Temporal and Spatial Interactions Modulate the Soybean Microbiome

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Key words: Soybean, ITS region, 16S rRNA gene, Plant Microbiomes, Microbiome succession

Running title: Soybean microbiome succession

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Abstract

Managed agricultural ecosystems are unique systems where crops and microbes are intrinsically linked. This study focuses on discerning microbiome successional patterns across all plant organs and tests for evidence of niche differentiation along temporal and spatial axes. Soybean plants were grown in an environmental chamber till seed maturation. Samples from various developmental stages (emergence, growth, flowering, and maturation) and compartments (leaf, stem, root, and rhizosphere) were collected. Community structure and composition were assessed with the 16S rRNA gene and ITS region amplicon sequencing. Overall, the interaction between spatial and temporal dynamics modulated alpha and beta diversity patterns.

Time lag analysis on measured diversity indices highlighted a strong temporal dependence of communities. Spatial and temporal interactions influenced the relative abundance of most abundant genera, whilst Random Forest predictions reinforced the observed localisation patterns of abundant genera. Overall our results show, spatial and temporal interactions tend to maintain high levels of biodiversity within the bacterial/archaeal community, whilst in fungal communities, OTUs within the same genus tend to have overlapping niches.

Introduction

The plant microbiome consists of the combined microbial communities that reside on and within the plant; these communities have an intrinsic relationship with their plant hosts (Berg *et al.*, 2016). The plant microbiome is an assemblage of archaea, bacteria, micro-eukaryotes, and virus communities that inhabit various plant organs with little overlap in taxonomic and functional composition (Turner *et al.*, 2013, Berg *et al.*, 2014, Rout, 2014). The distribution of these communities across space and time and how they are influenced by plant genotype (Lundberg *et al.*, 2012), plant species (Copeland *et al.*, 2015), climate change (Compant *et al.*, 2010), and plant nutrient status (Dakora & Phillips, 2002) was extensively studied. These studies highlighted the existence of spatial and temporal niches and how these are partitioned amongst and within communities.

Evidence of niche differentiation in creating a spatially heterogeneous environment and subsequently distinct archaea, bacteria and eukaryote communities is well documented in the root-soil interface of model plant systems (Bulgarelli *et al.*, 2012, Duran *et al.*, 2018). However, compared to the root-soil interface, the existence of clear niche differentiation in other plant organs and non-model plant species and their influence on microbiome succession and diversity is poorly understood. Recent advances have shown the existence of niche differentiation within the plant endosphere of poplar (Beckers *et al.*, 2017), and begun highlighting how plant organs and biogeography strongly influence microbial niches in crops (Coleman-Derr *et al.*, 2016).

In agricultural systems, seasonality and interactions within and between microbial communities are essential factors influencing foliar microbiome succession and diversity

(Copeland *et al.*, 2015, Ottesen *et al.*, 2016). It seems in part that niches created by changes in plant metabolism significantly influence the diversity and structure of the microbiome, and temporal dynamics are a robust discriminatory axis on which these communities are distributed (Shade *et al.*, 2013, Tkacz *et al.*, 2015). Although widely accepted, plant-mediated selection of the microbiome does not fully expound on the observed immense diversity and composition of these communities.

In field experiments, soybean rhizosphere bacterial communities were shown to be sharply delimited by soil type and developmental stages (Xu *et al.*, 2009), and spatial heterogeneity (rhizosphere-bulk soil) (Mendes *et al.*, 2014). Contrariwise, fungal communities are relatively stable over a growing season, but noticeable differences were observed between rhizosphere and bulk soil (Sugiyama *et al.*, 2014). Additionally, similar spatial and temporal dynamics have been highlighted within the leaf endophytic community of transgenic and conventional soybean cultivars (Montanari-Coelho *et al.*, 2018). In the case of soybean, it seems that the influence of cultivar on microbiome diversity and succession is negligible (Xu *et al.*, 2009, Copeland *et al.*, 2015). These studies have shed light on microbial dynamics within the soybean rhizosphere. However, the role of spatial and temporal dynamics in influencing the entire soybean microbial community succession is lacking

