFA-AFRI, program priority area: *Agricultural Microbiomes in Plant Systems and Natural Resources.* This **Seed Proposal** aims to determine to monitor changes in root microbial communities after the invasion of fungal pathogens and identify beneficial indigenous microbiota that suppresses them. The proposed work will apply the methods and strategies developed for *Xylaria* sp. to other soil-borne pathogens (*Rhizoctonia* sp. and *Sclerotium rolfsii*), causing root and seedling diseases of great economic interest to MS and national soybean growers.

Opportunities for training and professional development provided by the project:

1. Training of undergraduate students in microbiology, molecular, and plant biology techniques:
    - Tyrikus Hayes
    - Joshua Mitchell
    - Joseph Chromiak
    - Slade Smith

- Sean McGrath

2. Training of graduate students in metagenomics and lab techniques for biocontrol:
   - Jasmine Uyen Wesser, Master student
   - Philip Berg, Ph.D. student

Products and dissemination of results to communities of interest:

1. Poster presentations at the 82nd Meeting of Southern Section of the American Society of Plant Biologists (SS-ASPB) that took place on April 16-18, 2021, by two MSState Undergraduates working in the PI's lab:

   Joshua Mitchell: '*Common Soil Bacteria as Possible Antifungal Biological Control Agents Against Xylaria sp. and Taproot Decline'*

   Aja Black: '*Bacterial Influence on Xylaria sp. Growth'*

2. Oral presentations at the 82nd Meeting of Southern Section of the American Society of Plant Biologists (April 16-18, 2021) by Jasmine Uyen Wesser, a Master's Student in the PI's lab.

   Jasmine Uyen Wesser*: 'Characterization of Soybean Root Endophytes with Protective Activity Against the Soil-Borne Fungal Pathogen Xylaria sp.'*

3. Online posting on MS Soybean Promotion Board website: Joshua Mitchell's video presentation presented at the SS-ASPB meeting In April ,2021.

4. The abstracts of the posters and presentations from the SS-ASPB meeting were published in the Conference Program booklet.

Impact of our research on soybean production and growers:

Fungal disease outbreaks have increased steadily over the past two decades and are recognized as critical threats to food security in the US and worldwide. Seedling diseases caused by emerging pathogens, such as *Xylaria* spp, and established soil-borne pathogens, can generate up to 75% yield loss for many economically important crops such as soybean. Despite considerable research, these fungal pathogens still threaten a broad range of economically important crops and remain challenging to control. Very few varieties offer resistance, and broad-spectrum fungicides are essentially not effective. Additionally, extensive use of fungicides can be toxic to humans and ecosystems and lead to resistant fungal isolates. These problems have intensified efforts to obtain a biocontrol that is an alternative to conventional approaches. If successful, the results from this project will help fill significant knowledge gaps in

characterizing agricultural microbiomes and microbiome functions in agricultural production systems and the need for sustainable, chemical-free management strategies of soil-borne diseases.

Soybean is an essential crop, a source for feedstock, protein, vegetable oils, and biofuel production in the US and worldwide. A key focus in the ongoing soybean research has been to identify strategies to deter diseases. The total estimated economic loss due to soybean diseases is considerable (*e.g.*, between 1996-2016, $80.89 billion for Northern US and $14.59 billion for the Southern US). Diseases caused by soil-borne fungal pathogens include vascular/root rot in seedlings, early and advanced vegetative stages of the plant, causing significant damage. Although very diverse, soil-borne pathogens use similar mechanisms to invade plants through roots and reach above-ground plant organs. Seedling diseases caused by soil-borne pathogens have a long history as a significant constraint to soybean production, with consistent top ranking among diseases and pests that reduced yields in the US and worldwide. Losses due to stem/root diseases at the seedling stage have been consistently higher than other disease categories; across 21 years (1996-2016) and 28 soybean-producing states in the US, seedling diseases were estimated to be the 3rd most economically damaging per hectare.

