TITLE:

The phytobiomes of a three-crop rotation: Influence of land management, host, and plant organ on microbial diversity

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ABSTRACT:

Microbiomes from maize and soybean were characterized in a long-term crop rotation research site, under four different land management strategies, to begin unraveling the effects of common farming practices on microbial communities. The fungal and bacterial communities of leaves, stems, and roots in all crops were characterized across the growing season using amplicon sequencing. The wheat rotation has been previously characterized and included here for comparison. Communities differed across plant growth stages and organs, and these effects were most pronounced in the bacterial communities of wheat and maize above-ground organs. Roots consistently had the highest number of unique bacterial OTUs compared to above-ground organs, whereas the alpha diversity of fungi was similar above- and below-ground. Network analyses identified putatively influential members of the microbial communities of the three crops. The fungal OTUs specific to roots, stems or leaves were examined to determine if the specificity reflected their life histories based on previous studies. The analysis suggests that fungal dispersal mechanisms are drivers in organ specificity of the fungal community. Understanding the microbial community structure across the organs of crop plants and identifying influential taxa will provide a critical resource for researchers in future manipulations of naturally occurring microbial communities to reduce plant diseases and to increase crop yields. The ability to predict how these microbes will be dispersed to the target organ will enhance our ability to introduce them sustainably.

INTRODUCTION:

Maize, soybean, and wheat are major crops in the United States, with approximately 88, 83, and 47 million acres planted in the US annually, respectively (USDA 2016). For these crops, fungal and bacterial diseases are the major causes of losses (Mueller et al. 2017). Historically, studies of host-microbe interactions have focused on microbial communities of a single crop (Karlsson et al. 2017), and often are studied in a field or greenhouse (Granzow et al. 2017; Johnston-Monje et al. 2016; Liu et al. 2017). However, in the Midwestern US, these three crops are frequently grown in rotation. If winter wheat is in the rotation, continuous ground cover throughout the year helps stem soil erosion and retain moisture. Effects of crop rotations on crop microbiomes have not been well studied and our knowledge in this area will inform our ability to manipulate microbiomes for increased yield and protection against plant disease.

Plant scientists have used high-throughput sequencing technologies (such as Illumina MiSeq) to investigate microbial communities of plants; these technologies have expanded our understanding of the function of individual microbes and microbial communities in association with various plant organs. Root-associated microbiota can fix nitrogen (Bhuvaneswari et al. 1980; van der Heijden et al. 1998), leaf-associated microbiota play roles in carbon cycling (Abanda-Nkpatt et al. 2006, Vorholt 2012), and stimulate plate defenses (Vogel et al. 2016). Assembly of microbial communities is dictated by a combination of host genetics and stochastic factors (Chen et al. 2020; Hassani et al. 2020; Maignien et al. 2014).

Surveys of microbial communities on wild plants and agricultural systems have been conducted but the studies of agricultural plants have been limited in scope. Many have focused on root-associated microbiota (for example Nettles et al. 2016; Peiffer et al. 2013; Rojas et al. 2017), and many have focused on bacterial communities only (for example Mashiane et al. 2017; Rascovan et al. 2016). Studies of fungal communities in row crops have often focused on one plant organ, most commonly roots or rhizosphere, and less commonly leaves or phyllosphere (for example Karlsson et al. 2017; Sapkota et al. 2017). Several studies have looked at microbial responses to various land management (Longley et al. 2020) or rotation strategies, but these often use field sites that are not a nominal distance, so observed community differences are confounded by field location (Peiffer et al. 2013).

In the present study, we aimed to bridge the shortcomings in other studies by conducting a comprehensive community analysis. We investigated the fungal and bacterial communities of soybean, wheat, and maize in a three-year rotation cycle under different land management strategies. The investigation elucidated the microbial communities associated with each of the plant organs (root, stem, leaf) and the change in communities across growth stages. To achieve this goal, we analyzed the fungal and bacterial microbial communities of one-hectare replicate plots in a random distributed managed for over thirty years as organic, conventional till, no-till and low input (Robertson 2015). We compared these communities to our previous study of the wheat microbial community at the same site (Gdanetz & Trail 2017). Our analysis of organ associated microbiomes revealed fungi that were specific to a single plant organ and revealed factors that resulted in specificity of fungi to above- or below-ground plant organs.

MATERIALS AND METHODS:

Site Description and Sample Collection

The Michigan State University W. K. Kellogg Biological Station Long-Term Ecological Research site (KBS-LTER) is located in Hickory Corners, Michigan, USA (42.411085, -85.377078). The KBS-LTER Main Cropping System Experiment site has been planted in a wheat-maize-soybean rotation series since 1993, and is organized in randomized, replicated plots under four land management strategies with six replicate plots for each strategy (24 total plots): T1 = conventional till, T2 = no-till, T3 = reduced chemical inputs with red clover cover crop, T4 = organic with red clover cover crop (Robertson 2015). Average annual precipitation for this site is 1,005 mm, with approximately half falling as snow (NCDC 1980-2010 climate normals for the Gull Lake Biological Station, https://lter.kbs.msu.edu/research/site-description-and-maps/general-description/climate-normals). The annual precipitation was 1177 mm, 932 mm, and 1153 mm during wheat, maize, and soybean growing years, respectively. Detailed site and management information can be obtained from https://lter.kbs.msu.edu/research/.

For the 2013 harvest, wheat seeds of the cultivar, Pioneer 25R39 soft red winter wheat (Pioneer High-Bred International, Inc., Johnston, IA, USA), were planted in all plots, but wheat seeds for conventional, no-till, and reduced inputs plots were coated with Gaucho insecticide at purchase (Bayer Crop Science, Research Triangle Park, NC, USA). These have been previously analyzed and reported (Gdanetz and Trail 2017), but the data were used here as a comparison for the three-crop rotation. For the 2014 harvest, a commercial maize hybrid cultivar, Dekalb DKC52-59 maize hybrid (Monsanto

Company, St. Louis, MO, USA), was planted in plots T1-T3; and an organic-approved cultivar, Blue River Hybrids 25M75 organic maize (Blue River Organic Seed, Ames, IA, USA), was planted in T4 plots. For the 2015 harvest, the commercial soybean cultivar, Pioneer P22T69R (Roundup Ready®) soybean seed (Pioneer High-Bred International, Inc., Johnston, IA, USA), was planted in T1-T3 plots; and Viking Organic Soybean Seed, Variety 0.2265 (Albert Lea Seed, Albert Lea, MN, USA), was planted in T4 plots. The conventional, no-till, and low input management used fungicide- and insecticide-treated maize and soybean seed. Plants were collected at three analogous developmental stages for each crop: late vegetative growth, inflorescence, and early seed/ear/pod development (Table S1). At each developmental stage, three intact plants were removed from each of the 24 plots. Fine and thick roots, and above-ground organs (stems and leaves) from each plant were placed in separate sterile sample collection bags (Nasco Whirl-Pak®, Fort Atkinson, WI, USA) and maintained on ice during transport. Roots were rinsed to remove loosely attached soil, and plants were stored at -80°C, then lyophilized. Lyophilized plants were stored at room temperature under a desiccant until grinding and DNA extraction.

Sample and Sequence Processing

Lyophilized plants were separated by organ (root, stem, and leaf) before grinding with a Retsch Oscillating Mill M400 (Verder Scientific, Newtown, PA, USA) and DNA was extracted using the Mag-Bind® Plant DNA Plus Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's protocols with the KingFisher™ Flex (ThermoFisher Scientific, Waltham, MA, USA). Fungal ITS2 and bacterial 16S rRNA gene libraries were generated as described previously and sequenced using Illumina MiSeq 2x250 bp chemistry (Table S1) (Gdanetz & Trail 2017; Kozich et al. 2013; Toju et al. 2012). Sequences are available with the National Center for Biotechnology Information Small Read Archive project. Accession numbers for wheat, maize, and soybean are SRP102192, SRP102245, and SRP120500, respectively.

Forward and reverse read pairs from each library were merged with USEARCH (version v8.1.1861) (Edgar 2010; Edgar et al. 2011; Edgar & Flyvbjerg 2015). Low quality reads, (fastq expected error set to 1.0), and read pairs without mates were discarded (read length was set at 250 bp for 16S and 380 bp for ITS sequences). The 16S and ITS2 libraries from all three crops were concatenated into one large dataset for each barcode region. Sequences were processed as described previously (Gdanetz & Trail 2017). Briefly, the USEARCH pipeline was implemented for chimera filtering and OTU assignment, with the cluster threshold set to 97% similarity. The Ribosomal Database Project Classifier was used for taxonomic assignment, with the 16S and the UNITE ITS training sets for bacteria and fungi, respectively, customized to include the plant hosts (Cole et al. 2013; Deshpande et al. 2016; Wang et al. 2007). OTUs matching plants, mitochondria, and chloroplasts, unidentified at the Kingdom or Domain level, or matching non-target organisms were discarded. Samples were filtered to include OTUs that occurred in at least five samples and this trimmed dataset was used for all downstream analyses.

Analysis of Organ-Specific OTUs

Organ-specific fungal OTUs were identified during core OTU analysis. To reduce the number of OTUs that were associated with an organ due to chance, such as the bloom of fruiting bodies near plant collection sites, organ-specific lists were further filtered to include only OTUs found in a minimum of five replicate plots at the field site. Initial examination of the organ specific fungal OTUs suggested that there may be specificity of some taxa to one organ. Therefore, an indicator species analysis was conducted to determine which fungal OTUs were significantly associated with a crop organ.

A large volume of historic literature, driven mainly by dispersal of fungal plant pathogens, has shown that spore morphology and dispersal mechanisms play a defining role in distribution of fungal propagules. Therefore, we grouped our OTU list based on propagule qualities; size (length on longest side), cell motility, presence of yeast forms, melanization, and means of dispersal. For spore size, we

chose three categories (up to 10 µm, 10-100 µm, and greater than 100 µm) based upon the natural breaks in spore size occurring between large taxonomic groups Motile spores and basidiospores were not placed in size-based groups, but formed separate groups due to their special modes of dispersal in the former, and the unique size continuum in the latter which is dictated by size limitations not identified in other fungal taxa (Fischer et al. 2010). Spore pigmentation is also characteristic of some types of spores, melanization was included when known. Dispersal mechanisms included aerial dispersal, water dispersal (due to rain, pools, or flowing water), soil dispersal and hypogeous. Finally, yeasts are often present on plant surfaces, and we distinguished those species that are often in a yeast form. The category "other" was composed of fungi which had less than 5 representative significant OTUs in the indicator species analysis or categories with greater than 0.2% mean relative abundance, which included; spores up to 10 µm dispersed by water in the soil or aerially, spores up to 10 µm melanized and dispersed aerially, spores up to 10 µm hypogeous, spores 10 to 100 µm dispersed by insects, soil, water, or were hypogeous, spores greater than 100 µm dispersed by water or aerially, and yeasts dispersed aerially or by water.

To perform the indicator species analysis, samples with less than 1000 reads were dropped, then read counts were normalized using the cumulative sum scaling technique with 'metagenomeSeq' package (Paulson et al. 2013). Indicator species analysis was performed with the R package 'indicspecies' (version 1.7.6) to identify OTUs significantly associated with crop organs (De Caceres & Legendre 2009). Rare taxa with a mean relative abundance below 10^{-5} within each crop species were not included in the analysis and a Benjamini-Hochberg p-value correction was used for multiple testing. Following indicator species analysis, the compositional abundance of each OTU was plotted in a ternary diagram using 'ggtern' (Hamilton & Ferry 2018) and colored by spore dispersal category.

Community Analysis

Venn diagrams and tables for core taxa analysis were generated using the gplots package (Warnes et al. 2016) in the R statistical computing environment (version 3.3.3). Before generating bar plots, taxa were merged at Class level. Taxa that were present in less than 3% of the samples were removed. Relative abundances of taxa were calculated as a percentage of total sequences in each sample. OTUs per sample were normalized with variance-stabilizing normalization and Bonferroni-corrected p-values were used to identify significantly different OTUs across samples with the 'DESeq2' package (McMurdie & Holmes 2014).

To determine the effect of management strategy on microbial community diversity, comparison between crop species, organs with and between each growth stage, and comparisons between management were made for each crop. Alpha diversity statistics (abundance transformation, observed OTUs, and Shannon's Index) were calculated with the 'phyloseq' package (McMurdie & Holmes 2013). Non-rarefied data were used to calculate the Shannon Diversity Index (H'), an alpha diversity metric that measures species diversity within a sample (McMurdie and Holmes 2014), and analysis of variance (ANOVA) and Tukey's honest significant difference test (HSD) were used to determine significance. Non-metric multidimensional scaling (NMDS) ordination analysis was conducted using Bray-Curtis distance values, a measure of species diversity between communities. Permutational analysis of variance (PERMANOVA) tests were completed using the adonis function in the 'vegan' R package (Oksanen et al. 2016). Heterogeneity of the variances was calculated using the betadispersion function of the 'vegan' package (Oksanen et al. 2016). Figures were generated with the 'ggplot2' package (Wickham 2009).

Hub taxa, species that influence the presence of others in an environment, were identified as highly connected nodes based on calculations by Agler et al. Fungal and bacterial OTU tables were concatenated before calculating the co-occurrence network, leaf- and stem-specific OTUs were grouped together as phyllosphere samples and filtered to include OTUs with ≥20 reads in ≥10 samples using the SpiecEasi package in R (Kurtz et al. 2015). Network statistics and network plots were generated using

Cytoscape (version 3.5.1, http://www.cytoscape.org). Network statistics, specifically log-transformed degree (number of connections between nodes), betweenness centrality (measure of nodes with shortest connections to other nodes), and closeness centrality (measures distances between nodes), were fit to a normal distribution, and OTUs in the 75th and 95th percentile across all three statistics were identified as putative hub taxa.

RESULTS:

Observed and Core Taxa

We analyzed bacterial and fungal communities of maize and soybean grown under four management strategies over a two-year period (maize in 2014 and soybean in 2015) using fungal (ITS2) and bacterial (16S) rRNA gene amplicon sequencing. To investigate the effect of crop management strategy (organic, conventional, low input, no-till) on microbiomes of leaf, stem, and root; samples were collected across replicate plots throughout the season at three growth stages (Table S2). The analysis included the same amplicon sequencing for wheat from the 2013 field season (Gdanetz & Trail 2017) to include one full rotation cycle from this field site.

Following filtering and quality control, high quality reads were identified for fungi and bacteria, respectively, with means of 21,245 fungal and 42,166 bacterial reads per sample (Table S3). No fungal sequences from soybean T4 stems and roots passed quality control (Table S4) and thus were not included in the rest of the analysis. A total of 4,739 fungal and 8,942 bacterial high quality OTUs were identified for maize and soybean combined. Excluding off-target kingdom and domain matches, the combined data from all three crops revealed 7,233 fungal and 12,673 bacterial OTUs (Table S3).

Unique and shared OTUs were compared among the three crops and plant organs (roots, stems, leaves). Highly abundant bacterial Classes Actinobacteria, Alphaproteobacteria, Bacilli, Betaproteobacteria, and Gammaproteobacteria were generally shared across all crops (Fig. 1). Of the bacterial and fungal OTUs, fewer OTUs were unique to soybean plants (Figs. S1a-b). Bacterial communities were similar across all management strategies within a crop at the Class level (Fig. S2), however we observed differences among crops and organs (Fig. 1).

OTUs on maize and soybean showed organ-specificity, similar to what was observed for wheat from the KBS-LTER site (Gdanetz & Trail 2017). Roots consistently had the most organ specific bacterial OTUs, with higher numbers of bacterial OTUs unique to roots than shared among organs in maize and soybean (Fig. 1). Soybean roots contained 88% of the host-specific bacterial taxa, compared with 93% in wheat and 87% in maize (Fig. S1). In maize roots, 246 OTUs were significantly different in abundance across management strategies and growth stages ($p<0.01$, Table S5). In total, 255 bacterial OTUs from roots were identified as significantly different across management strategy and growth stage ($p<0.01$, Table S5). The phyllosphere organs of each crop, leaves and stems, revealed similar Classes of bacterial taxa when compared with the roots of the same crop (Figs. 1, S2). Bacterial community composition of wheat and maize above-ground organs changed with growth stage (Fig. 1). OTUs found in maize stems were more consistent between no-till and low input samples than other management strategies (Fig S2). The dominance of Alphaproteobacteria in soybean roots of all management strategies was not surprising since this Class includes Rhizobia.