Earlier efforts to manipulate the plant microbiome identified the molecular mechanisms that were influencing the plant-microbe interaction (Fray, 2002). However, even these earlier studies highlighted the role of interactions in plant microbiomes as important starting points for microbiome engineering. A recent review systematically outlines evidence to highlight the role of

plant circadian rhythm, temperature, and nutrient needs (Cheng *et al.*, 2019) on plant microbiomes. It seems then that, the key to improving attempts at engineering the plant microbiome ought to consider the following: 1) influence of spatial and temporal interactions between and within plant microbiomes on composition and structure, 2) influence of environmental conditions on the plant microbiome, and 3)influence of plan metabolic processes on the plant microbiom

Methods

Plant growth conditions

Soybean plants (Pioneer: P19T01R) were grown with an 18-hour photoperiod at 25 °C followed by a 6-hour dark period at 20°C in a Conviron growth chamber (Winnipeg, Canada). Plants were supplied with a modified Hoagland's plant nutrient solution biweekly (Moscatiello *et al.*, 2013). Plants were destructively sampled at each developmental stage V1 (emergence), V3 (growth), R1 (flowering), and R3 (maturation). Samples were collected from rhizosphere, root, stem (between 1st internode and second internode), and leaf (youngest and oldest trifoliate) for a sample. A total of five plants were destructively sampled at each developmental stage, and DNA extraction was performed right after sampling. Samples were collected from rhizosphere, root, stem, and leaves. The soil used was collected from an experimental field that had been ploughed with no history of agricultural practice at the Institut National de la Recherche Scientifique (Laval, QC, Canada).

Microbiome sampling and sequencing

At each sampling period, the rhizosphere samples were considered as all the soil that was directly attached to the root surface. The entire epiphytic community (leaves, stem, and roots) was extracted using a modified protocol from Qvit-Raz *et al.* (2008). Briefly, the samples were placed in sterile 50 ml plastic Falcon test tubes (Corning, Tewksbury, MA, USA) and filled with sterile phosphate-buffered saline (PBS 0.1M, pH 7.4). The samples were then placed in sonication tub (Fisher FS20, Fisher Scientific, Waltham, USA) for 15 min and then vortexed for 10 s. The samples were then transferred into a new tube containing PBS and rinsed twice. The wash was pooled and spun down in a centrifuge at 2,000 g for 20 min, and the resulting pellet was considered to be the

epiphytic community. The endophyte community was considered to be all the remaining microbes after the sonication and rinse treatment. Plant tissue was then pulverised in liquid nitrogen using a sterile pestle and mortar. For each sample, 0.25 g was added to the bead tubes from the Qiagen Power Soil DNA kit (Hilden, Germany) and DNA was extracted following the manufacturer's instructions. For all sample, the presence of DNA was visualised by electrophoresis in 1% agarose gels.

The bacterial/archaeal V2-V3 hypervariable regions of the 16S rRNA gene was amplified using 520F and 799R primer pairs which were shown to exclude chloroplast sequences (Edwards *et al.*, 2008). Whereas fungal ITS region was amplified using ITS1F and 58A2R (Martin & Rygiewicz, 2005). Average length of 16S amplicon sequences were of approximately 280 bp and ITS sequences ranged between 250 and 493 bp. Briefly, extracted DNA was used to construct sequencing libraries according to Illumina's "16 S Metagenomic Sequencing Library Preparation" guide (Part # 15044223 Rev. B), with the exception of using Qiagen HotStar MasterMix for the first PCR ("amplicon PCR") and halving reagent volumes for the second PCR ("index PCR"). The first PCR ("amplicon PCR") was carried out for 25 cycles with annealing temperatures of 55 °C. The resulting amplicons were pooled together and sequenced at the McGill University and Genome Québec Innovation Center (MUGQIC). Diluted pooled samples were loaded on an Illumina MiSeq and sequenced using a 500-cycle (paired-end sequencing configuration of 2x250 bp) MiSeq Reagent Kit v3. Bacterial and fungal sequences were processed bioinformatics using the AmpliconTagger pipeline (Tremblay *et al.*, 2015, Tremblay & Yergeau, 2019). Briefly, raw reads were scanned for sequencing adapters and PhiX spike-in sequences and remaining reads were merged using their common overlapping part with FLASH (Magoc & Salzberg, 2011).