APPENDIX I

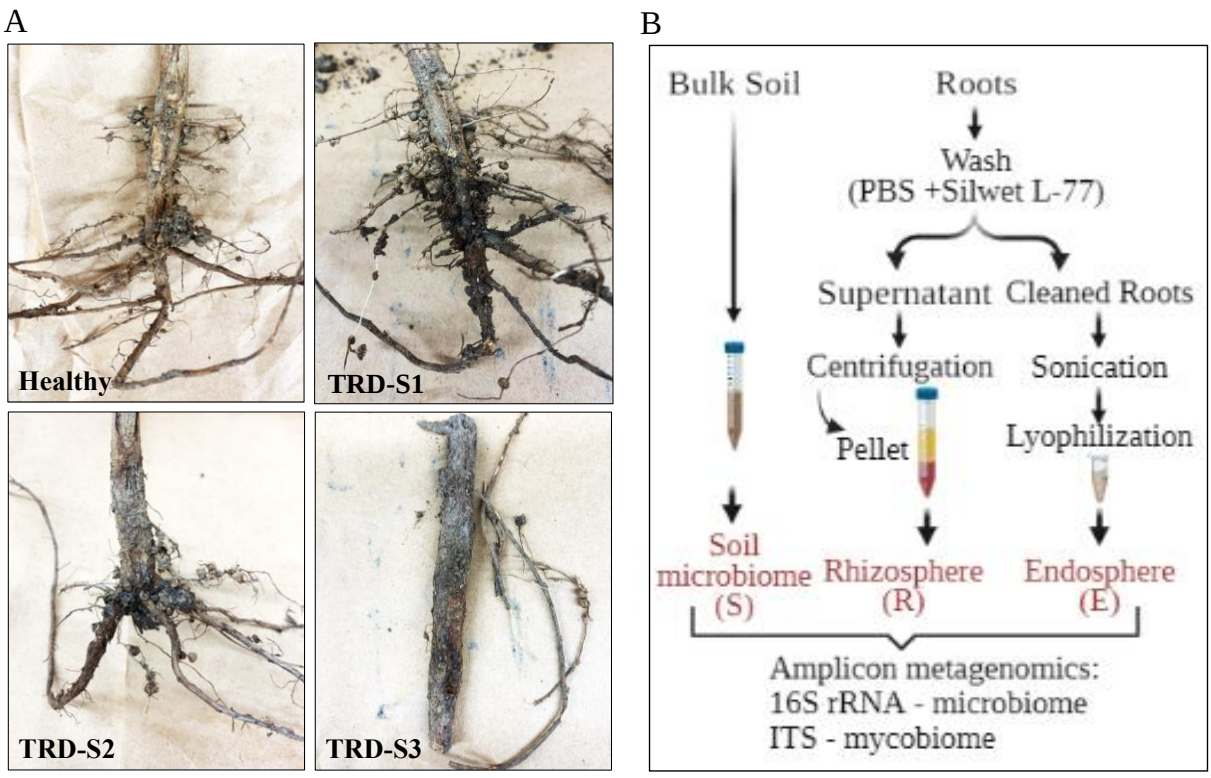


Figure 1. Root material and analysis scheme to investigate the microbiome and micobiome of healthy and diseased soybean roots. A) Soybean roots were collected from healthy plants and plants showing symptoms of taproot decline (TRD) at incipient (S1), moderate (S2) and advanced (S3) stages of infection with *X. necrophora*. Representative images of the roots before processing are shown. **B)** Root samples and bulk soil collected from the same field site were processed to obtain the microbial and fungal communities of the soil (S), root rhizosphere (R) and endosphere (E). The composition of all samples was investigated using amplicon metagenomics (16S rRNA sequencing for V1-V4, and internal transcribed spacer (ITS, regions 1-4) sequencing).