Fungal OTUs were more uniformly represented across crops than bacteria. At the OTU level, 266 fungal OTUs (5% of total OTUs) were identified as significantly different ($p<0.05$) across management strategies and growth stages (Table S5). The Classes Dothideomycetes, Leotiomycetes, and Sordariomycetes occupied the highest percentages of OTUs for each of the crop organs and growth stages (Fig. 2). Most notable were the Dothidiomycetes, commonly containing plant pathogens and melanized endophytes, which dominated all growth stages and treatments (Fig. 2, S3). Root communities of maize and soybean differed from those found on aboveground organs. Fungal root communities of wheat also differed from aboveground, but these observations were dependent on

plant age (Fig. 2). Wheat root communities were more similar to aboveground communities during vegetative growth (Gdanetz & Trail 2017). Root samples of all crops and stages, except the wheat vegetative stage, had a higher abundance of Agaricomycetes than did the phyllosphere organs (Figs. 2, S3). The Basidiomycota Class Tremellomycetes, predominantly yeast forms, were highly represented in phyllosphere organs on maize, but at very low levels in other samples (Figs. 2, S3). The Glomeromycetes (the group containing the arbuscular mycorrhizal fungi) and Orbiliomycetes (nematode trapping fungi) were more common and abundant in soybean roots than other host plant species (Figs. 2, S3).

Organ-Specific OTUs

We identified unique fungal OTUs for each plant organ across all crops (Fig. 3a-b; Table 1). Although many OTUs were shared across organs (Figs. 1A, S1), there was identifiable organ-specificity to the plant-associated fungal taxa. Across all crops, 2591 root-, 112 stem-, and 2257 leaf-specific fungal OTUs were identified. A total of 1506 root-, 112- stem and 1789- leaf specific OTUS were assigned a taxonomic classification specific enough to determine their spore and dispersal qualities (Table S6); the remaining OTUs could not be assigned spore categories. Spore categories were chosen based on spore size, apparent means of dispersal, type of spore when the type affected dispersal. Spores from 10 to 100 µm comprised 60% of the spores of all taxa identified, and 74% of leaf-specific taxa, 43% of root-specific taxa and 65% of stem-specific taxa, the majority of spore types in every category were associated with taxa that contained melanized spores and were at least in part aerially distributed. These taxa, together with the taxa producing spores greater than 100 µm formed the predominant root-specific fungi (65%). Taxa forming spores up to 10 µm formed 10% of the leaf-specific taxa, 18% of the root-specific taxa, and 11% of the stem-specific taxa.

To visualize the data from the three organs, we used indicator species analysis combined with ternary plots to illustrate the distribution of the spore categories for each of the crops (Fig. 3c, Table S6) and to illustrate the distribution of each of the categories alone (Fig. S4, Table S6). It was apparent that the microbiomes of the maize, soybean, and wheat organs differed in important ways. The taxa forming spores larger than 100 µm were found at relatively high numbers in maize roots and stems, less so in soybean, and were highly reduced in wheat roots. Both soybean and wheat roots and leaves were populated with aerially distributed and melanized taxa from 10-100 µm. The zoospore forming taxa were almost exclusively in roots, with wheat harbored the most out of all three plant hosts. Maize harbored the greatest numbers of leaf-specific basidiospore producing taxa. Finally, maize appeared to have the most discrete stem-specific taxa and harbored more yeast-producing taxa on the leaves and stems than the other crop hosts. The plant associations of those OTUs that showed significant association with a specific organ but could not be assigned a taxon are shown as pale grey in Figs. 3c and S4 (n.d./n.d.), where their distribution across the different organs followed that of all of the organ specific OTUs.

Community Diversity

Within a growing season, plots under all management strategies were planted with the same crop species. For low input and organic plots, a cover crop was planted after harvest. During the maize and soybean years, all of the plots except organic were planted with transgenic cultivars. Transgenic and conventional cultivars did not differ significantly in alpha diversity (as measured by *H*') of fungal and bacterial communities when comparing the same growth stage and plant organ (Fig. S5, Tables S7-S8). The effect on presence or absence of cover crops on fungal and bacterial communities inhabiting each of the organs (leaf, stem, or root) was compared for each collection period and crop species. *H'* of fungal communities were significantly different between plots with and without cover crops in soybean stems during seed development stage only (Fig. S6, Tables S7-S8). *H'* of root bacterial communities during flowering of wheat (Fig. S6, Tables S7-S8) was significantly lower in plots with cover crops ($p < 0.001$).

There were no other observed differences in alpha diversity between crops with and without cover crops (Fig. S6, Tables S7-S8).

Effect of management strategies on the microbial community within organs was assessed. Overall, bacterial alpha diversity of wheat, maize, and soybean roots was significantly higher than *H'* of leaves and stems of these crops, but there was not a significant difference in *H'* among fungal communities of crop organs (Figs. 4c-d, Tables S7-S8). Bacterial *H'* of maize roots was higher than that of above-ground organs at all growth stages and under all management strategies, this was a significant increase except in leaves and stems of conventional, no-till, and reduced input of maize at the vegetative stage (Figs. 4c, S7b, Tables S7-S8). The bacterial diversity of soybean roots was more variable than maize and decreased across the growing season (Figs. 4d, S7b). Bacterial communities of soybean leaves and stems during pod development from all management strategies had significantly higher *H'* compared to roots, except stems from the organic management strategy, due to the dominant presence of Rhizobia OTUs (Tables S7-S8). The mean fungal diversity of maize roots trended lower than maize above-ground organs at the vegetative stage but was significantly lower when compared with fungal diversity of maize leaves from conventional plots (Fig. 4a, Tables S7-S8). Whereas fungal diversity in soybean roots decreased as plants aged, this trend was only significant within the low input plots (Fig. 4b, Tables S7-S8).

Beta diversity of microbial communities was visualized with NMDS plots generated from Bray-Curtis distances (Figs. 5, S8). Global analysis revealed clusters for each crop species and segregation was clear for above-ground versus below-ground samples in fungi (Figs. 5a, S8a, S8c). At the vegetative growth stage, wheat bacterial communities from all samples formed a clearly defined cluster, while maize and soybean communities shared a segregation between above- and below-ground; however, the separation of wheat from the other crop species was lost in the later growth stages (Fig. 5b). Within maize (Figs. S8a-b) and soybean communities (Figs. S8c-d), there was clustering of fungal and bacterial communities by plant organs. Centroids of clusters were significantly distinct as determined by PERMANOVA, and variances were also significantly different ($P < 0.001$, Table S9). Within each growth stage, there was not clear clustering of fungal and bacterial communities by land management strategy; although, root samples formed clusters separate from above-ground organs (Fig. S8). NMDS plots of management strategies had significant centroids ($p < 0.01$); significantly different variances were observed for fungal communities ($p < 0.05$) but were not significant for bacterial communities (Table S9). Ordination analysis of the effect of cover crop or cultivar on microbial communities, showed similar patterns to ordination analysis of management strategy; points segregated by above- and below-ground samples (Figs. S10-S11, Table S9).

Microbial Networks

Microbial hubs, species that influence the presence of others in an environment, are defined as nodes with a high degree of connectivity, betweenness centrality, and closeness centrality. Networks included OTUs from all management strategies, and growth stages. Networks from individual growth stages of one crop did not meet filtering requirements, therefore all growth stages were analyzed together. Across all three crops, 111 hubs were identified, and using more stringent criteria (95th versus 75th percentiles), the number of hub taxa was reduced to 17 (Table 2). Of the hub taxa, nine bacterial hubs were shared between wheat roots and the phyllosphere (Table 2, Figs. 6, S12). All hub taxa in wheat roots were correlated with at least one other hub taxon in the network, and six of these hubs had at least one negative correlation with another node (Fig. 6). *Metarhizium* was the only hub taxon in the wheat phyllosphere not correlated with other hub taxa (Fig. S12). Of the wheat phyllosphere hub species, *Acidovorax* sp., *Discula destructiva, Helvella dovrensis, Luteolibacter* sp., *Metarhizium* sp., *Methylophilus* sp., *Pedobacter* sp., and *Taibaiella* sp. did not have any negative co-occurrence (Fig. S12).

The bacterial hub taxa in maize roots were frequently positively correlated with other bacterial species. However, bacterial hubs had negative correlations with fungal nodes, with the exception of *Myxococcaceae* sp. and *Pseudonocardineae* sp., which were negatively correlated with each other (Fig. 7a). Glomeromycota, a group of fungi which form endomycorrhizal symbioses with plants, formed a small isolated network not connected with the main maize root network (Fig. 7a). Hub taxa in the maize phyllosphere were largely identified as fungi, with only four hubs identified as bacteria (Fig. S13).

Hub taxa were shared across crop organs, and they were also shared across crop species. *Discula destructiva* was identified as a hub taxon in both wheat and maize phyllospheres, no hub taxa were shared with the soybean phyllosphere. Four hub taxa were shared across maize and soybean roots (Table 2). Only one hub taxon, *Neosetophoma* sp., was shared between soybean root and phyllosphere networks (Figs. 7b, S14). Closely related taxa, such as several nodule-forming bacterial OTUs (*Bradyrhizobium*, *Mesorhizobium*, *Neorhizobium*) frequently occurred together in the networks. *Fusarium* sp. was present in all the co-occurrence networks and was identified as a hub taxon in the wheat phyllosphere.

DISCUSSION:

Here we present an in-depth comparative analysis of fungal and bacterial microbial communities that colonize the three major US row crops (wheat, maize, and soybean) and have assessed the microbiomes across different management strategies (no-till, conventional, organic, and low input) that have been in place for 30 years. Although these plant hosts share microbial taxa, the communities also harbor distinct OTUs for each crop species, as determined by core taxa analysis and diversity metrics. Global analysis revealed distinct community clusters for above-ground versus below-ground organs. Furthermore, we identified a set of OTUs specific to each plant organ: leaves, stems and roots. The majority of taxa that uniquely colonize specific plant organs as identified in the current work, and which were classified to the genus or species level, were associated with spore morphologies that would maximize dispersal to those organs. In a composite analysis of all treatments combined, we demonstrate that a subset of fungi were specifically and uniquely associated with a single plant organ: leaf, stem, or roots. These organ-specific taxa represent a small subset of the fungal microbiome of the three-crop rotation. However, they provide some clues as to the assembly of the microbiome, likely driven by delivery of propagules. For most fungi, the spores are the agents of dispersal, having evolved to optimally distribute propagules to desirable areas (Fischer et al. 2010; Pringle et al. 2016). Therefore, we hypothesized that a major determining factor for organ specificity is the dispersal mechanism of each of these taxa.

Fungi are niche-adapted to effectively disperse, with highly evolved modes of release and distribution (Deacon 1980) and the impact of fungal adaptation to dispersal is suggested by these results. Modes of dispersal can be illustrated among the spore types of the major taxa found uniquely on specific plant organs. Generally, size differentials are apparent among fungi unique to above and below-ground organs. Root-associated fungi such as arbuscular mycorrhizal fungi are associated with extremely large (greater than 100 µm), generally spherical spores, that evolved to be transported by animals, and remain in the soil where produced (Watkinson 2016). Fungi that dominate in the phyllosphere and are leaf pathogens and/or endophytes typically produce large spores (10 to 100 µm) that are airborne and often melanized, to protect from UV damage and desiccation (Eisenmann & Casadevall 2012). These are represented in our study by the rusts (mainly urediniospores), notable leaf pathogens with spores known for very long-range dispersal (Brown & Hovmøller 2012; Golan & Pringle 2017), and *Alternaria tenuissima*, a pigmented airborne leaf-infecting fungus (Pastor & Guarro, 2008). The distribution of basidiospores to roots as well as leaves may reflect their abundant distribution under the leaf canopy, with basidiocarps forming on the moist soil surface through colonization of rotting plant tissue, and firing spores using the requisite available moisture (Webster et al. 1984).

Contrary to expectations, there was no substantial evidence that management altered microbial diversity more than plant organ or crop species. Previous studies that explored differences in microbial communities among management strategies (such as conventional vs. organic management) analyzed rhizosphere soil communities or conducted comparisons of fields that were large distances apart (for example Bernard et al. 2012; Hartmann et al. 2014; Peiffer et al. 2013; Souza et al. 2015). Studies which surveyed plant microbiomes of the same genotypes across multiple locations found that geography and environment have a stronger influence on microbial community than management and plant genotype (for example Escobar Rodríguez et al. 2018; Finkel et al. 2011). The regional microbial reservoir of the soil may be the biggest influencing factor on the final microbial community composition. Based on the present study, Longley *et al.* (2020), and previous studies, land management affects soil and rhizosphere microbial community composition, but appears to have a weaker impact on plant and phyllosphere microbial community composition (Bálint et al. 2013; Bonito et al. 2014; Coleman-Derr et al. 2015; Naylor et al. 2017; Sapkota et al. 2015).

Although the conventional, no-till, and low input management used fungicide- and insecticide-treated seed, there were no controls for these processes to assess the direct effect of fungicides on the microbiome. Nettles *et al*. (2016) have demonstrated that fungicide seed treatments affect phyllosphere microbiomes of crops tested during the vegetative growth phase. It is not known if this observation would persist to mature plants. Fungicides have also been known to affect mycorrhizal associations and reproduction (Marx & Rowan 1981). However, Plante (Plante 2017) argues that crop management strategies, such as applications of fertilizers and pesticides, are not perturbation events in the traditional ecological sense, which would alter the microbiota found on plants, and thus application of pesticides may be a chronic stress for the native microbiota instead of a perturbation event. However, the data presented in the current study indicate that fungicides may be secondary influencers of microbial community composition, with geography and genotype as the primary influencing factors.

We demonstrate that root-associated microbial communities are distinct from those of the phyllosphere or above-ground organs in the three-crop rotation at KBS-LTER. Studies that analyze microbial communities across multiple organs and tissues on the same plant often show that there are distinct communities across these niches (Coleman-Derr et al. 2015). Yet, there is a bias in the literature toward analyzing soil or rhizosphere microbial communities more often than the phyllosphere (de Souza et al. 2016; Copeland et al. 2015). Although the rhizosphere has been found to have a higher abundance and diversity of plant-associated bacteria and fungi compared to the phyllosphere (present study, Ottesen et al. 2013), microbes inhabiting other plant organs or compartments are also important to plant health (Remus-Emsermann et al. 2018, Ryffel et al. 2015, Vorholt 2012). Research on model systems shows that phyllosphere microbes can influence disease occurrence and nutrient cycling (Delmotte et al. 2009). Neglecting above-ground plant organs, in favor of the soil and rhizosphere, during microbial community analyses excludes microbes that have a life cycle that is not captured by a soil or root-associated phase, for example, important plant pathogens such as rust fungi (Webster & Weber 2007).

Our findings indicate that maize and soybean fungal diversity decreases across the growing season while bacterial diversity remains constant in these two crops. Previous studies examining changes in plant-associated microbial communities in a variety of crops over the course of a growing season have not identified a consistent trend. Some studies found increasing community diversity over time (in apples (Shade et al. 2013) and wheat (Gdanetz & Trail 2017)) whereas decreases have been documented in soybean and sugarcane crops (Copeland et al. 2015; de Souza et al. 2016), or no significant change in wheat (Sapkota et al. 2017). Further studies are needed to examine the plant microbial community dynamics over time to determine if the observed differences throughout the growing season are driven by maturation of the host plant or environmental factors, such as decreasing light. Some aspects of microbial diversity, specifically the alpha-diversity described in this study, appear

to vary temporally across the seasons, but this may also be an artifact of seasonal changes such as moisture (Lauber et al. 2013). Further investigation should be conducted to determine the degree which random colonization by microbes affects the end community composition. Computer modelling of the plant microbiome suggests the initial colonizers influence final community structure (Evans et al. 2016). An understanding of the influence of the initial colonizers on final community composition will have implications for community manipulation in field crops and synthetic environments such as greenhouses or hydroponic systems.