Merged sequences were filtered for quality such that sequences having an average quality (Phred) score lower than 27 or one or more undefined base (N) or more than 10 bases lower than quality score 15 were discarded. Remaining sequences were clustered at 100% identity and then clustered/denoised at 99% identity (DNACLUST v3) (Ghodsi *et al.*, 2011). Clusters having abundances lower than 3 were discarded. Remaining clusters were scanned for chimeras with VSEARCH's version of UCHIME denovo (Rognes *et al.*, 2016), UCHIME reference (Edgar *et al.*, 2011), and clustered at 97% (DNACLUST) to form the final clusters/OTUs. Bacterial and fungal OTUs were then assigned a taxonomic lineage with the RDP classifier (Wang *et al.*, 2007), using the AmpliconTagger 16S and ITS training sets (Tremblay, 2019), respectively. The RDP classifier gives a score (0 to 1) to each taxonomic depth of each OTU. Each taxonomic depth having a score >= 0.5 was kept to reconstruct the final lineage. Taxonomic lineages were combined with the cluster abundance matrix obtained above to generate a raw OTU table. For cross sample comparisons, ten iterations were performed on a random subsample of 1,000 reads rarefactions and the average number of reads of each OTU of each sample was then computed to obtain a consensus rarefied OTU table. A multiple sequence alignment was then obtained by aligning OTU sequences on a Greengenes core latest reference alignment (DeSantis *et al.*, 2006) and using the PyNAST v1.2.2 aligner (Caporaso *et al.*, 2010). Alignments were filtered to keep only the hypervariable region of the alignment. Alpha (observed species) and taxonomic summaries were then computed using the QIIME v1.9.1 software suite using the consensus rarefied OTU (Caporaso *et al.*, 2010, Kuczynski *et al.*, 2011). Total numbers of quality-checked sequences and OTUs are provided in supplementary material.

Statistical analyses

The OTU abundance tables for both communities were normalised such that the summed relative abundance of all OTUs of each sample was equal to one. All statistical analyses were performed using R version 3.5.0 (Team, 2015)

Alpha diversity patterns

OTU accumulation curves were constructed to determine if the sampling effort (number of samples) could recover most of the taxa, whilst the Preston log-normal curves were used to evaluate the estimated richness and occurrence of rare taxa across all samples (the presence of a normal distribution indicates a higher probability of rare taxa being represented). A three-way ANOVA was performed to evaluate the effects of developmental stage, plant organ (rhizosphere, root, stem, and leaves), and location (endophyte or epiphyte) on the number of observed OTUs (S_{obs}) and Shannon diversity indices for both communities. Pairwise comparisons were performed using Tukey's HSD tests, and where data was not normally distributed Kruskal-Wallis pairwise Wilcox test with Bonferroni correction applied. To determine the influence of temporal autocorrelation on diversity Augmented Dickey-Fuller (ADF) and Kwiatkowski-Phillips-Schmidt-Shin (KPSS) tests were performed using S_{obs} and Shannon diversity as input variables.

Microbial community structure

Principal Coordinate Analysis (PCoA) was used to visualise the community structure of the bacterial and fungal communities using the Bray-Curtis dissimilarity matrix. Multivariate dispersion and homogeneity across all samples were quantified and confirmed using ANOVA with 999 permutations. A Permutational Multivariate Analysis of Variance (PERMANOVA) test was performed with 999 permutations to assess the relative significances of temporal (developmental stage) and spatial dynamics (plant organ and location) on the community structure. Multivariate

dispersion in the community data was evaluated and confirmed using ANOVA with 999 permutations.

Taxonomic profiles and Random Forest Models

The relative abundance of microbial taxa at the phylum and genus taxonomic levels were evaluated across developmental stages and plant organs. In both communities, random forest algorithms were used to predict the prevalence of taxa associated with each developmental stage at the genus and OTU taxonomic levels. Random forest prediction were made using the randomForest algorithm (Liaw & Wiener, 2002) and implemented on the MicrobiomeAnalysis pipeline (Chong *et al.*, 2020)

Data availability

The 16S and ITS rDNA raw reads from the microbiota analyses have been deposited at the NCBI BioProject repository under study accession number PRJNA601979.