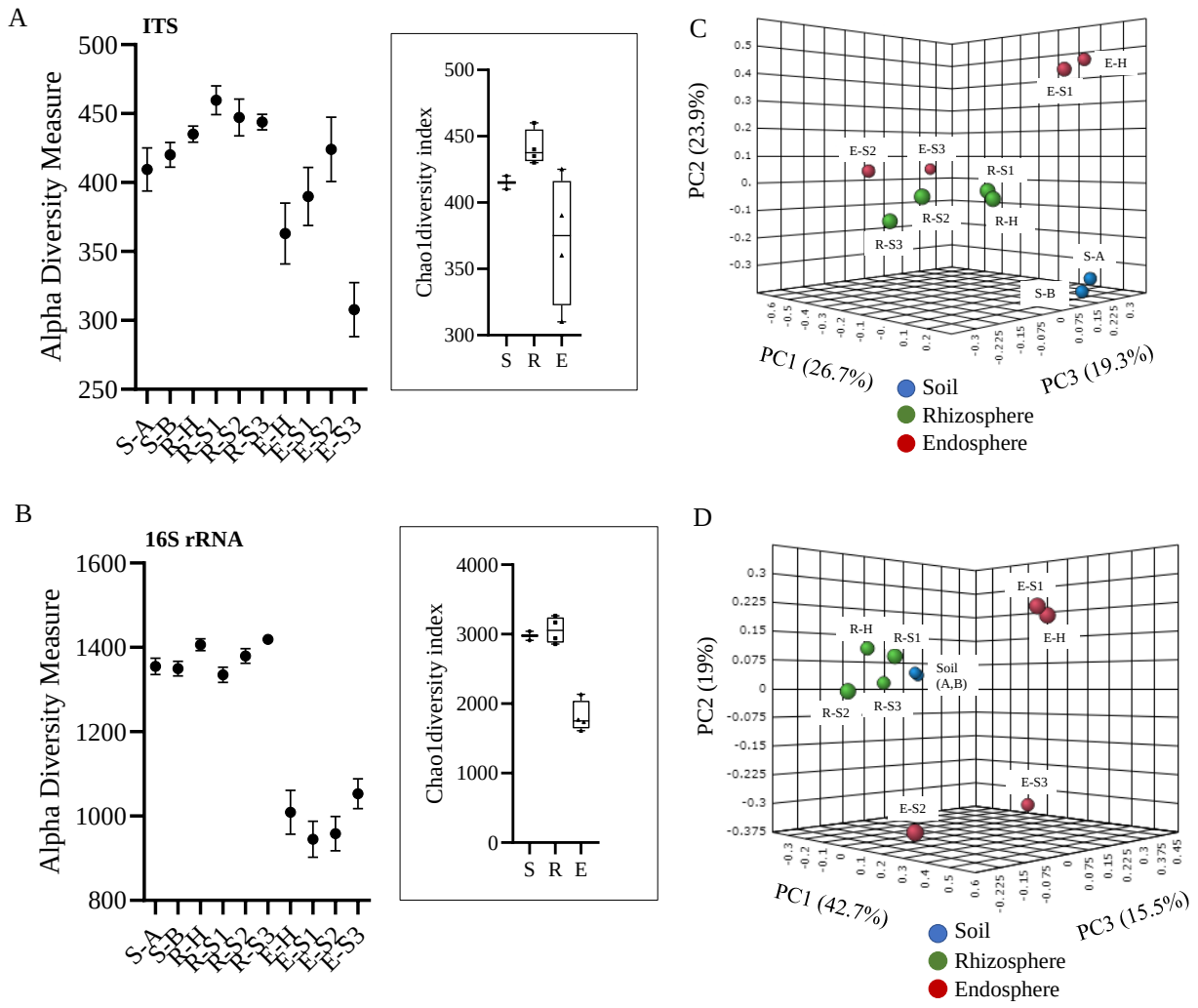


Figure 2. Alpha and beta diversity of the mycobiome and microbiome of soybean roots. A and **B)** Alpha diversity calculated using Chao1 species diversity index for ITS (A) and 16S rRNA (B) datasets using the Chao1 diversity measure. Significance of the alpha diversity profiling was assessed through T-test ANOVA (p-value < 0.05). Inserts show cumulative measures for soil (S), four rhizosphere samples (R) and four endosphere samples (E). **C** and **D**) Constrained Analysis of Principal Coordinates (PC) of ITS (C) or 16S rRNA (D)diversity in the soil (S-A and S-B), and rhizosphere (R-H, R-S1 to S3) and endosphere (E-H, E-S1 to S3) healthy or at diverse stages of taproot decline disease. Cumulative-sum scaling transformed reads were used to calculate Bray-Curtis distances. Significance of the PC analysis was assessed through Permutation MANOVA (PERMANOVA) (p-value < 0.05).