Hub taxa are important members of the plant-associated microbiota that can influence final community composition and plant health (Agler et al. 2016). Identification of hub taxa and understanding their influence on final community composition will be important for community-scale microbial manipulation to improve agriculture. In the current study, hub taxa identified in the plant organs of one crop were not shared across crops. However, these hub taxa are commonly occurring OTUs found across all crops and management strategies in this study. The fungal hub species identified in the current study included pathogens of other plant species. For example, *Discula destructiva,* a hub species of both the wheat and maize phyllospheres, causes dogwood anthracnose. The presence of non-host pathogen hubs can prevent the infection of another pathogen, as demonstrated by (Agler et al. 2016; Chen et al. 2018). As observed in this study and previously described (Berry & Widder 2014), closely related OTUs such as species of Rhizobia, wood rot fungi, or mycorrhizal fungi frequently co-occur in network analyses. Also demonstrated here and as others have shown previously with inferred networks (Agler et al. 2016), microorganisms interact with each other across Kingdoms. Putative hub species need verification by experimental and functional methods to determine their role in microbial communities.

This study shows that multiple organs (root, leaf, and stem) on the same plant often have distinct microbial communities. The microbial reservoir of the soil and land management strategy are factors which can influence the plant-associated microbial communities; however, plants are still able to select for specific microbes. This selection may be in the form of root exudates that recruit microbes (Wu et al. 2015), or leaf chemistry that suppresses spore germination. But in addition, as we show here, fungi evolve to place their spores where they will thrive, so spore dispersal also plays a role in developing the fungal phytobiome community. A detailed understanding of the composition of plant-associated microbial communities will allow growers to add in the influence of the microbiota when calculating pesticide or fertilizer applications in precision agriculture approaches (Tackenberg et al. 2016). Because only a single year was examined for each host, the role of host versus environment in selecting microbiome species is unresolved. Additional years to examine the three-crop rotation will resolve some of these questions. Our long-term goal is to monitor this system to view the stability of the microbiomes across the years, particularly in the light of climate change.

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Author Contributions:
FT and KG designed the experiments. KG managed the field collections, sample preparation and performed the data analysis. ZAN conducted ternary analysis. KG and FT interpreted the data and wrote the manuscript.

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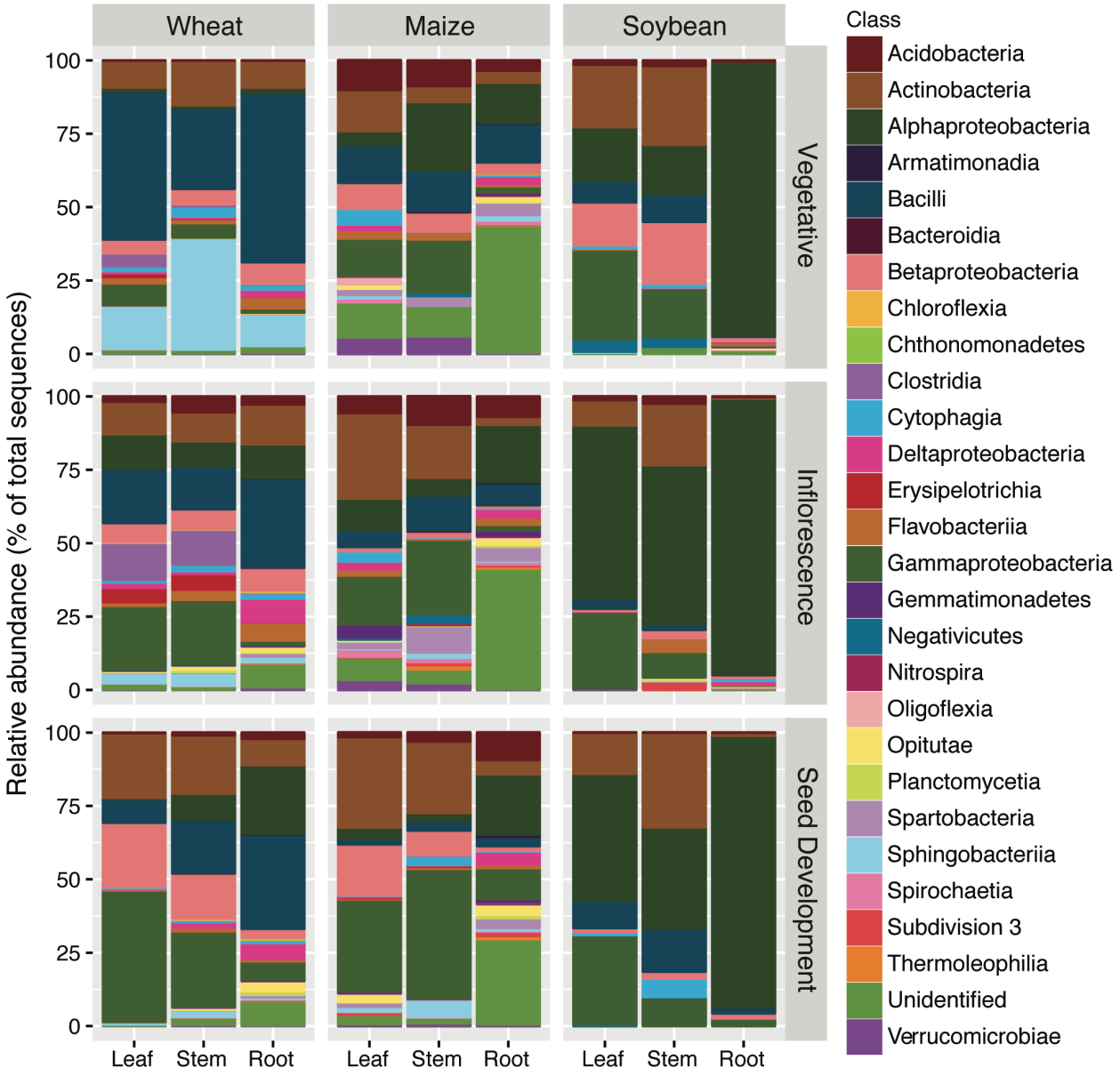

Figure 1. Class-level relative abundance of operational taxonomic units in bacterial communities across crop, organ, and growth stage.

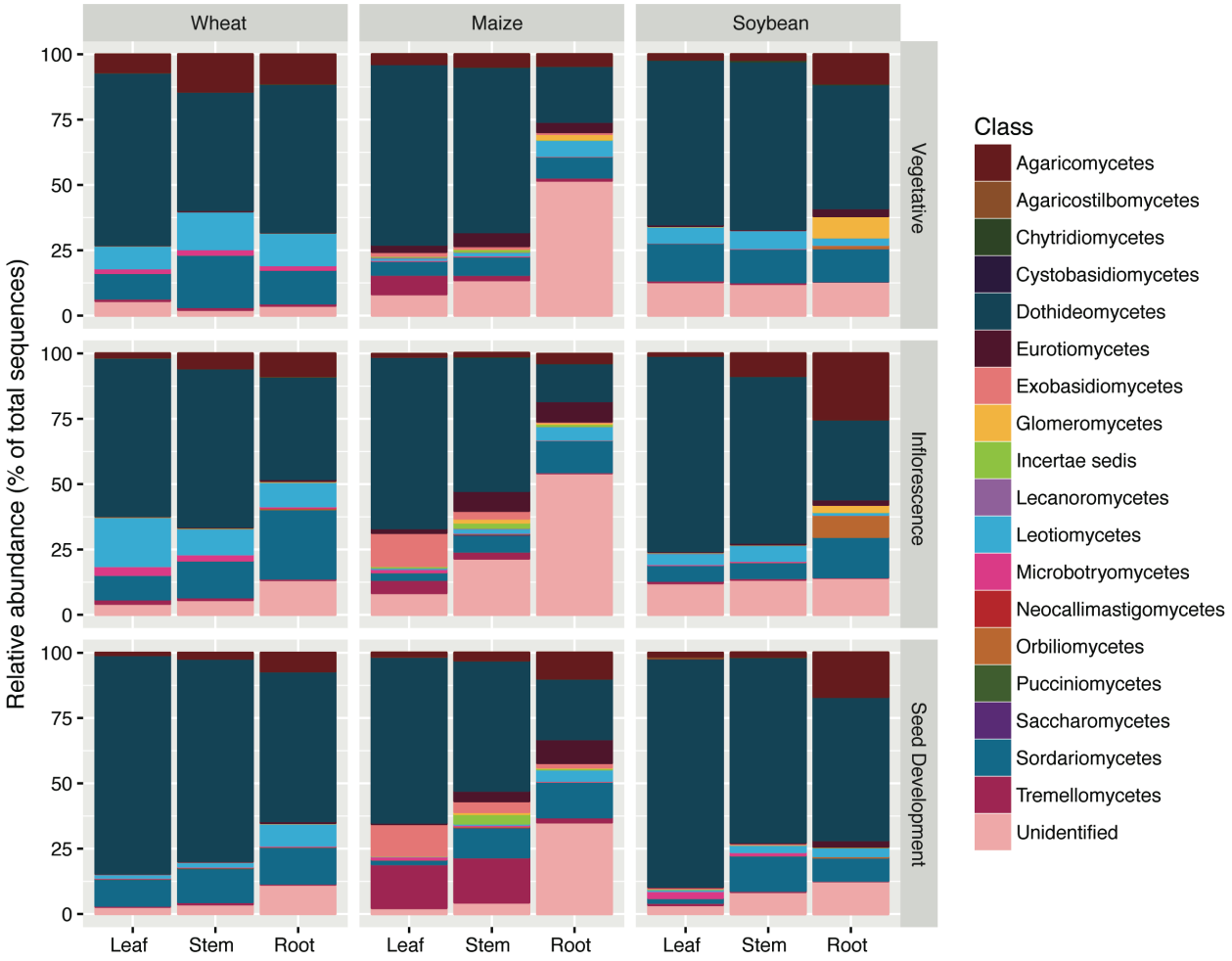

Figure 2. Class-level relative abundance of operational taxonomic units in fungal communities across crop, organ, and growth stage.

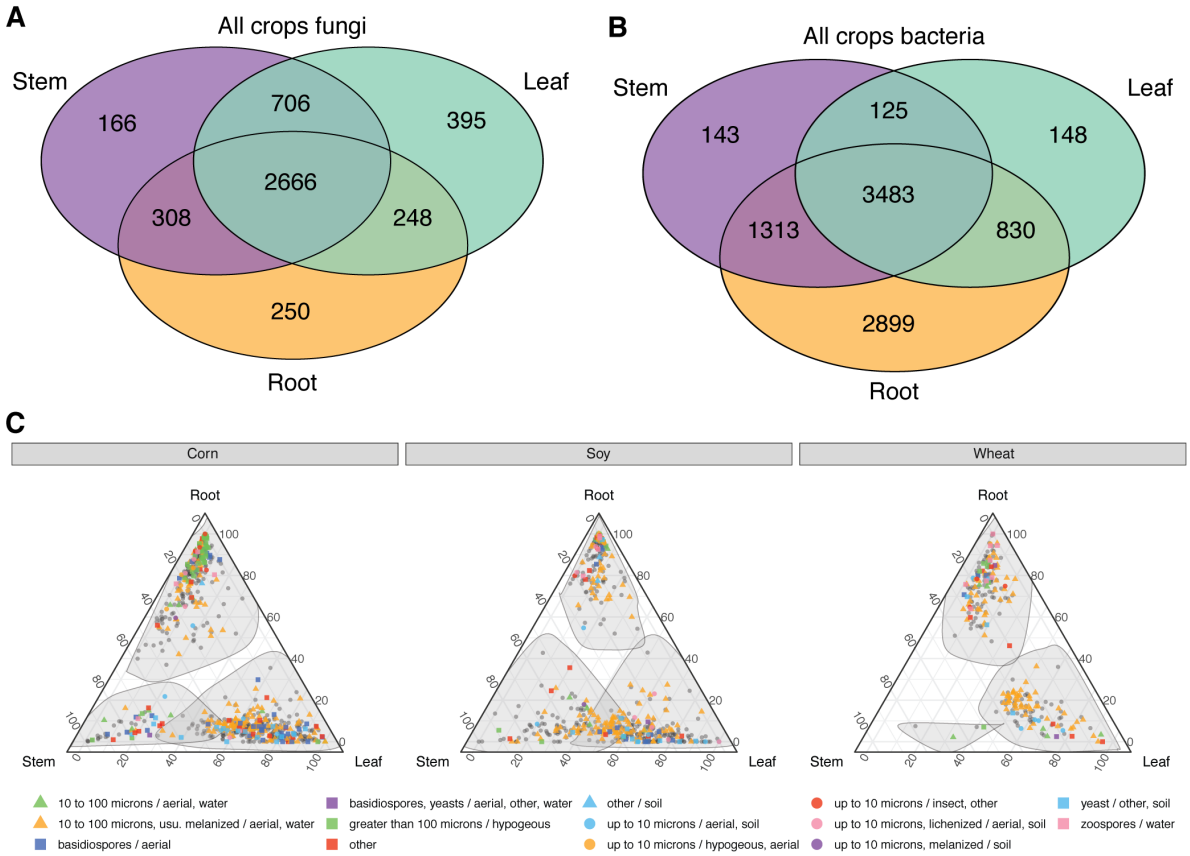

Figure 3. Microbes associated with plant organs. (A) Fungal and (B) bacterial shared and unique OTUs among plant organs. Additional information on spore dispersal and function can be found in Table S5. (C) Ternary plots illustrating the composition of each fungal OTU in each plant organ-crop combination. Colored points were significant indicators of a particular plant organ with a Benjamini-Hochberg corrected P-value ($\alpha = 0.05$). Shaded areas encircle the OTUs that are significant indicators for a plant organ. Points are colored by spore category to illustrate the distribution of spore categories within each plant organ. The distribution of spore categories across crops is shown in Fig. S4.