Results

Alpha diversity patterns

In both cases, OTU accumulation curves indicated that a substantial amount of microbial OTUs was recovered and the sampling effort was enough to reach saturation or near saturation (Fig.S1). Furthermore, Preston log-normal curves for both taxa were shown to be a near-complete bell-shape indicating that samples were sequenced enough to detect rare and low-abundance taxa. (Fig.S1). Overall, these indicate that the sampling effort was enough to capture not only the most abundant but also rare taxa and thus giving a comprehensive outlook of the community.

Overall, the interactions between these spatial and temporal components significantly influenced the abundance and diversity of fungal and bacterial/archaeal community (Fig.1; Fig.S2). Typically, the aboveground and belowground microbial communities are assembled and influenced by different ecological processes. As such, in addition to analysing the total community, the aboveground and belowground communities were analysed separately. At the root-soil interface, developmental stage significantly influenced the abundance and diversity of fungal (Observed OTUs χ^2=15.66***; Shannon diversity χ^2 =13.48*) and bacterial/archaeal (Observed OTUs χ^2=13.42**; Shannon diversity χ^2 =11.96*) communities (Fig.1; Fig.S2). Although there was a significant influence of the developmental stage on the abundance and diversity on both communities, pairwise Wilcox test with Bonferroni correction did not reveal any differences between the developmental stages. There were significant influences of sample site (rhizosphere, rhizoplane, and root endosphere) on the abundance of fungal (Observed OTUs χ^2=40.31***; Shannon diversity χ^2 =38.01***) and bacterial/archaeal (Observed OTUs χ^2=42.23***; Shannon diversity χ^2 =43.32***) communities (Fig.1; Fig.S2). In both communities, the rhizosphere consistently had the highest abundance and was significantly different to both rhizoplane and root

endosphere (p<0.05), whilst only the bacterial/archaeal rhizoplane and root endosphere communities were marginally different from each other (p=0.08). Both developmental stage and sample site influenced alpha diversity in both communities. In the aboveground compartment, developmental stage significantly influenced only diversity of the fungal community (Shannon diversity F value =3.77*) and influenced the abundance and diversity bacterial/archaeal (Observed OTUs χ^2=9.52*; Shannon diversity χ^2 =11.36**) communities (Fig.1; Fig.S2). Fungal alpha diversity significantly differed between the emerging and maturation stage (p<0.01; Tukey's HSD; Fig. S2). The bacterial/archaeal alpha diversity varied significantly between the emergence and flowering stage ($p\leq$ 0.05; Wilcox test with Bonferroni correction; Fig.1; Fig.S2). Lastly, within the aboveground communities there seem to be no influence of samples site (leaf endophyte, leaf epiphyte, stem endophyte, and stem epiphyte) on both fungal and bacterial/archaeal communities.

Lastly, both analyses for temporal autocorrelation (ADF and KPSS) indicated that in both microbial communities Sobs and Shannon diversity index were temporally autocorrelated. The tests both rejected the null hypothesis that the datasets were stationary (p>0.05). Both tests independently arrived at the same conclusion that there is an existence of a root unit, highlighting the presence of a strong seasonal trend in microbial a-diversity patterns and indicating that these patterns are dependent upon one another.

Beta diversity patterns

For both communities, the Bray-Curtis dissimilarity matrix was used to infer community beta-diversity patterns and structure. Firstly, homogeneity and multivariate dispersion analysis were used to determine the relative influence of between community composition (between-sample variation) and within community composition (variation within replicates) on overall microbial community structure. In both communities, there was increased multivariate dispersion across plant organs and not developmental stages except the control (soil and seed) and pod samples (Fig.S3). When considering developmental stage, both communities displayed significant heterogeneous dispersion (bacteria: F value= 14.48, $p<0.001$; fungi: F value= 5.58, $p<0.001$; Fig.S3). Similarly, different plant organs highlighted that both communities displayed significant heterogeneous dispersion (bacteria: F value= 17.74, $p<0.001$; fungi: F value= 20.94, $p<0.001$; Fig.S3). Lastly, when considering location, i.e. endophyte or epiphyte, the multivariate dispersion analysis indicated that the endophytic and epiphytic communities had variable dispersion (bacteria: F value= 4.12, $p<0.05$; fungi: F value= 12.66, $p<0.01$).