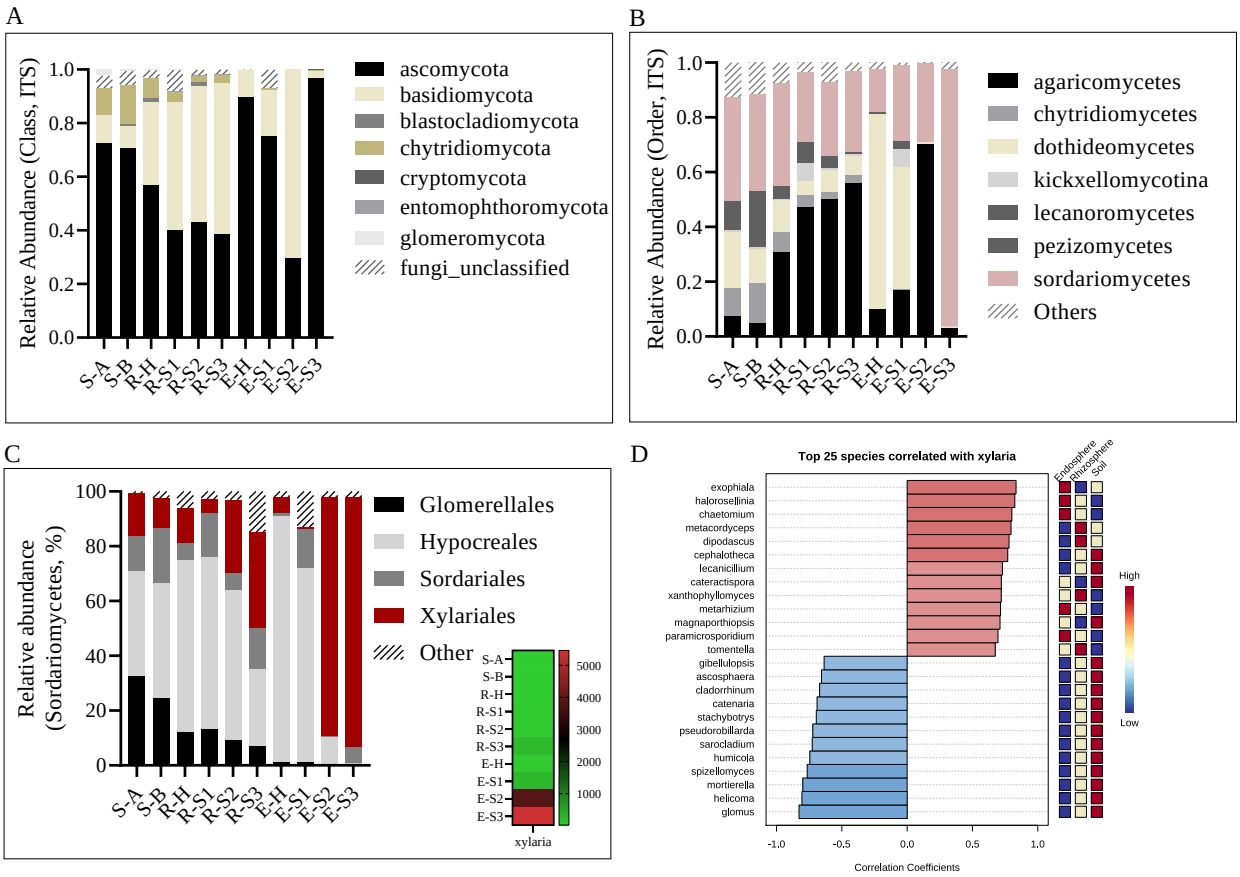


Figure 3. The effect of taproot decline disease on the soybean root mycobiome. A-C The bar graphs report the relative abundance of fungal taxa at the class level (A), order level (B) and Sordariomycetes (C) in bulk soil (S-A and B), rhizosphere and endosphere mycobiomes of healthy and TRD-symptomatic soybean, showing taxa with the highest abundance. Th inset in C shows a heat map of *Xylaria sp*. in all samples analyzed based on total counts. D) Pattern search to identify species significantly correlated with *Xylaria*. Using the distance measure SparCC.

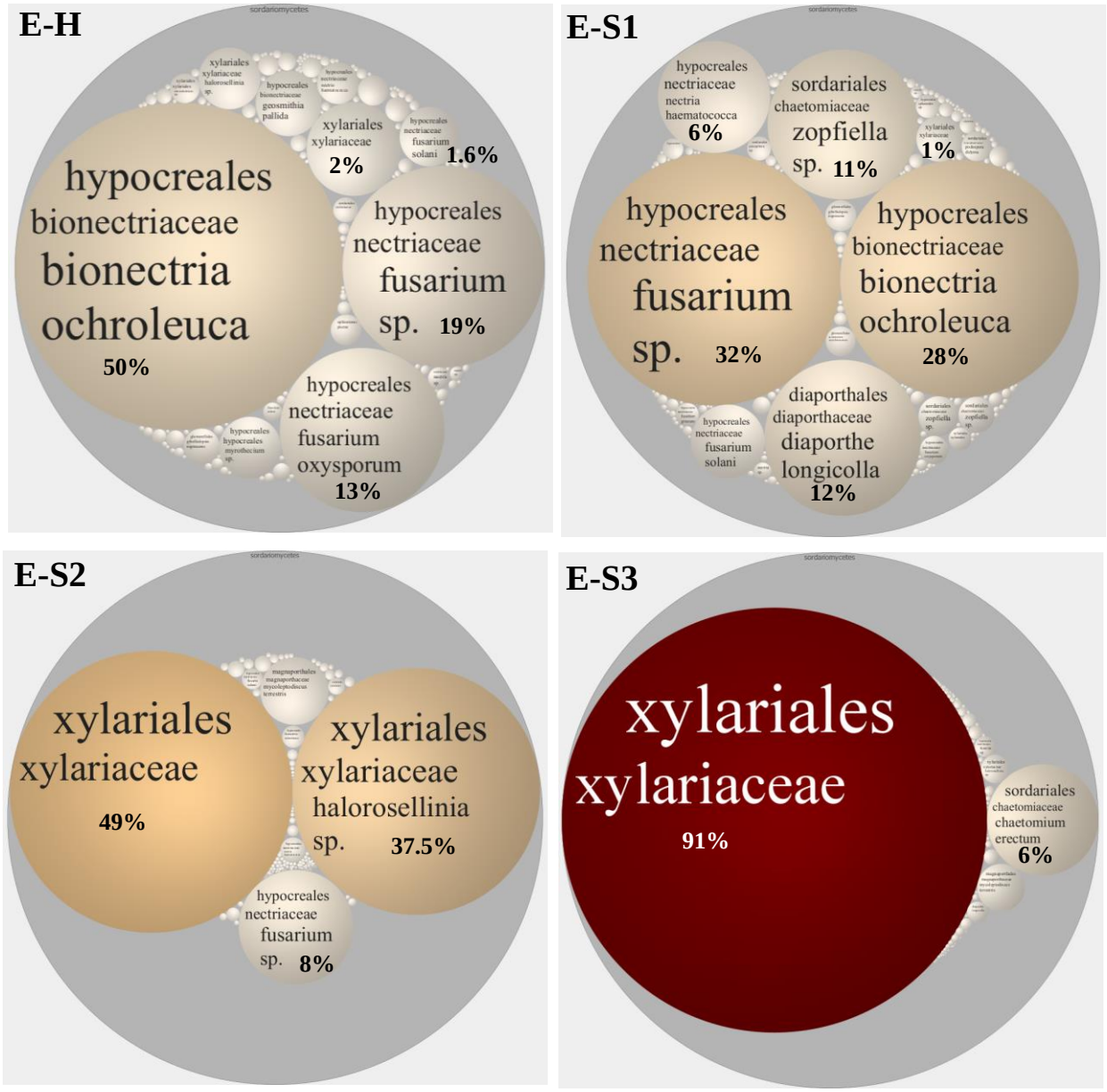


Figure 4. Taxonomic classification map of the Sordariomycetes endophytes in healthy and TRD symptomatic roots. Average reads numbers based on ITS sequencing were grouped by class (Sordariomycetes). Labels show order, family, and species. Numbers depict percentages of selected taxonomical categories. Size of map circle is proportional to reads number.