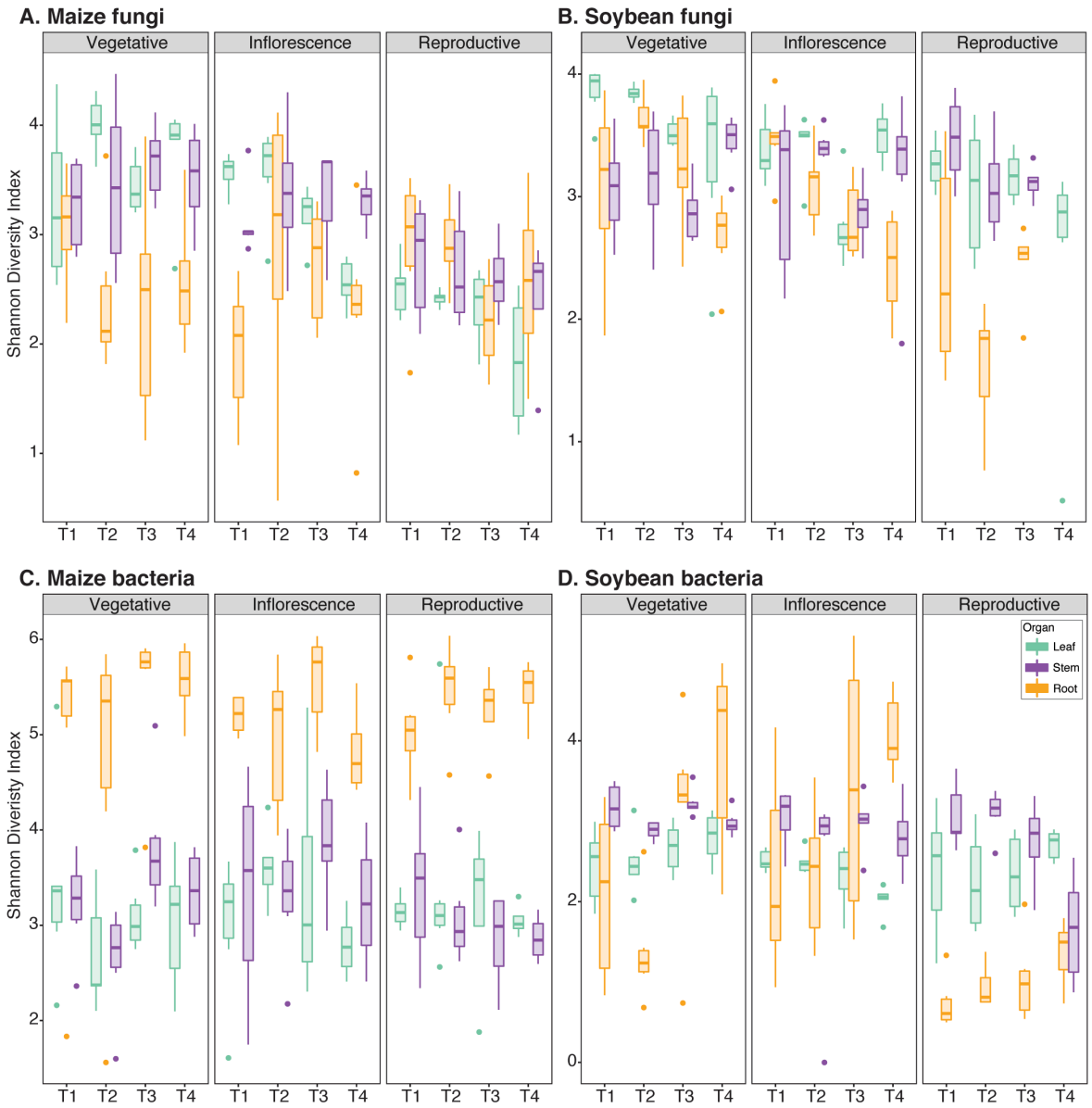

Figure 4. Alpha diversity maize fungi (A), soybean fungi (B), maize bacteria (C), soybean bacteria (D) found on different plant organs across growth stages, and under four different management strategies, estimated by Shannon Diversity Index. T1=conventional, T2=no till, T3=low input, T4=organic. Reproductive growth stage indicates early ear development, and early pod fill for maize and soybean, respectively. Data are represented by six replicates from each stage-management-organ combination. Center line of boxes represents median of samples. The upper and lower sides of the boxes represent the third and first quartiles, respectively. Whiskers represent ± 1.5 times the interquartile range. Data points beyond whiskers represent outliers. Analysis of variance and Tukey's honest significant difference were used to test significance ($P < 0.05$). Statistical support is detailed in Table S5.

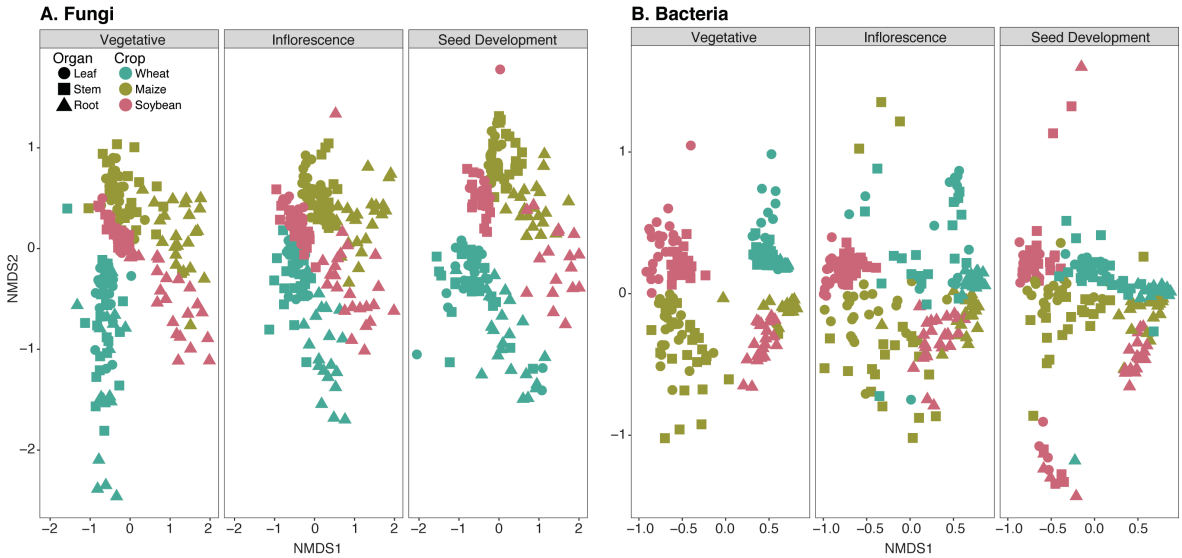

Figure 5. Effect of growth stage on beta diversity of fungal (A) and bacterial (B) communities on all crops. Points colored by crop species, shapes indicate plant organ. Non-metric multidimensional scaling (NMDS) calculated by Bray-Curtis distance. Statistical support is detailed in Table S9.

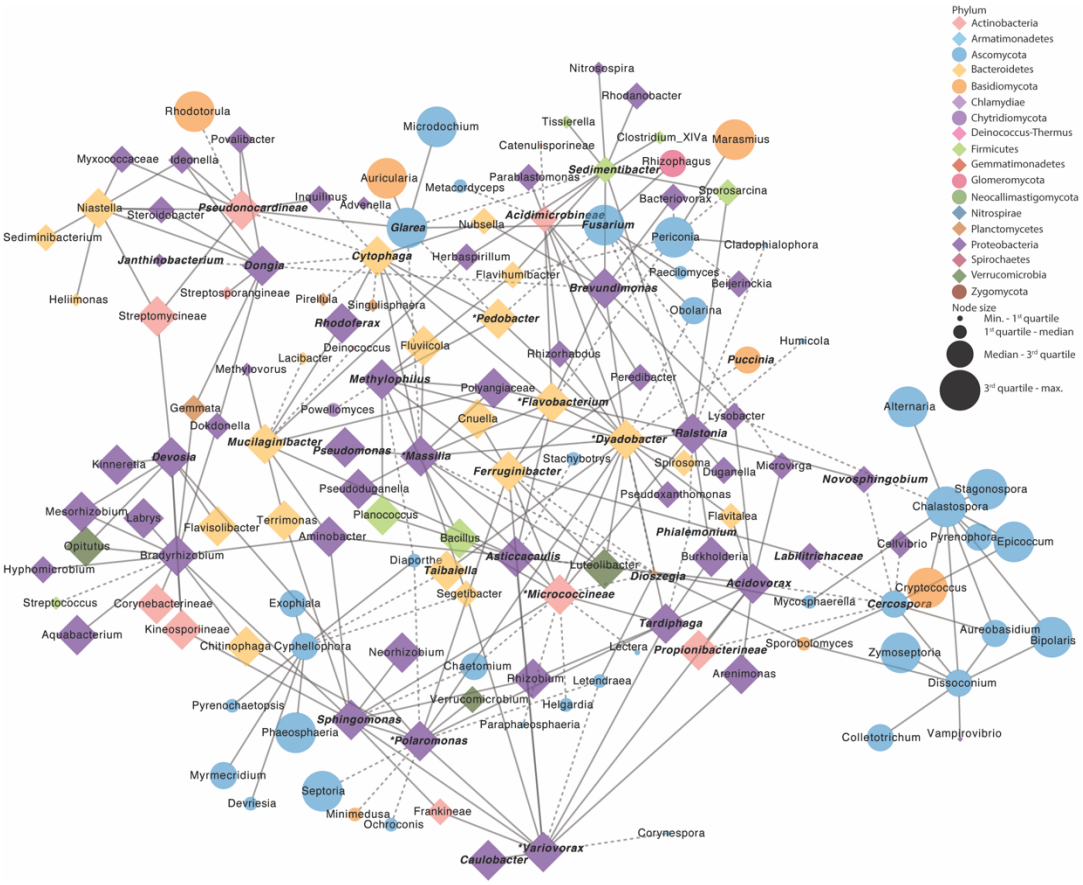

Figure 6. Co-occurrence network of taxa in wheat roots. Bacterial operational taxonomic units (OTUs) are represented by diamond shapes, fungal OTUs are represented by circles, and nodes are colored by class. Solid lines indicate positive correlation, and dashed lines indicate a negative correlation between OTUs. Node size indicates relative abundance, bolded node labels indicated hub taxa at the 75th percentile, node labels marked with an asterisk indicate hub taxa at the 95th percentile.

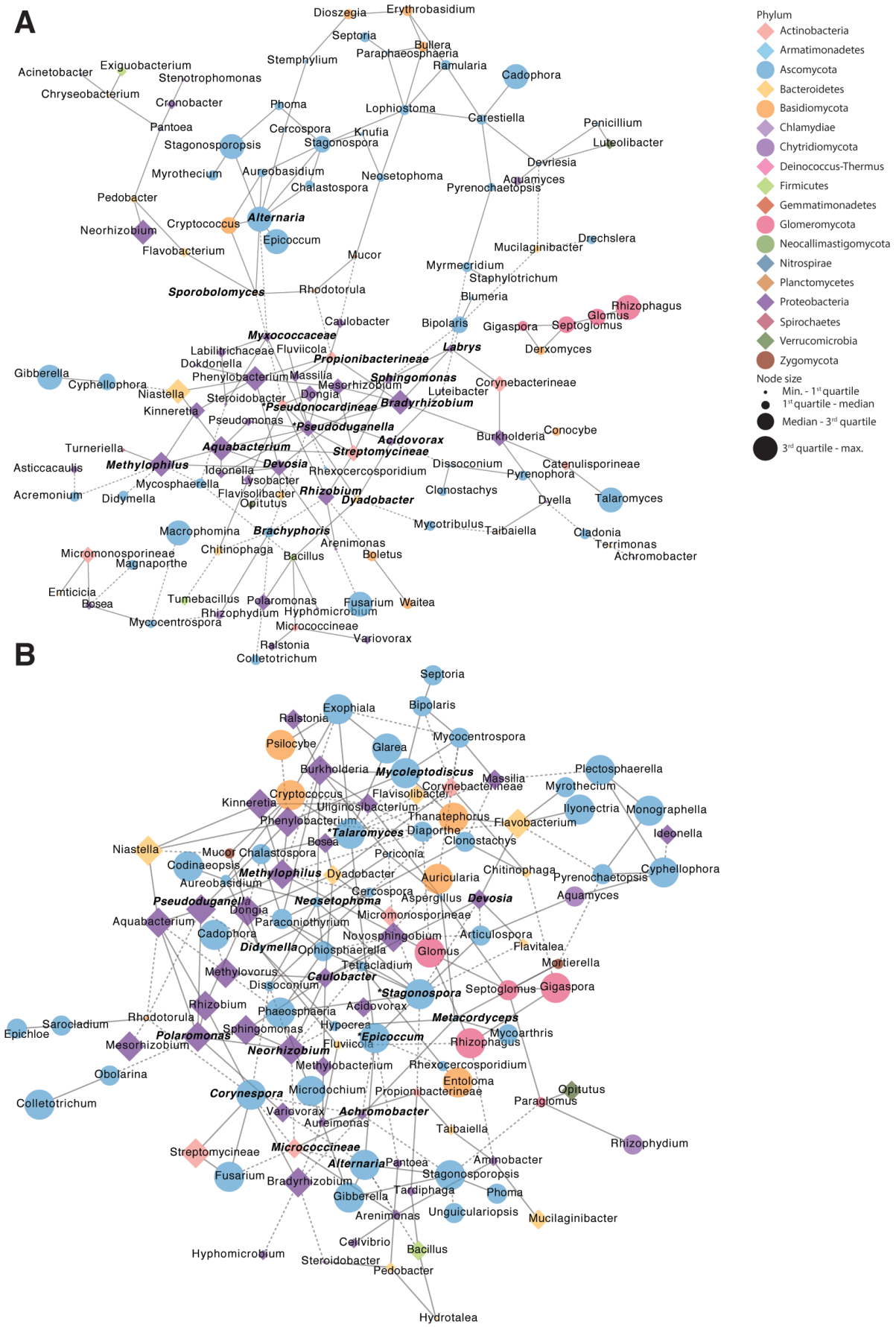
A
B
Phylum
Actinobacteria
Armatimonadetes
Ascomycota
Bacteroidetes
Basidiomycota
Chlamydiae
Chytridiomycota
Deinococcus-Thermus
Firmicutes
Gemmatimonadetes
Glomeromycota
Neocallimastigomycota
Nitrospirae
Planctomycetes
Proteobacteria
Spirochaetes
Verrucomicrobia
Zygomycota
Node size
Min. - 1st quartile
1st quartile - median
Median - 3rd quartile
3rd quartile - max.
Dioszegia
Erythrobasidium
Septoria
Bullera
Paraphaeosphaeria
Stemphylium
Ramularia
Cadophora
Acinetobacter
Exiguobacterium
Stenotrophomonas
Chryseobacterium
Cronobacter
Phoma
Lophiostoma
Carestiella
Pantoea
Cercospora
Knufia
Penicillium
Stagonosporopsis
Stagonospora
Luteolibacter
Devriesia
Myrothecium
Aureobasidium
Neosetophoma
Aquamyces
Chalastospora
Pyrenochaetopsis
Pedobacter
Alternaria
Cryptococcus
Neorhizobium
Epicoccum
Drechslera
Flavobacterium
Mucor
Mucilaginibacter
Myrmecridium
Staphylotrichum
Sporobolomyces
Rhodotorula
Blumeria
Rhizophagus
Glomus
Caulobacter
Bipolaris
Gigaspora
Septoglomus
Myxococcaceae
Labrys
Derxomyces
Labilitrichaceae
Fluviicola
Propionibacterineae
Dokdonella
Gibberella
Phenylobacterium
Massilia
Sphingomonas
Cyphellophora
Mesorhizobium
Corynebacterineae
Niastella
Steroidobacter
Dongia
Luteibacter
Kinneretia
*Pseudonocardineae
Bradyrhizobium
Pseudomonas
*Pseudoduganella
Acidovorax
Burkholderia
Conocybe
Turneriella
Aquabacterium
Streptomycineae
Asticcacaulis
Methylophilus
Devosia
Ideonella
Rhexocercosporidium
Dissoconium
Catenulisporineae
Pyrenophora
Mycosphaerella
Lysobacter
Acremonium
Didymella
Flavisolibacter
Rhizobium
Clonostachys
Dyella
Talaromyces
Opitutus
Dyadobacter
Mycotribulus
Taibaiella
Macrophomina
Brachyphoris
Cladonia
Arenimonas
Terrimonas
Micromonosporineae
Chitinophaga
Bacillus
Boletus
Achromobacter
Magnaporthe
Emticicia
Tumebacillus
Polaromonas
Fusarium
Waitea
Bosea
Rhizophydium
Hyphomicrobium
Mycocentrospora
Micrococcineae
Variovorax
Ralstonia
Colletotrichum
Septoria
Exophiala
Bipolaris
Ralstonia
Mycocentrospora
Psilocybe
Glarea
Burkholderia
Mycoleptodiscus
Massilia
Plectosphaerella
Corynebacterineae
Myrothecium
Cryptococcus
Flavisolibacter
Kinneretia
Uliginosibacterium
Ilyonectria
Monographella
Phenylobacterium
Thanatephorus
Flavobacterium
*Talaromyces
Diaporthe
Ideonella
Bosea
Clonostachys
Niastella
Mucor
Chalastospora
Periconia
Codinaeopsis
Methylophilus
Dyadobacter
Auricularia
Chitinophaga
Pyrenochaetopsis
Cyphellophora
Aureobasidium
Cercospora
Aspergillus
Devosia
Aquamyces
Pseudoduganella
Neosetophoma
Dongia
Micromonosporineae
Aquabacterium
Paraconiothyrium
Cadophora
Novosphingobium
Articulospora
Didymella
Ophiosphaerella
Glomus
Flavitalea
Tetracladium
Mortierella
Methylovorus
Caulobacter
Dissoconium
*Stagonospora
Septoglomus
Gigaspora
Rhizobium
Phaeosphaeria
Acidovorax
Metacordyceps
Sarocladium
Rhodotorula
Hypocrea
Epichloe
Sphingomonas
*Epicoccum
Mycoarthris
Polaromonas
Rhizophagus
Mesorhizobium
Fluviicola
Neorhizobium
Methylobacterium
Rhexocercosporidium
Obolarina
Entoloma
Opitutus
Corynespora
Microdochium
Propionibacterineae
Paraglomus
Colletotrichum
Variovorax
Achromobacter
Aureimonas
Taibaiella
Rhizophydium
Micrococcineae
Streptomycineae
Alternaria
Pantoea
Aminobacter
Fusarium
Stagonosporopsis
Bradyrhizobium
Tardiphaga
Phoma
Gibberella
Arenimonas
Unguiculariopsis
Mucilaginibacter
Cellvibrio
Hyphomicrobium
Bacillus
Steroidobacter
Pedobacter
Hydrotalea

Figure 7. Co-occurrence network of taxa in maize (A) and soybean (B) roots. Bacterial operational taxonomic units (OTUs) are represented by diamond shapes, fungal OTUs are represented by circles, and nodes are colored by class. Solid lines indicate positive correlation, and dashed lines indicate a negative correlation between OTUs. Node size indicates relative abundance, bolded node labels indicated hub taxa at the 75th percentile, node labels marked with an asterisk indicate hub taxa at the 95th percentile.