Principle Coordinate Analysis (PCoA) was used to evaluate and visualise the microbial community structure of both communities with Bray-Curtis dissimilarity as input. For both communities, the interactions amongst variables had a significant influence on community structure (PERMANOVA, Table S1; Fig.S4). Multivariate analysis of variance of belowground and aboveground compartments recapitulated the influence of interactions in influencing community structure. Firstly, the belowground communities were significantly influenced by the interactions of developmental stage and sample site (Rhizosphere, Rhizoplane, and Root Endosphere) (Table 1). Similarly, the aboveground communities were also influenced by the interactions between developmental stage and sample site (Leaf Endophyte, Leaf Epiphyte, Stem Endophyte, Stem Epiphyte, and Pod) (Table 1). Additionally, PCoA highlighted a significant

spatial and temporal niche separation amongst aboveground communities (Table 1; Fig.2; Fig.3). There was pronounced niche separation of communities along a spatial and temporal axis within the belowground compartments for both bacterial/archaeal and fungal communities (Table 1; Fig.2; Fig.3). Overall, the aboveground and belowground communities are modulated by the same spatial and temporal interactions. However, it is within the belowground compartment that the influence of these interactions is pronounced.

Taxonomic profiles and Random Forest Models

Overall, there were variations in the relative abundance of dominant phyla across all samples for both microbial communities. For bacteria/archaea, *Proteobacteria* was the dominant phylum across all plant organs and developmental stages (Fig.S5). At the plant organ level, the relative abundance of dominant bacterial phyla was similar between the internal (endophyte) and external (epiphyte) with the noticeable increase of *Thaumarchaeota* in the root epiphyte, rhizosphere, and leaf endophyte. At the genus level, *Pseudomonas* had the highest the relative abundance in both the endophyte and epiphyte communities across all plant organs apart from the stem and pod organs (Fig.S5). There were similar taxonomic profiles at each developmental stage for the endophyte and epiphyte community at the phylum level. However, at the genus, there were divergences in the relative abundance of dominant taxa between the endophyte and epiphyte communities. Strikingly, although *Pseudomonas* was prevalent across all samples, there was a dominance of *Acinetobacter* at the growth developmental stage in the endophyte community (Fig.S5). The control seed taxonomic profile was divergent to both the immature and mature pods. However, there was a marked dominance of *Streptomyces* at the immature pods whilst the abundance of *Shigella* was higher in both mature seeds and control seed (Fig. S5). The relative

abundance of the most abundant genera was comparable between above and below compartments (Fig.4; Fig.S6).

In the belowground compartment, the relative abundance of *Novosphingobium* was exclusively modulated by developmental stage (Fig.4; χ^2=14.99**). For the rest, sample site significantly influenced their relative abundance: *Massilia* (Fig.4; χ^2=13.98***), *Niastella* (Fig.4; χ^2=42.56***), *Shigella* (Fig.4; χ^2=33.79***), *Gaiella* (Fig.4; χ^2=40.91***), *Bradyrhizobioum* (Fig.4; χ^2=11.40*), and *Acinetobacter* (Fig.4; χ^2=31.24***). *Pseudomonas* is one of two genera whose relative abundance was influenced by both developmental stage (Fig.4; χ^2=25.41***) and sample site (Fig.4; χ^2=11.60*). The other is *Streptomyces* whose relative abundance is influenced by developmental stage (Fig.4; χ^2=11.43*) and sample site (Fig.4; χ^2=43.53***). In the aboveground compartment the relative abundance of *Pseudomonas, Streptomyces, Novosphingobium, Shigella,* and *Acinetobacter* were significantly influenced by both factors (Fig.S6).