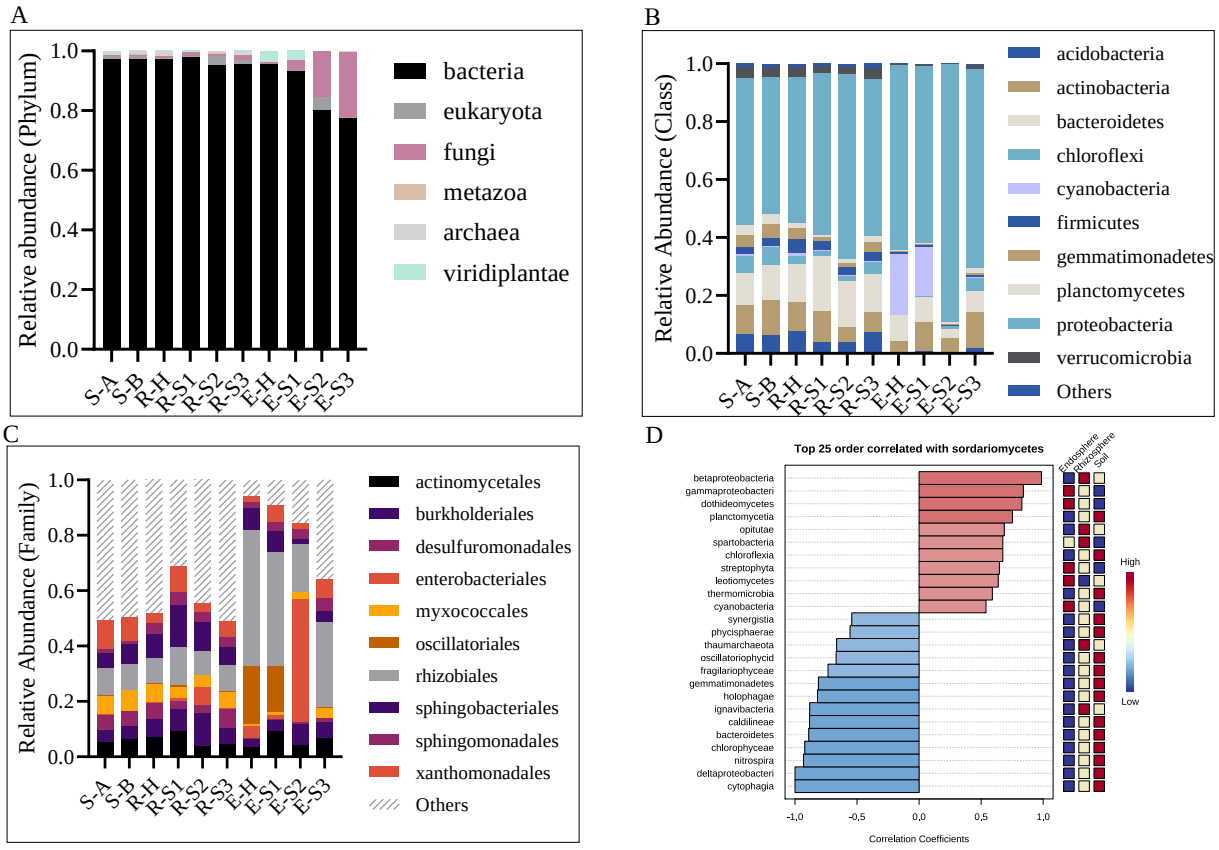


Figure 5. The soybean root mycobiome in healthy and taproot decline disease stages. A-C) The bar graphs report the relative abundance of microbiome taxa at the phylum (A), class (B) and family level (C) in bulk soil and root microbiomes of healthy and TRD-symptomatic soybean roots, showing taxa with the highest abundance. D) Pattern search to identify bacteria orders significantly correlated with *Xylaria*, using the distance measure SparCC.