Table 1. Counts of the number of fungal OTUs significantly associated with leaf, roots, or stems across corn, wheat, and soybean.

Spore Category[a]	Leaf	Root	Stem	Total	Mean % RA
10 to 100 microns, usu. melanized / aerial, water	206	102	35	343	14.191
greater than 100 microns / hypogeous	2	81	6	89	0.556
basidiospores / aerial	31	24	6	61	2.118
10 to 100 microns / aerial, water	10	9	10	29	1.359
yeast / other, soil	20	4	4	28	1.988
up to 10 microns / aerial, soil	13	5	2	20	0.888
zoospores / water	0	15	1	16	0.038
up to 10 microns / hypogeous, aerial	0	13	0	13	0.026
up to 10 microns, lichenized / aerial, soil	7	5	0	12	0.029
up to 10 microns / insect, other	0	10	0	10	0.031
basidiospores, yeasts / aerial, other, water	2	3	1	6	0.021
up to 10 microns, melanized / soil	0	1	1	2	0.256
other / soil	0	3	0	3	0.482
other	5	17	10	32	1.47

[a]Other category includes spore categories with fewer than five representative significant OTUs, or categories with mean percent relative abundance was less than 0.2

Table 2. Hub taxa of KBS LTER crops.

	Root	Phyllosphere
Wheat	*Acidimicrobineae* sp.	*Acidovorax* sp.
	Acidovorax sp.	*Aquabacterium* sp.
	Asticcacaulis sp.	*Devosia* sp.
	Brevundimonas sp.	*Discula destructiva*[a]
	Caulobacter sp.	*Dyadobacter* sp.
	Cercospora olivascens[a]	*Ferruginibacter* sp.
	Cytophaga sp.	*Flavobacterium* sp.
	Devosia sp.	*Helvella dovrensis*[a]
	Dioszegia hungarica[a]	*Luteolibacter* sp.
	Dongia sp.	*Massilia* sp.
	Dyadobacter sp.*	*Metarhizium anisopliae*[a]
	Ferruginibacter sp.	*Methylophilus* sp.
	Flavobacterium sp.*	*Micrococcineae* sp.*
	Fusarium pseudensiforme[a]	*Pedobacter* sp.
	Glarea lozoyensis[a]	*Polaromonas* sp.*
	Janthinobacterium sp.	*Rhizobium* sp.*
	Labilitrichaceae sp.	*Septoria glycinicola*[a]
	Massilia sp.*	*Taibaiella* sp.
	Methylophilus sp.	*Tardiphaga* sp.
	Micrococcineae sp.*	
	Mucilaginibacter sp.	
	Novosphingobium sp.	
	Pedobacter sp.*	
	Phialemonium globosum[a]	
	Polaromonas sp.*	
	Propionibacterineae sp.	
	Pseudomonas sp.	
	Pseudonocardineae sp.	
	Puccinia recondita[a]	
	Ralstonia sp.*	
	Rhodoferax sp.	
	Sedimentibacter sp.	
	Sphingomonas sp.	
	Taibaiella sp.	
	Tardiphaga sp.	
	Variovorax sp.*	

Maize	*Acidovorax* sp.	*Cladonia* sp.[a]
	Alternaria alternata[a]	*Diaporthe caulivora*[a]
	Aquabacterium sp.	*Discula destructiva*[a]
	Brachyphoris oviparasitica[a]	*Exophiala pisciphila*[a]
	Bradyrhizobium sp.	*Kinneretia* sp.
	Devosia sp.	*Kluyvera* sp.
	Dyadobacter sp.	*Lactococcus* sp.
	Labrys sp.	*Lophiostoma macrostomum*[a]
	Methylophilus sp.	*Ophiognomonia sogonovii*[a]
	Myxococcaceae sp.	*Paraconiothyrium hawaiiense*[a]
	Propionibacterineae sp.	*Ralstonia* sp.
	Pseudoduganella sp.*	*Schizophyllum commune*[a]
	Pseudonocardineae sp.*	*Stagonosporopsis loticola*[a]
	Rhizobium sp.	*Talaromyces aculeatus*[a]
	Sphingomonas sp.	
	Sporobolomyces coprosmae[a]	
	Streptomycineae sp.	
Soybean	*Achromobacter* sp.	*Articulospora* sp.[a]
	Alternaria alternata[a]	*Bensingtonia subrosea*[a]
	Caulobacter sp.	*Cercospora olivascens*[a]
	Corynespora cassiicola[a]	*Neosetophoma* sp.[a]
	Devosia sp.	*Rhodotorula ingeniosa*[a]
	Didymella phacae[a]	*Schizopora flavipora*[a]
	*Epicoccum nigrum**[a]	*Sporobolomyces linderae*[a]
	Metacordyceps chlamydosporia[a]	*Thanatephorus cucumeris**[a]
	Methylophilus sp.	
	Micrococcineae sp.	
	Mycoleptodiscus terrestris[a]	
	Neorhizobium sp.	
	Neosetophoma sp.[a]	
	Polaromonas sp.	
	Pseudoduganella sp.	
	*Stagonospora pseudovitensis**[a]	
	*Talaromyces aculeatus**[a]	

*95th percentile

[a]fungal OTU

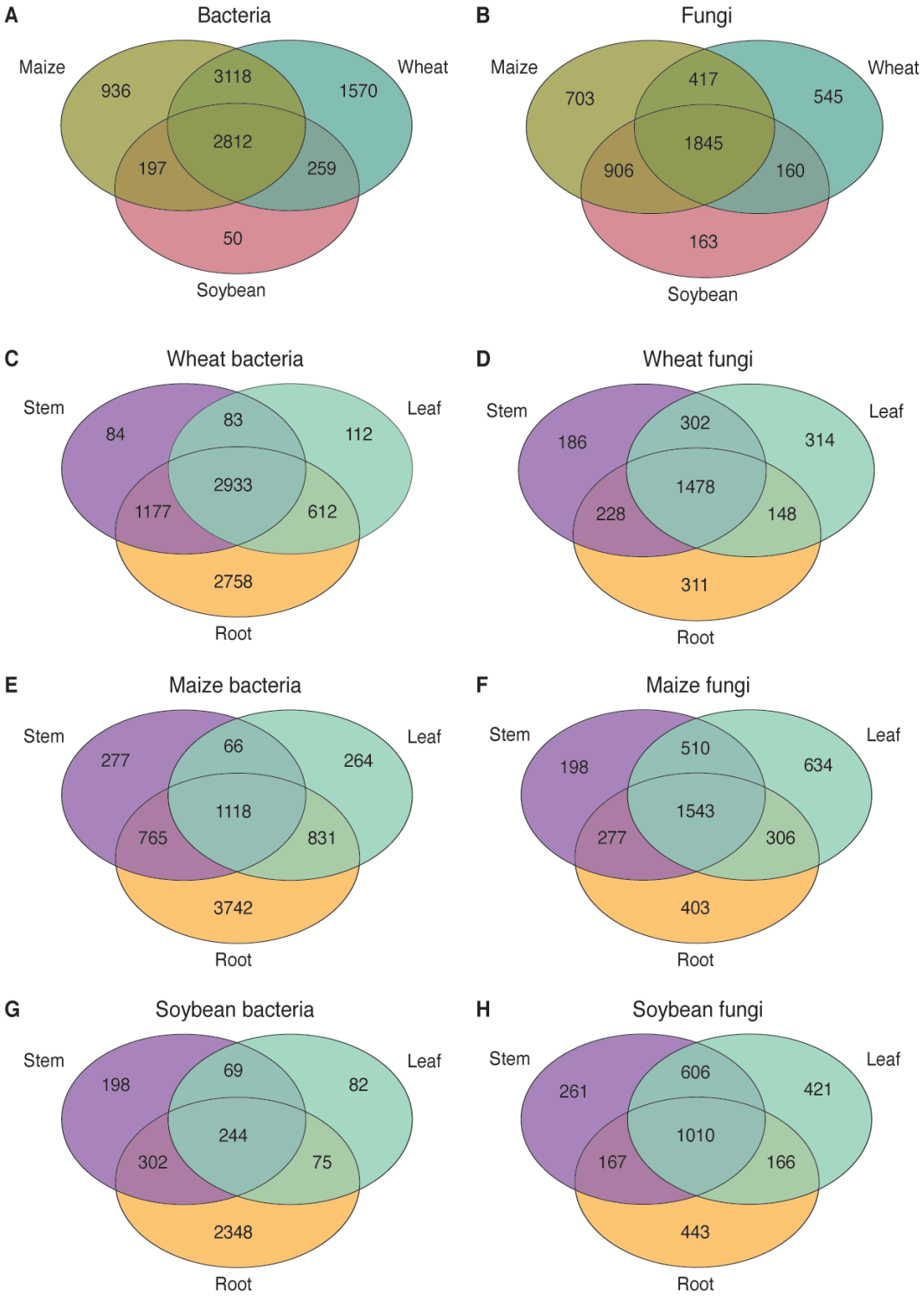

Figure S1 Distribution of OTUs across crops and organs. Shared and unique operational taxonomic units across whole plants (A, B) and organs. (C, E, G) Total bacterial OTUS in wheat, maize, and soybean respectively. (D, F, H) Total fungal OTUs in wheat, maize, and soybean, respectively.

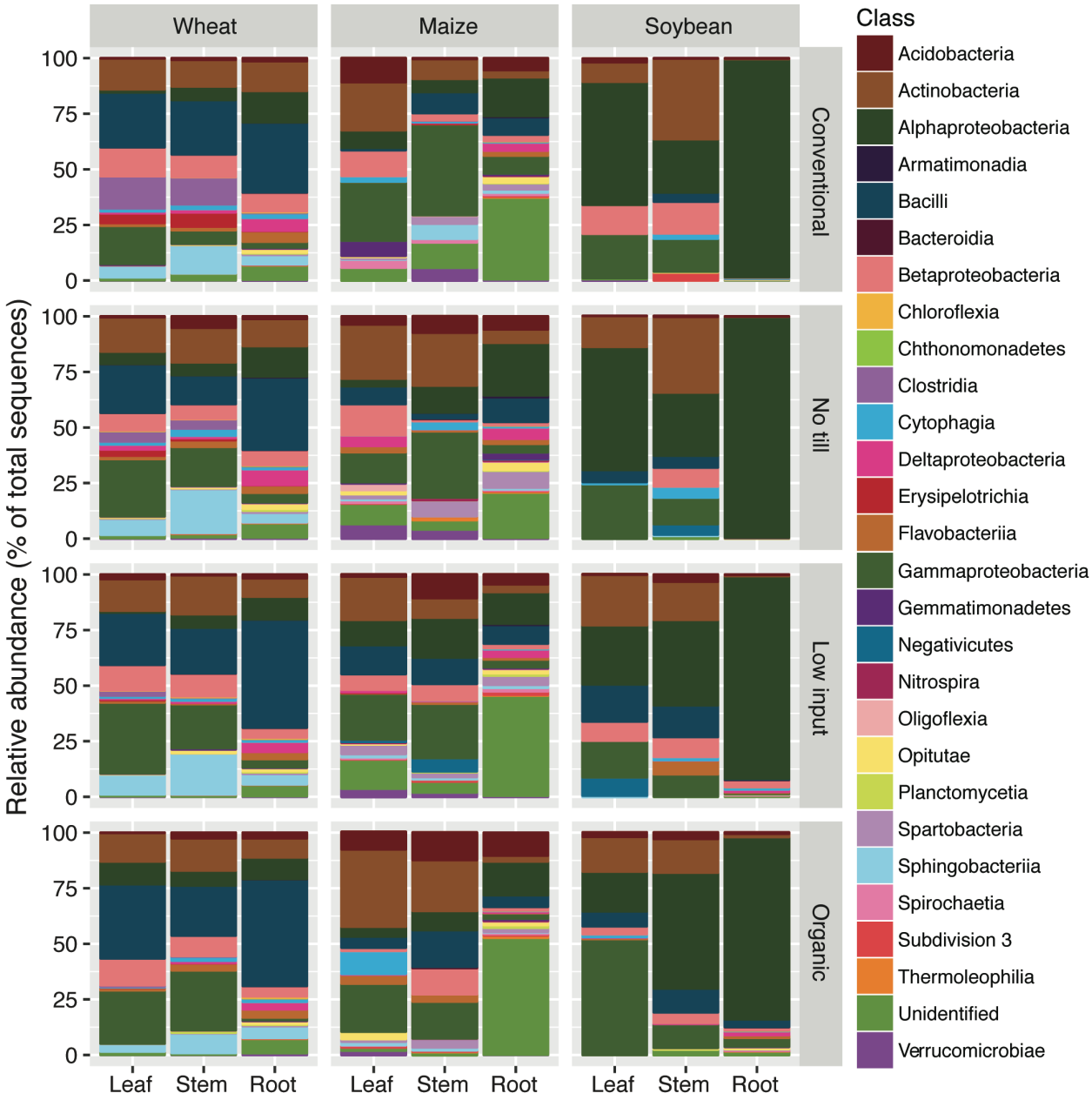

Figure S2. Class-level relative abundance of operational taxonomic units in bacterial communities across crop, organ, and management strategies.