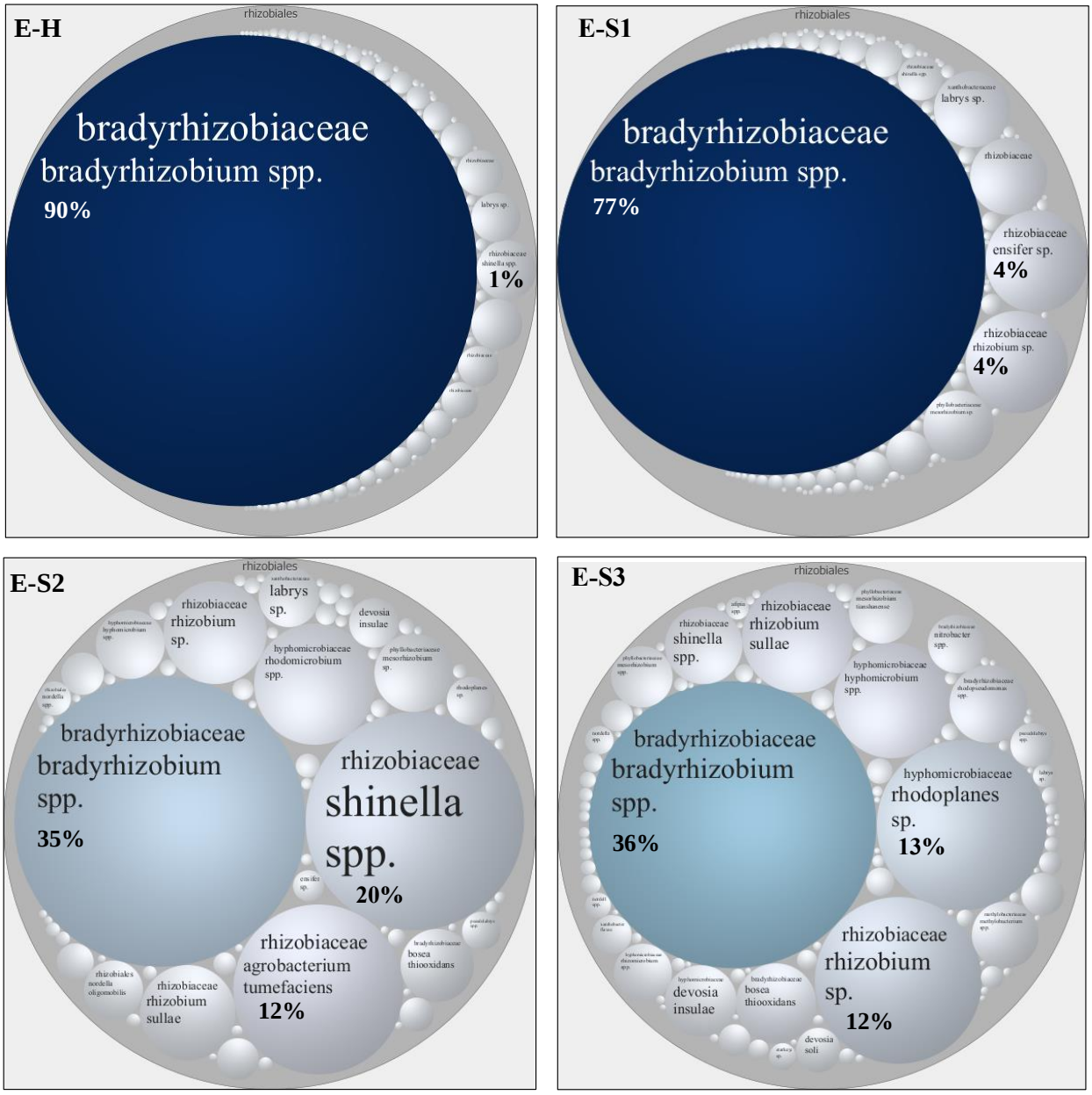
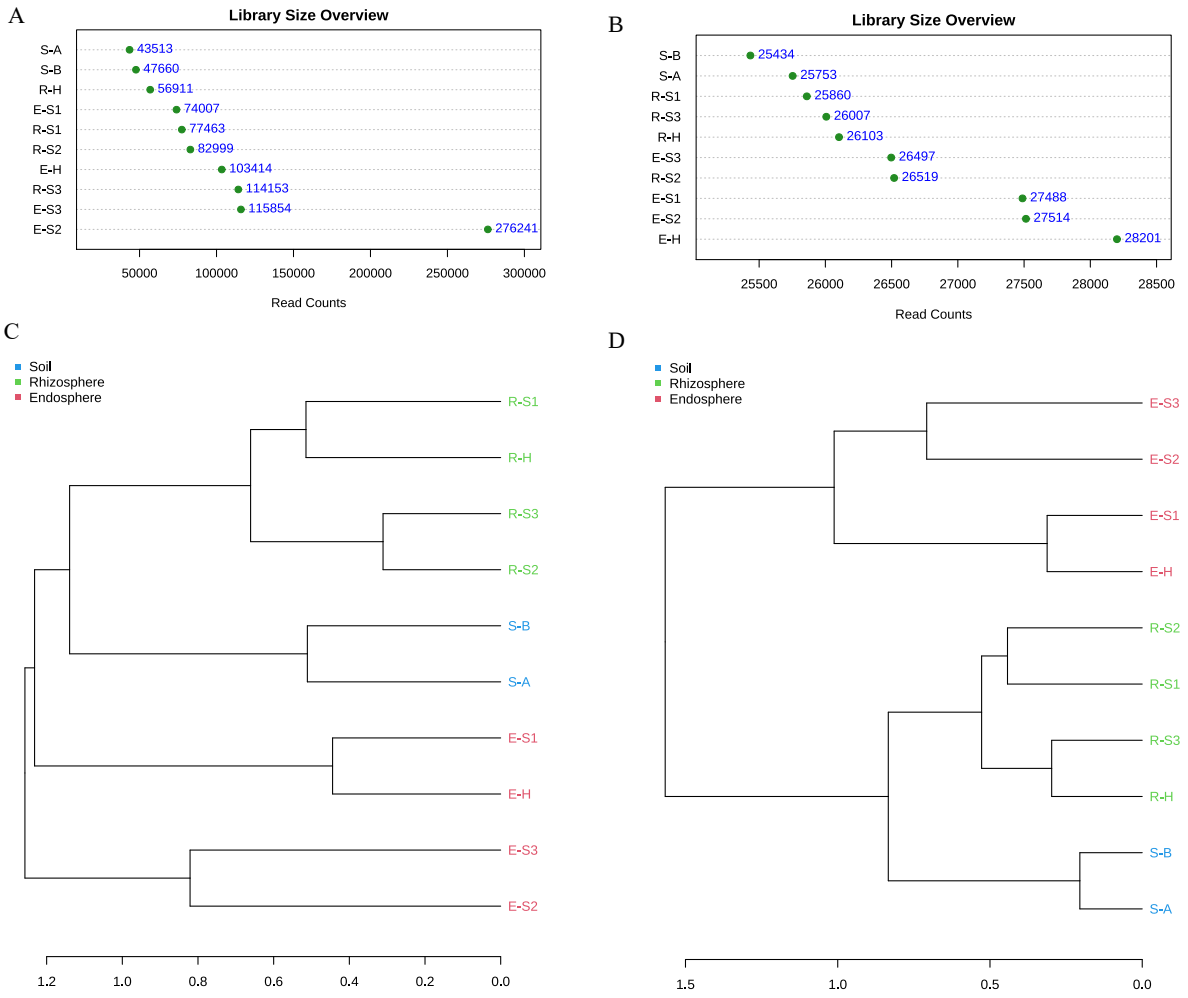
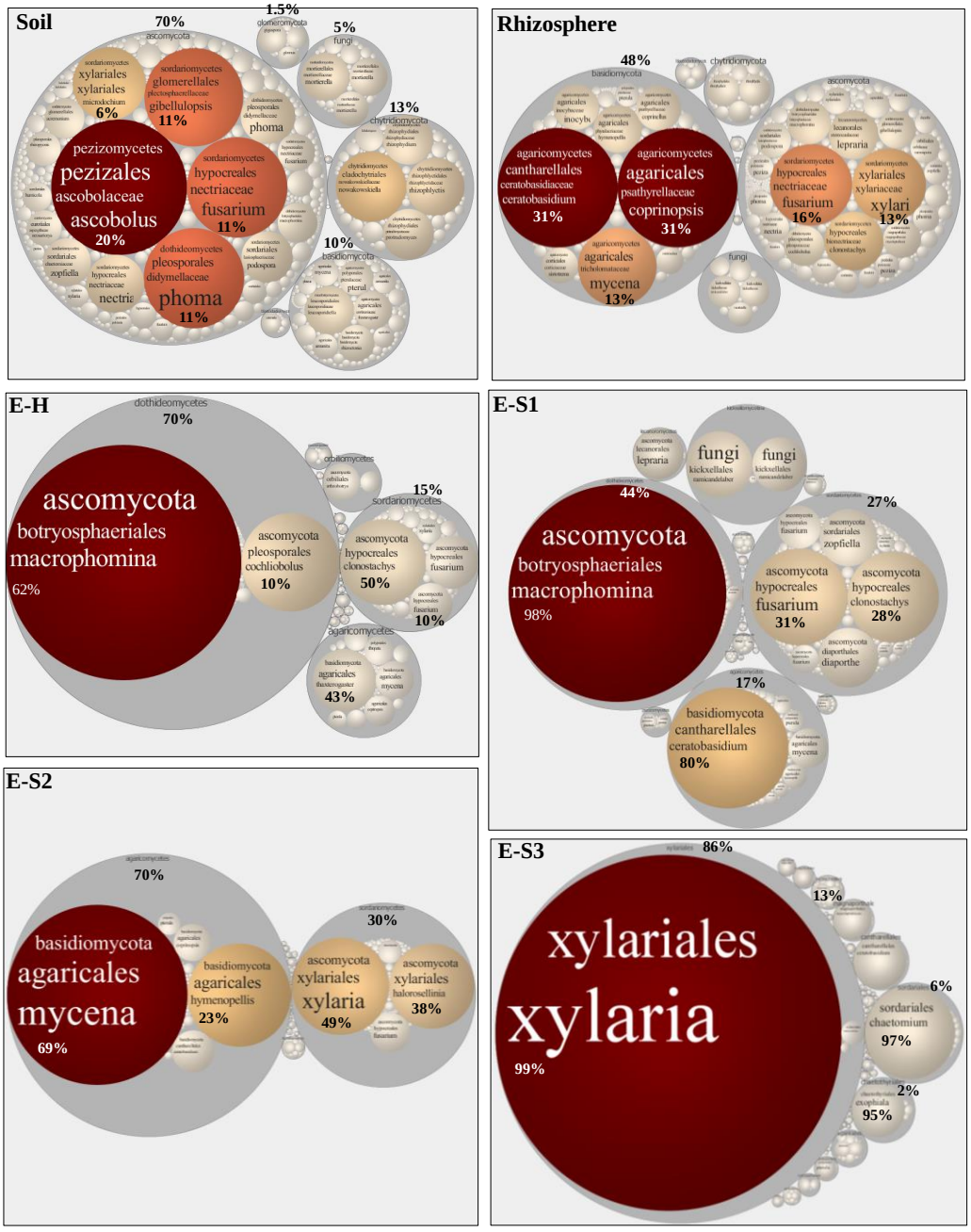