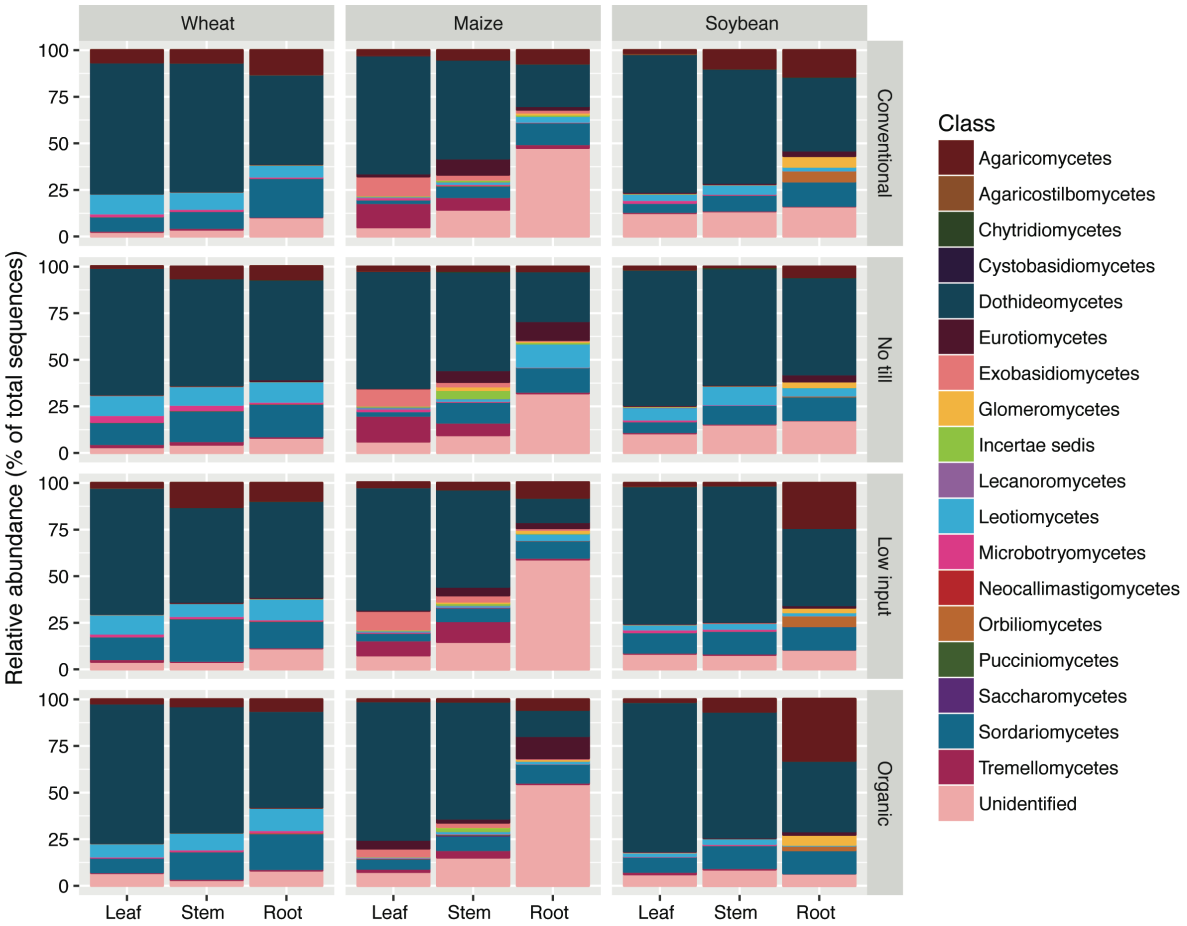

Figure S3. Class-level relative abundance of operational taxonomic units in fungal communities across crop, organ, and management strategies.

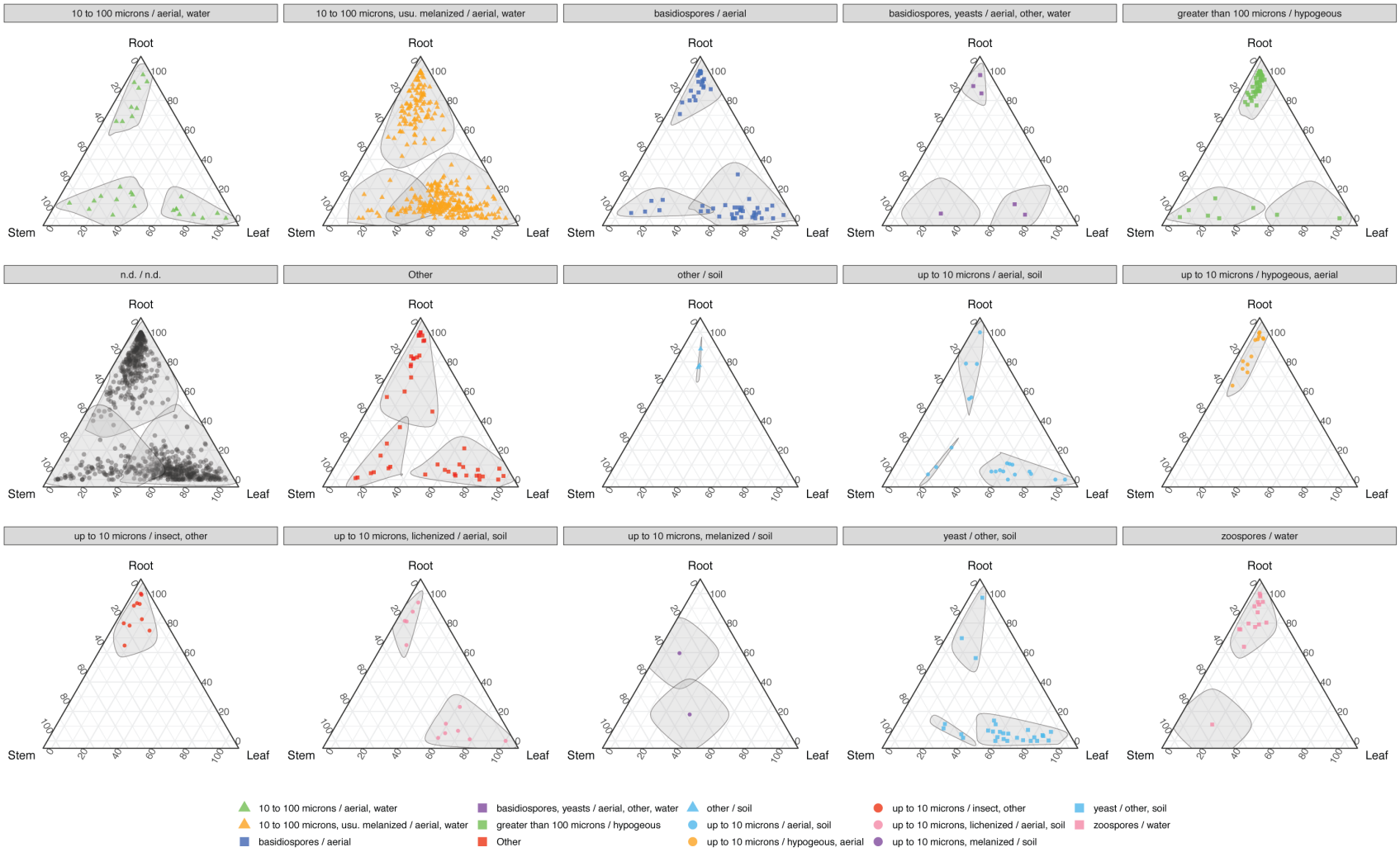

Figure S4. The distribution of spore categories across plant organs. Points are colored by spore category were significant indicators of a particular plant organ with a Benjamini-Hochberg corrected P-value (α = 0.05). Shaded areas encircle OTUs that are significant indicators for a plant organ. Grey points were significantly associated with a crop organ, but the spore category could not be determined (n.d. / n.d.).

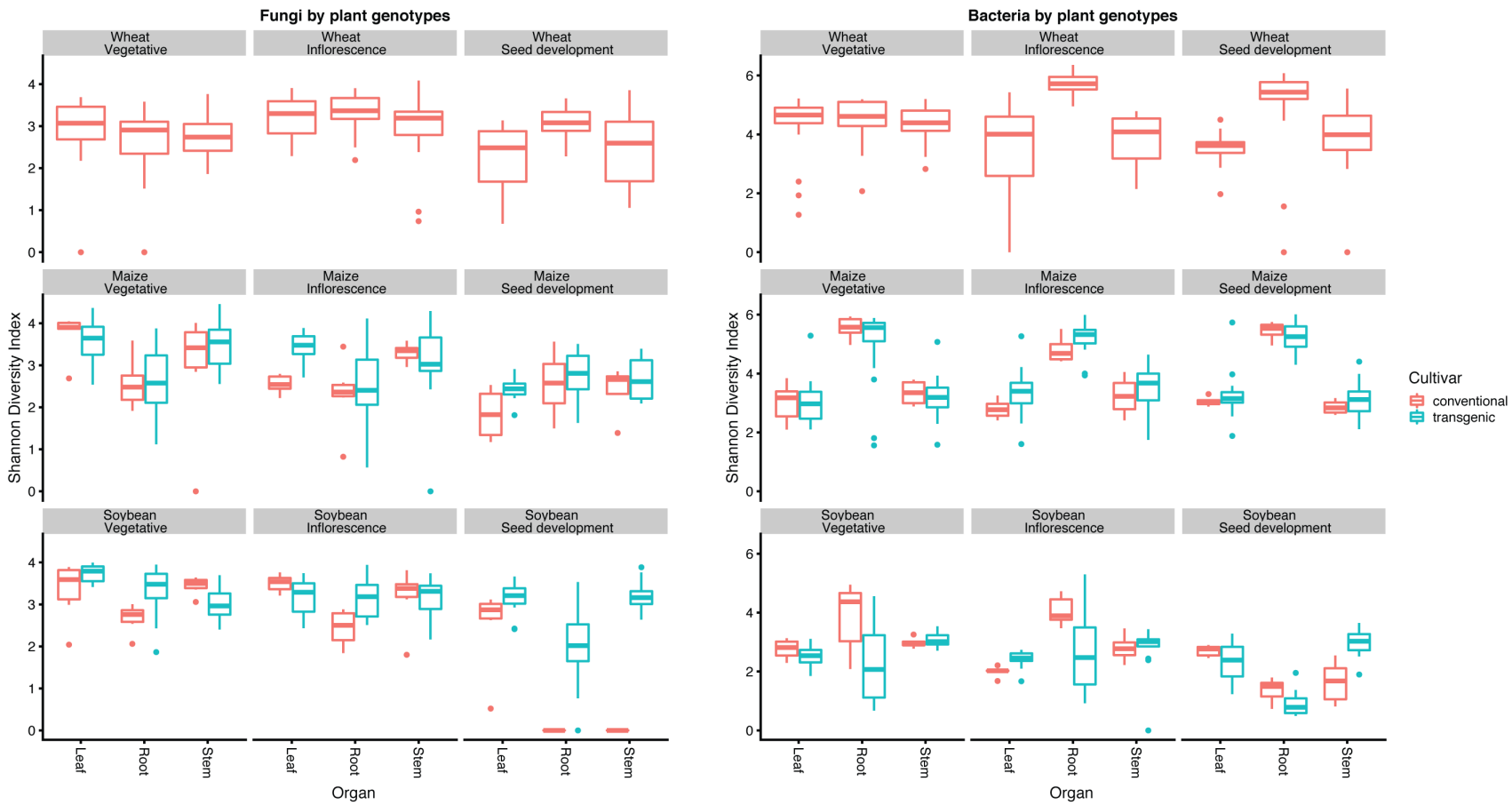

Figure S5. Alpha diversity of managements with (T1 = conventional, T2 = no-till, T3 = low inputs in maize and soybean) and without (T1-T4 wheat, T4 = organic in maize and soybean) transgenic cultivars; fungi (left panels) and bacteria (right panels). Comparisons with made between Shannon Diversity Index (H') of conventional or transgenic (maize and soybean only).

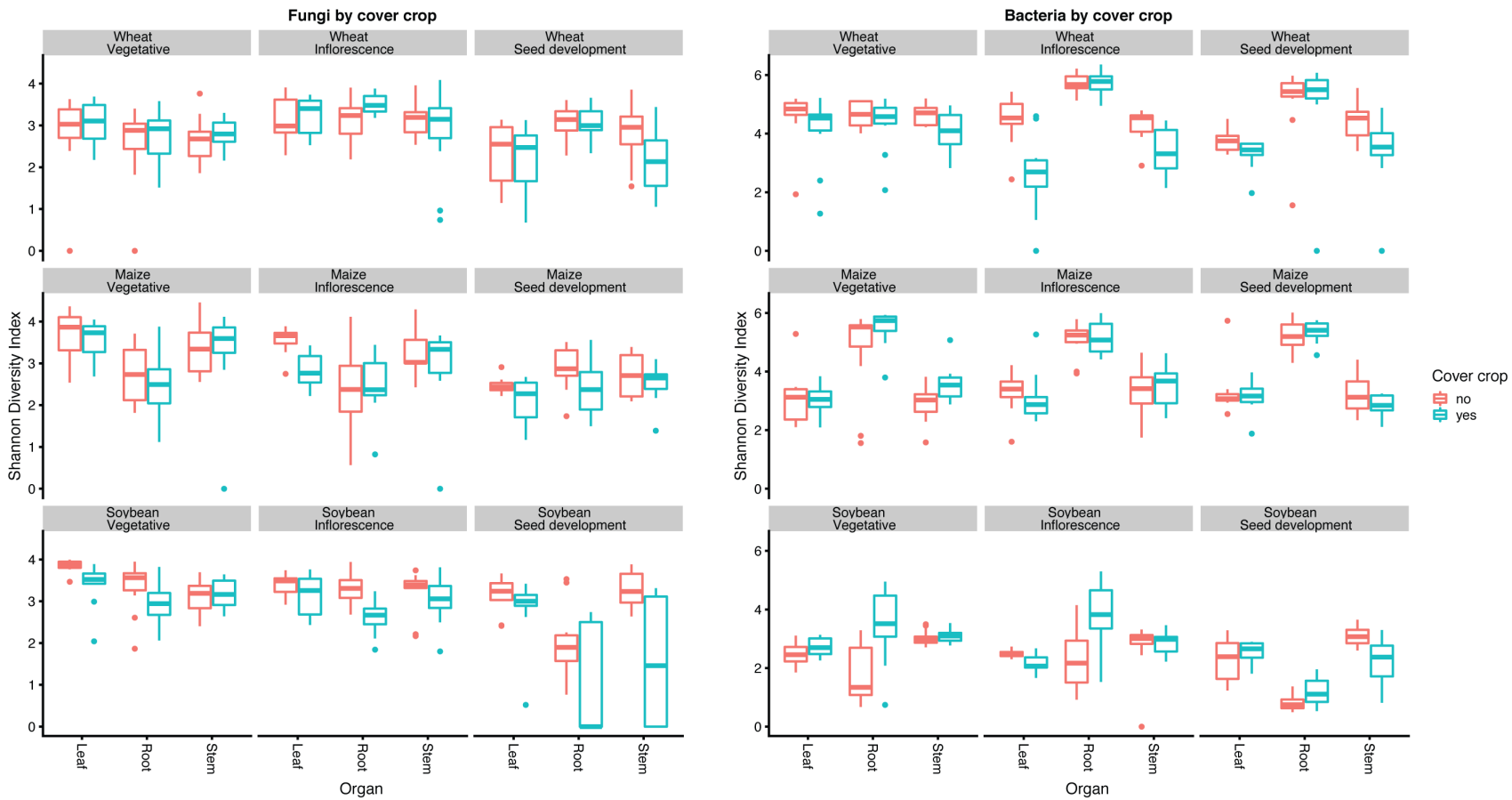

Figure S6. Alpha diversity of managements with (low inputs and organic) and without (conventional and no-till) cover crops; fungi (left panels) and bacteria (right panels). Comparisons were made within Shannon Diversity Index (H') Microbial communities inhabiting each of the organs (leaf, stem, or root) were compared for plots with and without cover crops within each collection period and host plant species.