Figure 6. Taxonomic classification map of Rhizobiales endophytes in healthy and diseased soybean roots. Average reads numbers based on 16S rRNA sequencing for the E-H (healthy) and TRD-symptomatic samples (E-S1, S2 and S3) were grouped by class. Labels show family and species. Numbers depict percentages of selected taxonomical categories. Size of map circle is proportional to reads number.

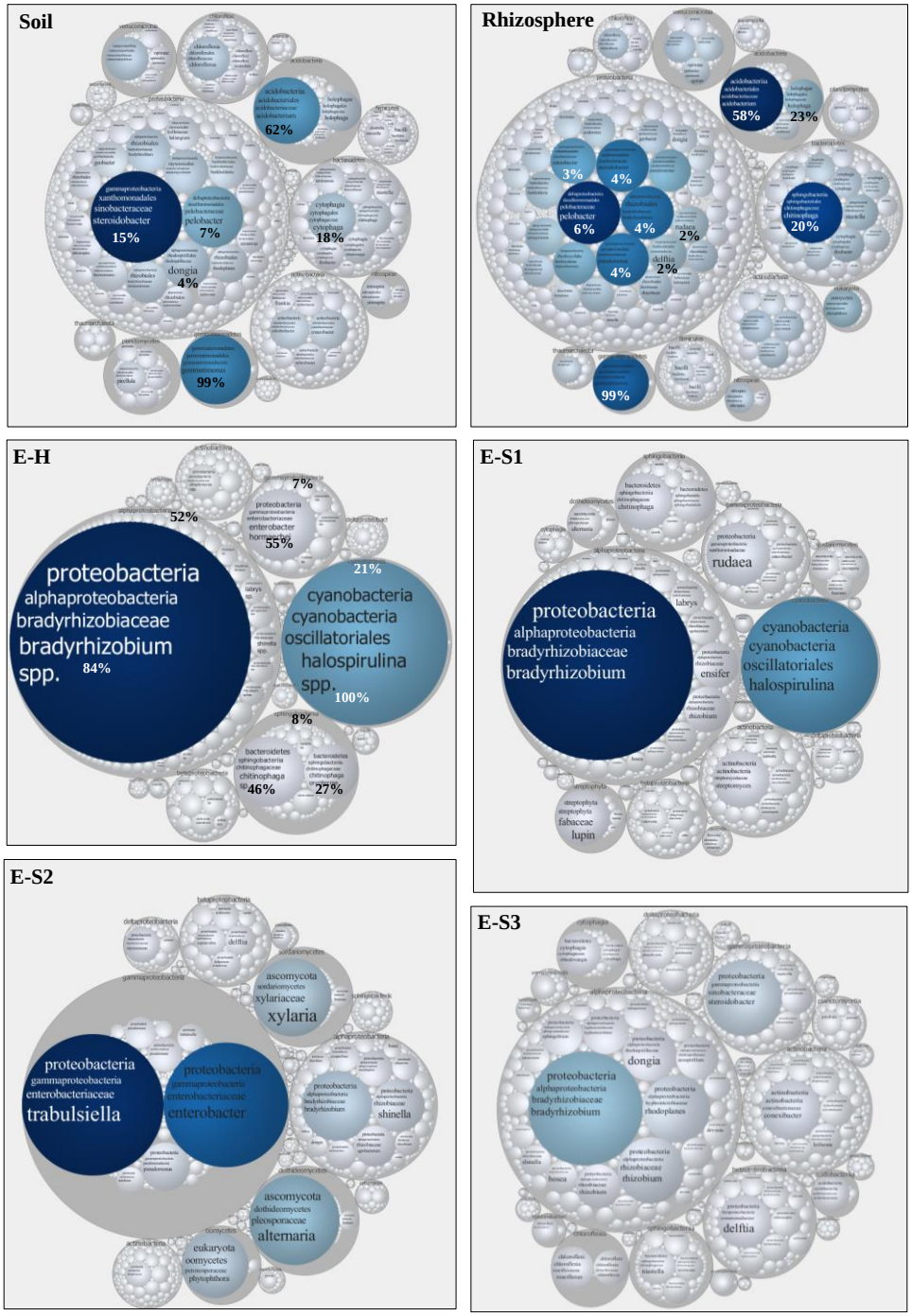
Supplementary figures



Supplemental Figure 1. Overview and distribution of reads extracted from the metagenome. A and B) Graphs summarize cumulative numbers of total high-quality reads for the ITS (A) and 16S rRNA (B) samples. R: rhizosphere fraction, E: endosphere fraction; S: bulk soil fraction; H: healthy roots; S1 to S3: roots at diverse stages of TRD. C and D) Dendrogram analysis. Hierarchical clustering of samples based on pairwise Bray Curtis Similarity (Ward clustering algorithm).



Supplemental Figure 2. Taxonomic classification maps of the healthy and diseased mycobiomes of soybean. Circular maps are reporting taxonomic differences in root endophyte communities from two soil samples, an average of four rhizosphere fractions, and the endosphere healthy fractions of soybean roots. Taxonomic differences are based on ITS sequencing and OTUs with the highest abundance (n > 0.2 %). The largest circles represent class; the inner circles show phylum, order, and genus. The size of the map circle is proportional to the reads number. Numbers indicate the relative percentage abundance of the most abundant categories for each condition.



Supplemental Figure 3. Taxonomic classification maps of the healthy and diseased soybean microbiomes. Circular maps reporting taxonomic differences in bulk soil and root microbial communities from two soil samples, an average of four rhizosphere fractions, and the endosphere healthy and TRD symptomatic plants. Taxonomic differences are based on 16S rRNA sequencing and OTUs with the highest abundance (n > 0.2 %). The largest circles represent class; the inner circles show phylum, order, and genus. The size of the map circle is proportional to the reads number. Numbers indicate the relative percentage abundance of the most abundant categories for each condition.