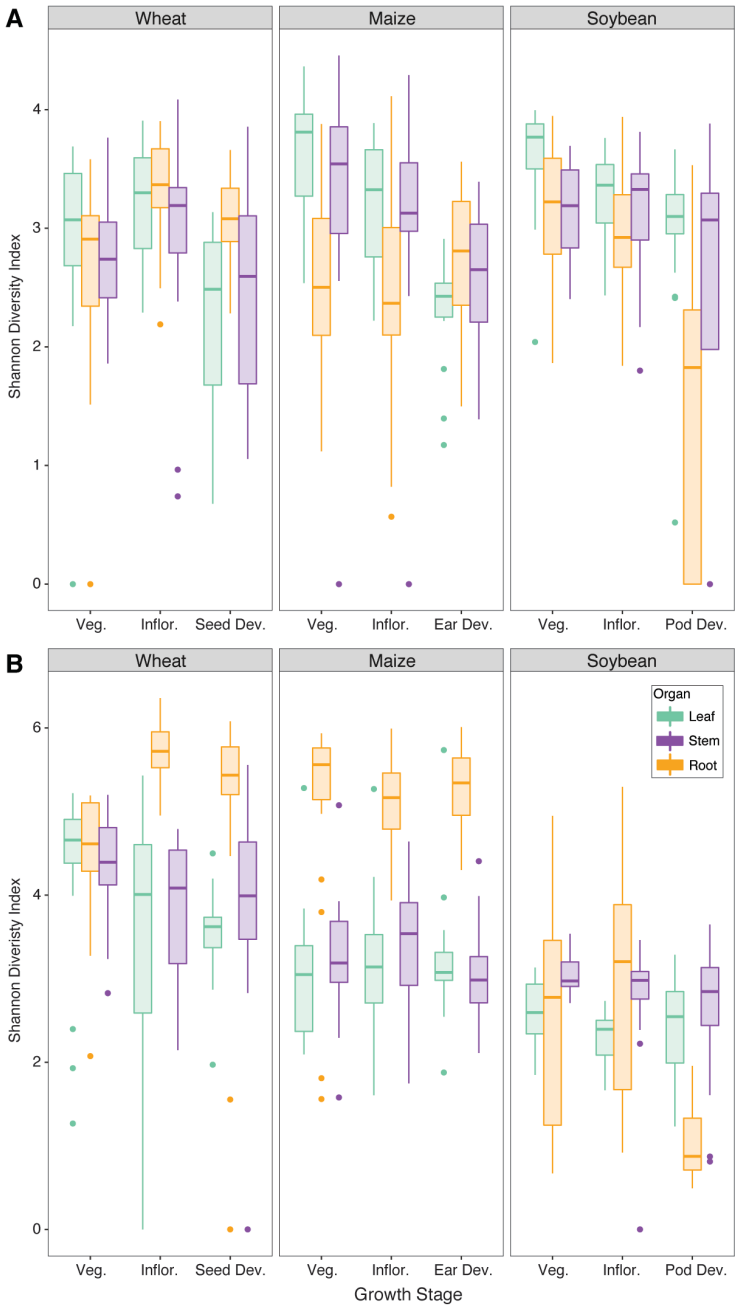

Figure S7. Global alpha diversity patterns calculated by Shannon Diversity Index (H'). All treatments and replicates were pooled within growth stages for (A) fungi and (B) bacteria. Data are represented by 24 replicates from each crop-growth stage-organ combination. Center line of boxes represents median of samples. The upper and lower sides of the boxes represent the third and first quartiles, respectively. Whiskers represent ± 1.5 times the interquartile range. Data points beyond whiskers represent outliers. Analysis of variance and Tukey's honest significant difference were used to test significance ($P < 0.05$). Statistical support is detailed in Table S7.

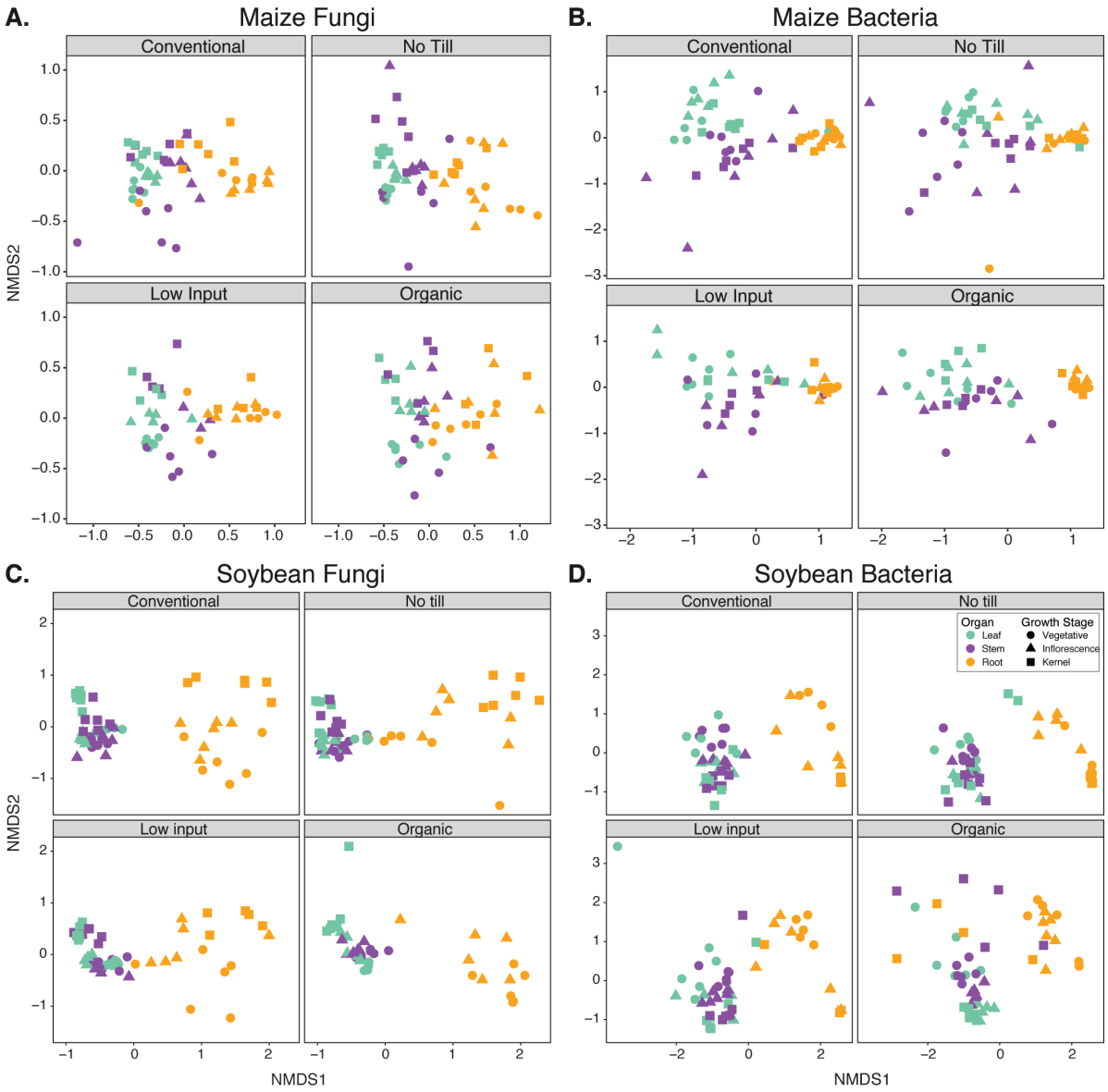

Figure S8. Influence of management strategies on beta diversity of maize fungal (A), maize bacterial (B), soybean fungal (C), soybean bacterial (D) communities originating from each plant organ. NMDS calculated by Bray-Curtis distance. Statistical support is detailed in Table S7.

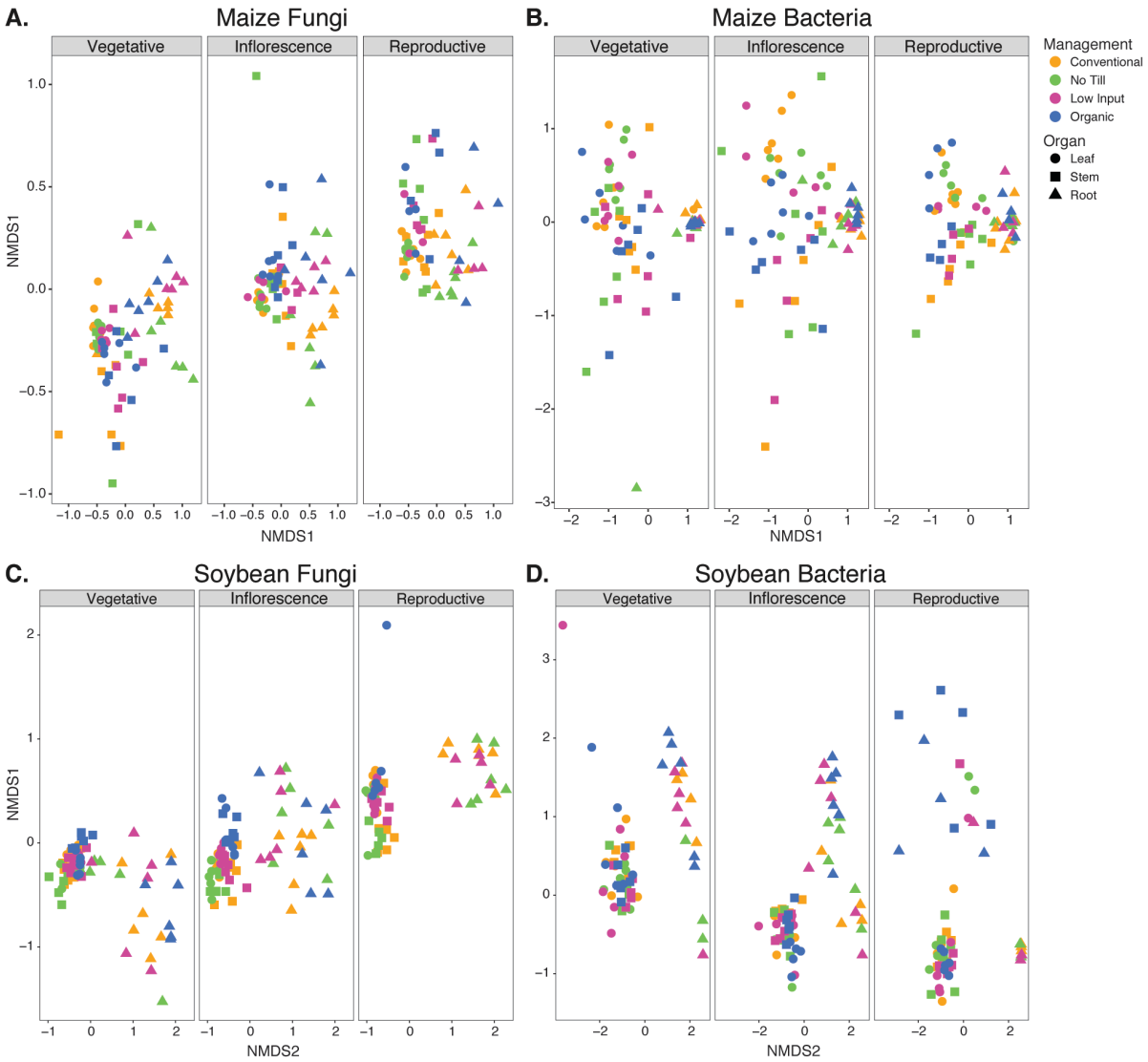

Figure S9. Effect of growth stage on beta diversity of maize fungal (A), maize bacterial (B), soybean fungal (C), and soybean bacterial (D) communities originating from each management strategy. NMDS calculated by Bray-Curtis distance. Statistical support is detailed in Table S9.

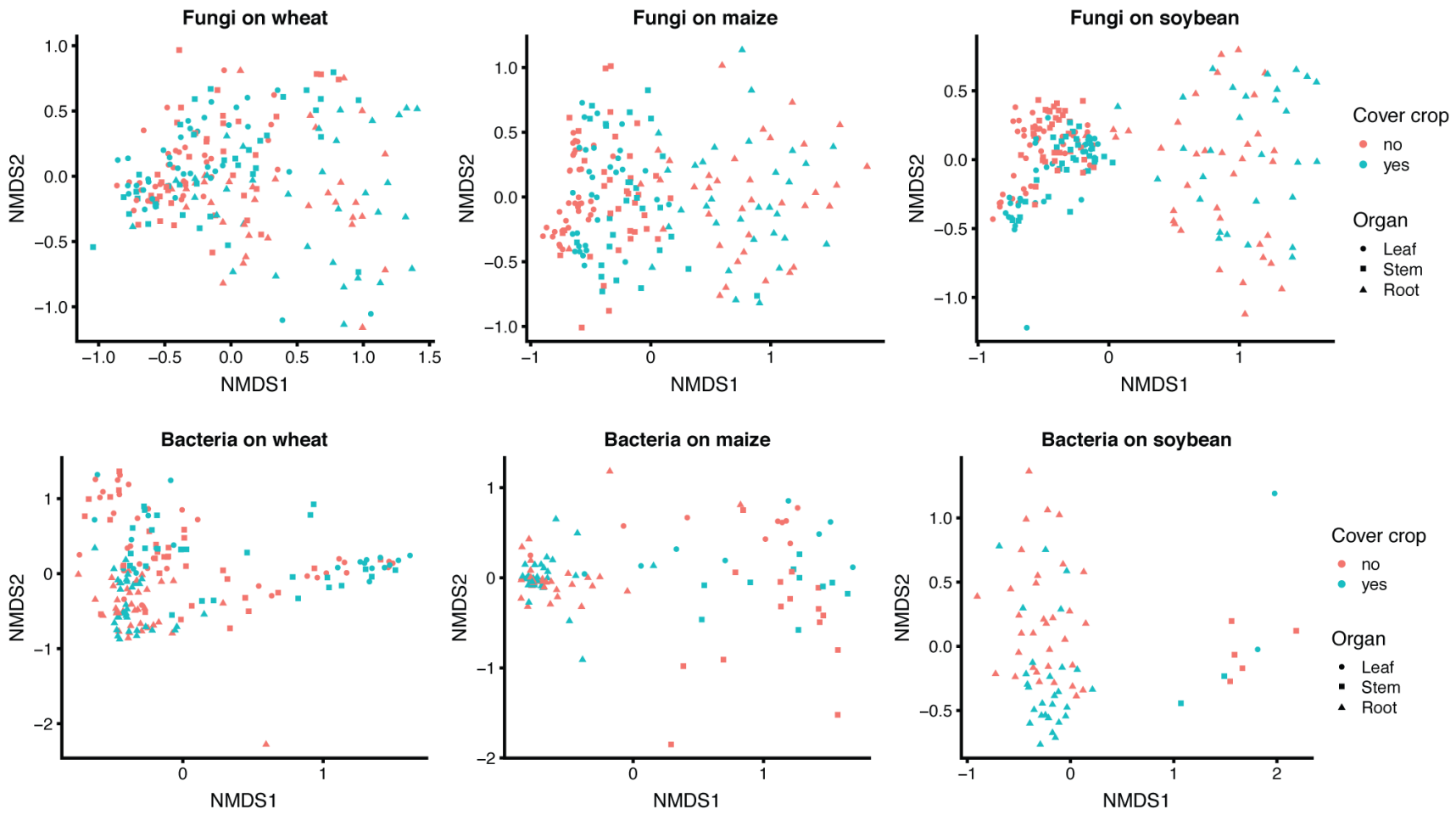

Figure S10. Effect of cover crop on beta diversity of fungal (upper row) and bacterial (lower row) communities. Low inputs (T3) and organic (T4) managements planted with red clover cover crops, and conventional (T1) and no-till (T2) received no cover crop. NMDS calculated by Bray-Curtis distance. Statistical support is detailed in Table S9.

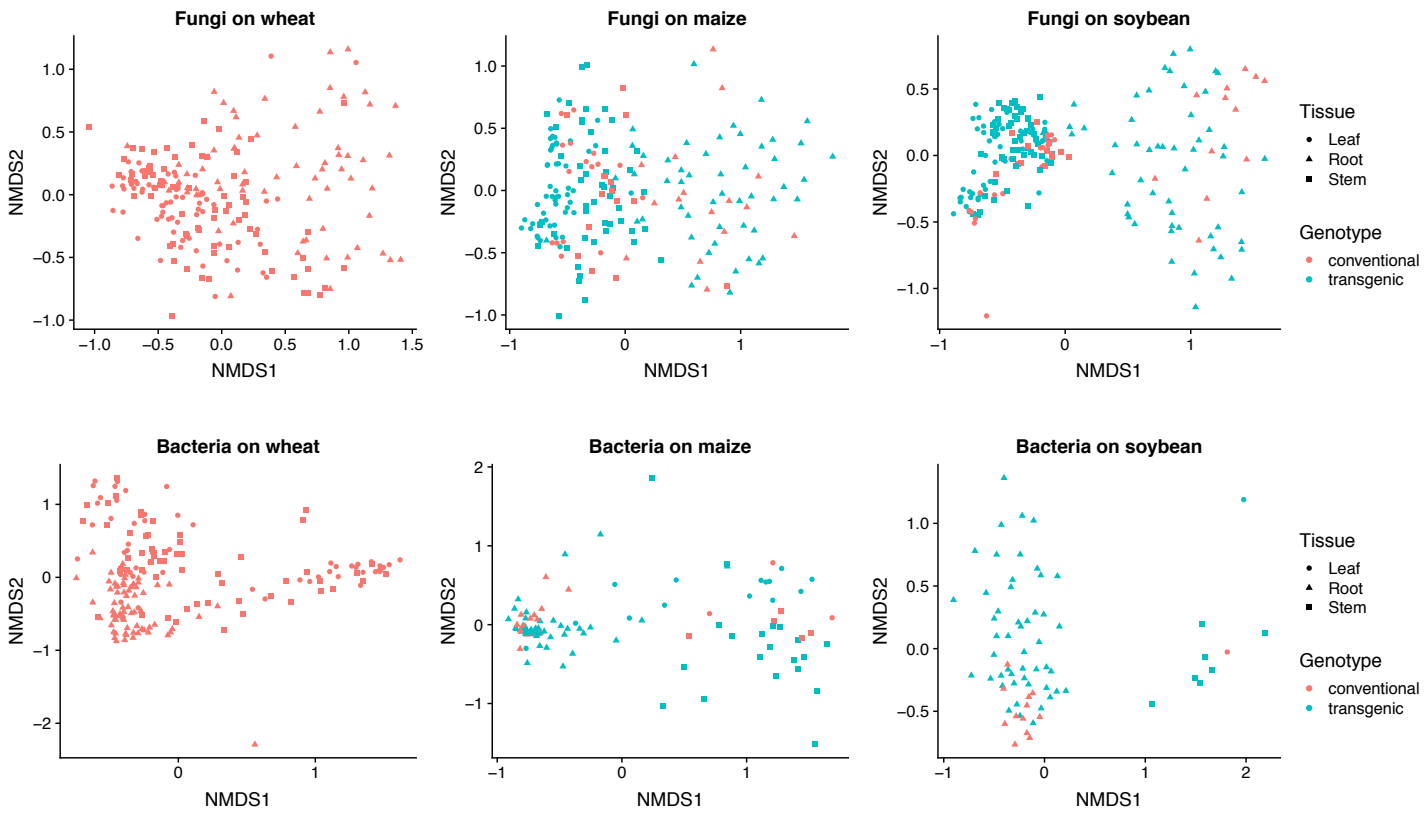

Figure S11. Effect of cultivar on beta diversity of fungal (upper row) and bacterial (lower row) communities. NMDS calculated by Bray-Curtis distance. Maize and soybean in T1 = conventional, T2 = no-till, T3 = low inputs were transgenic cultivars, and all wheat (T1-T4), T4 = organic maize, T4 and soybean were planted with conventional cultivars. Statistical support is detailed in Table S9.

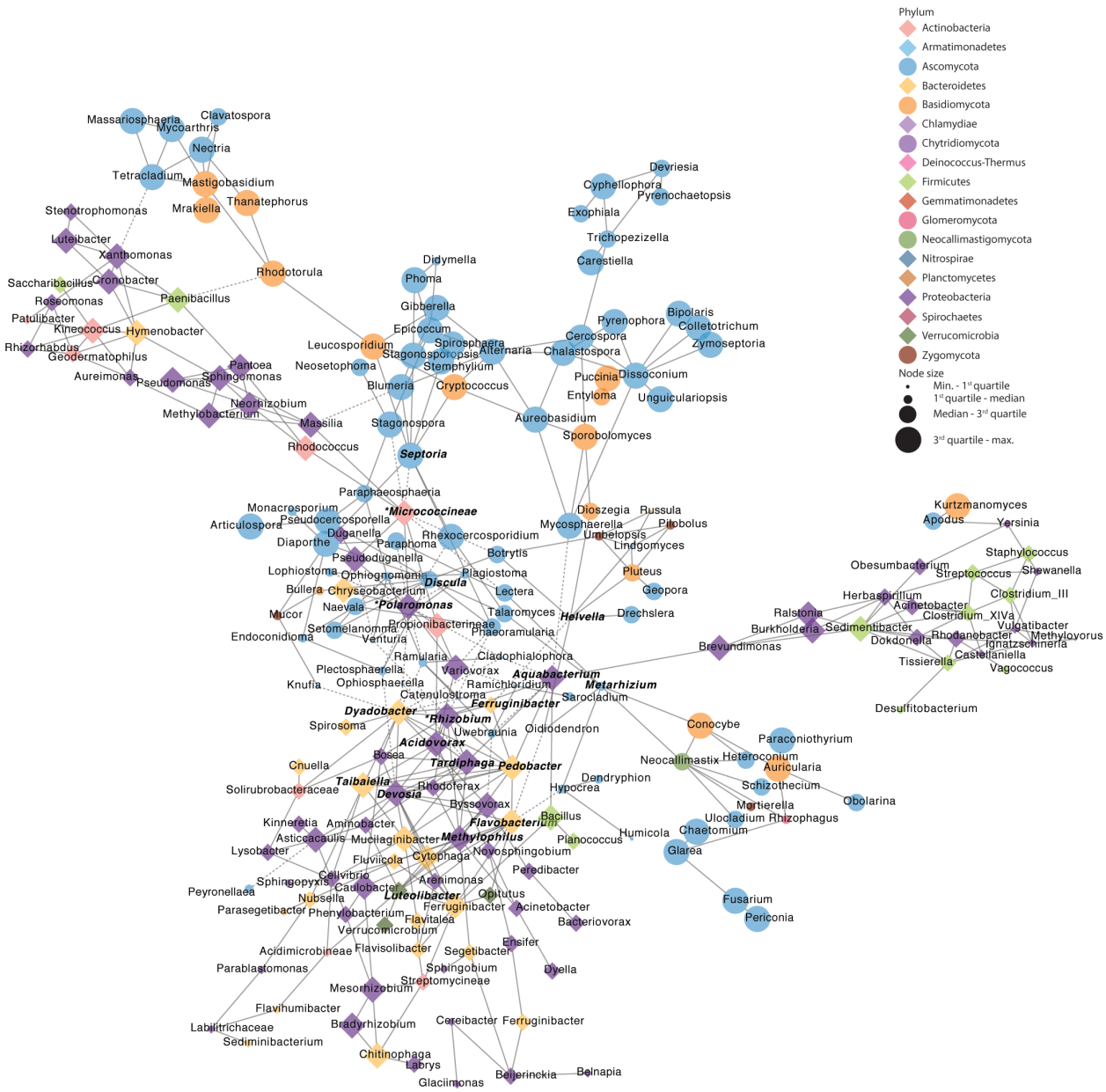

Figure S12. Co-occurrence network of taxa in wheat phyllosphere. Bacterial OTUs are represented by diamond shapes, fungal OTUs are represented by circles, and nodes are colored by class. Solid lines indicate positive correlation, and dashed lines indicate a negative correlation between OTUs. Node size indicates degree of connectivity. Node size indicates relative abundance, bolded node labels indicated hub taxa at the 75th percentile, node labels marked with an asterisk indicate hub taxa at the 95th percentile.

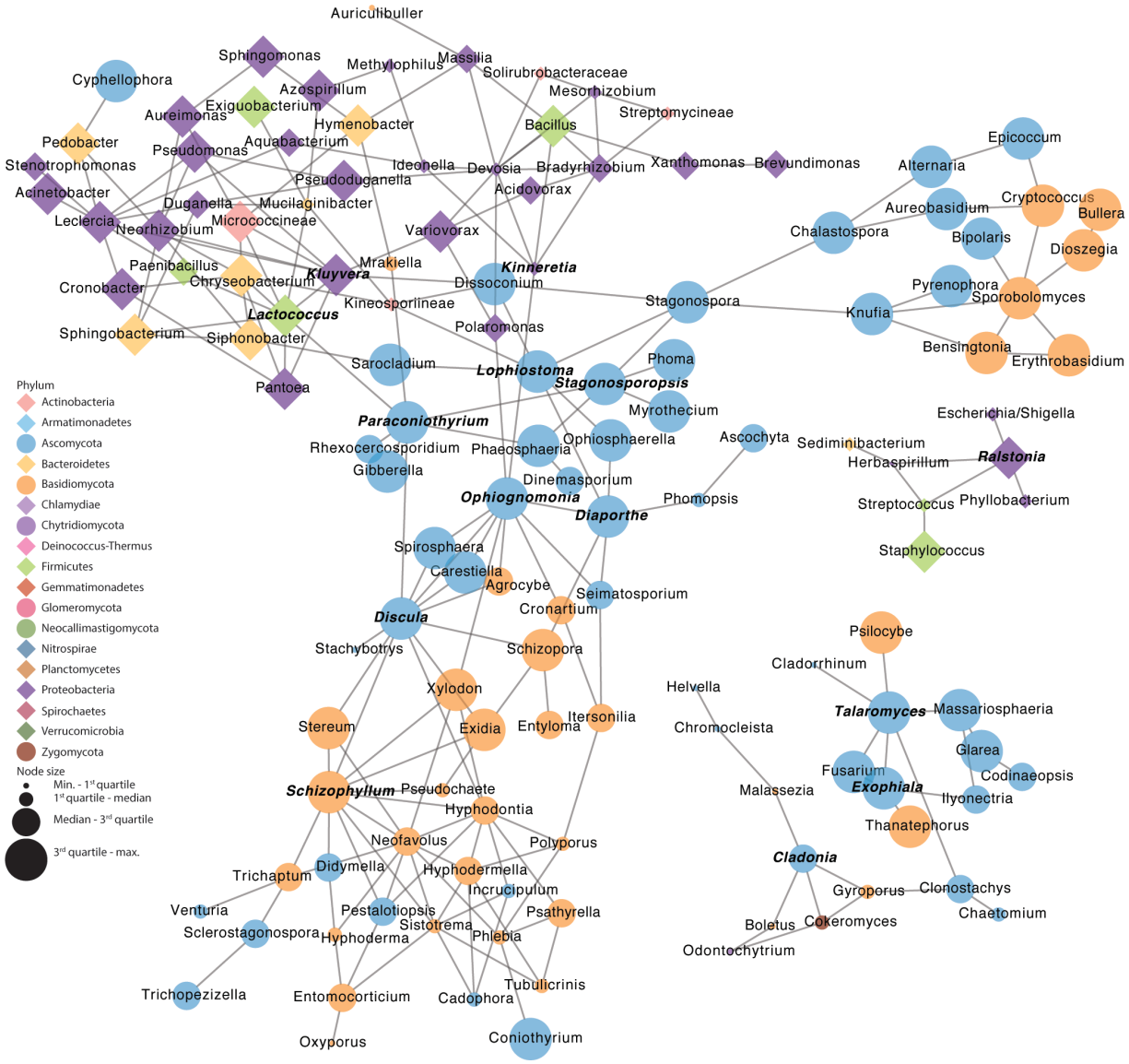

Figure S13. Co-occurrence network of taxa in maize phyllosphere. Bacterial OTUs are represented by diamond shapes, fungal OTUs are represented by circles, and nodes are colored by class. Solid lines indicate positive correlation, and dashed lines indicate a negative correlation between OTUs. Node size indicates degree of connectivity. Node size indicates relative abundance, bolded node labels indicated hub taxa at the 75th percentile, node labels marked with an asterisk indicate hub taxa at the 95th percentile.

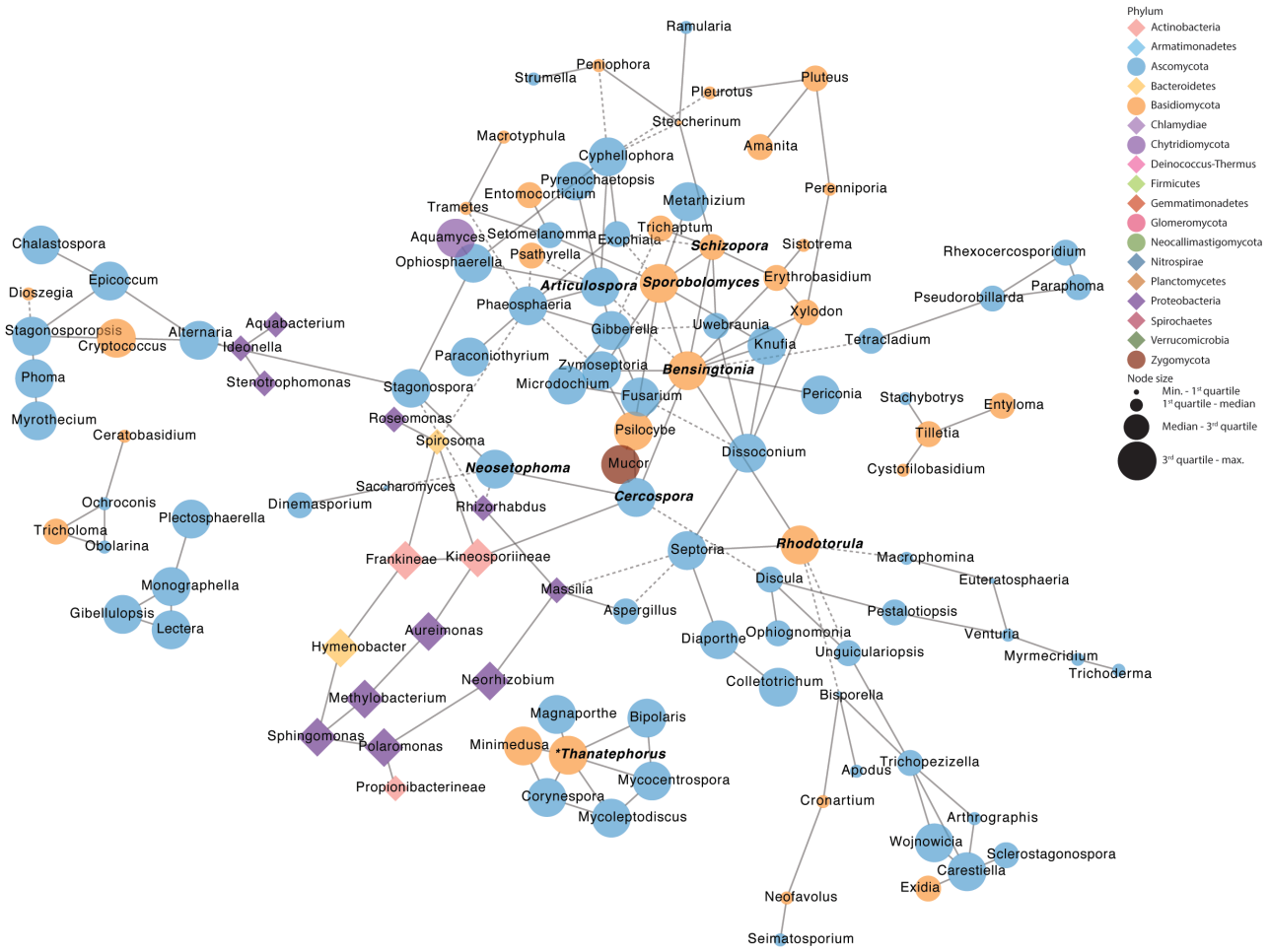

Figure S14. Co-occurrence network of taxa in soybean phyllosphere. Bacterial OTUs are represented by diamond shapes, fungal OTUs are represented by circles, and nodes are colored by class. Solid lines indicate positive correlation, and dashed lines indicate a negative correlation between OTUs. Node size indicates degree of connectivity. Node size indicates relative abundance, bolded node labels indicated hub taxa at the 75th percentile, node labels marked with an asterisk indicate hub taxa at the 95th percentile.

Table S1. Microbiome gene primer sequences.

Table S2. Collection dates of plant growth stages.

Table S3. Sequence processing statistics and OTU assignment statistics.

Table S4. Samples with zero high-quality reads

Table S5. Significantly different OTUs (Bonferroni adjusted p-value) identified with DeSeq2 across management strategies or growth stages of each crop.

Table S6. Spore categories of fungal OTUs significantly associated with a crop and/or organ.

Table S7. Mean estimated Shannon diversity ± standard deviation. Superscripts indicate significant differences within a growth stage and management style, after Tukey's HSD.

Table S8. Mean observed OTUs ± standard deviation. Superscripts indicate significant differences within a growth stage and management style, after Tukey's HSD.

Table S9. PERMANOVA results of Bray-Curtis values.