The relative fungal abundance was mainly dominated by *Ascomycota, Zygomycota*, and *Basidiomycota* all showed variation across developmental stages and plant organs (Fig.S7). Across all plant organs and in both endophyte and epiphyte communities, *Ascomycota* was the most prevalent phyla except in the pods- *Basidiomycota* was more abundant (Fig.S7). Relative abundances of most dominant genera in the epiphyte and endophyte communities were similar across plant organs, apart from the root where there was an increase in abundance of *Dactylonectria* and *Nectriaceae* (known soybean growth-promoting genera) in the endophyte community (Fig.S7). Across developmental stages, there was a clear dominance of *Ascomycota* in both the epiphyte and endophyte community. Noticeably, *Zygomycota* was the second most abundant phyla in the epiphyte community

whilst *Basidiomycota* dominated the endophyte community (Fig.S8). *Basidiomycota* was the second most prevalent phylum in the pods (immature and mature) and control seed (Fig.S7). At the genus level, the abundance of *Aspergillus* was more ubiquitous across all developmental stages and in the endophyte and epiphyte communities, except for pods (immature and mature) and control soil where it was nearly absent (Fig. S7). Interestingly, although the taxonomic composition at the maturation stage was similar in both communities (endophyte and epiphyte), there was a marked difference in the relative abundance of taxa. Noticeably, the relative abundance of *Aspergillus, Dactylonectria,* and *Monographella* were much higher in the endophyte communities, whereas *Mortierella* was nearly absent (Fig. S7).

In the belowground compartment, the relative abundance of fungal taxa were influenced by both developmental stage and sample site: *Penicillium* (Fig.5; developmental stage χ^2=30.35***; sample site χ^2=14.67**), *Fusarium* (Fig.5; developmental stage χ^2=20.53***; sample site χ^2=18.07*), *Mortierella* (Fig.5; developmental stage χ^2=25.83***; sample site χ^2=32.86***), *Dactylonectria* (Fig.5; developmental stage χ^2=26.73***; sample site χ^2=37.26***), *Metarhizium* (Fig.5; developmental stage χ^2=13.96**; sample site χ^2=43.83***), *Monographella* (Fig.5; developmental stage χ^2=20.21***; sample site χ^2=14.36***). The relative abundances of *Aspergillus* (χ^2=13.89*), *Gibberella* (χ^2=24.50***), and *Malassezia* (χ^2=11.56*) were solely influenced by developmental stage (Fig.5). In the aboveground compartments, *Aspergillus* and *Malassezia* were the only taxa whose relative abundance was influenced by both developmental stage and sample site (Fig.S8).

Random forest algorithms using a small training set were used to predict the likelihood of association of OTUs across developmental stages and samples sites. There were specific taxa whose abundance and presence were significantly associated with the developmental stage and

sample site. Of the predicted bacterial genera, four had the highest Mean Decrease Accuracy (taxa with a substantial value are more critical for the classification of the plant organ/ developmental stage). For instance, an OTU belonging to the genus *Azospirillum* were consistently shown to be associated with the root endosphere and stem communities (endophyte and epiphyte), whilst the other was highly associated mainly with root endosphere (Fig.6). Many of the OTUs associated belowground compartment were also associated with the stem compartment, except for a single OTU from the genus *Niastealla* was highly associated with leaf epiphyte and root endosphere (Fig.6). Across developmental stages, OTUs from the genus *Pseudomonas* and *Rhizobium* were highly associated with the flowering stage (Fig.6). Two OTUs from *Klebsiella* and *Paenibacillus* genera were associated with the maturation stage along with an OTU from family *Sphingomonadaceae* (Fig.6). The majority of OTUs were associated with the emergence stage.

Generally, random forest algorithms had increased accuracy when predicting the presence and abundance of fungal general across developmental stages and sample site. Firstly, four OTUs from the genus *Fusarium* were mainly highly associated with the belowground compartments and control soil (Fig.7). OTUs from *Dactylonectria* and *Tetracladium* were highly associated with the belowground compartments; also, an OTU from *Monographella* associated with the control seed. (Fig.7). The zoosporic *Olpidium* was only significantly associated with the emergence and growth developmental stages only (Fig.7). Five OTUs from genus *Mortierella* were highly associated with the flowering stage, whilst an OTU from genus *Gymnostellatospora* was highly associated with the emergence stage (Fig.7). Lastly, *Chaetomidium* was highly associated with the growth and to a lesser extend flowering developmental stage (Fig.7).

Discussion

To our knowledge, this is the first report that simultaneously provided evidence for the existence of microbial niches across spatial and temporal axes for a major crop. Here, our aim was to (1) highlight the influence of spatial and temporal dynamics on soybean microbiome and (2) testing for the existence of temporal niche spaces. Our results show that the interactions between spatial and temporal dynamics influence community structure and assert a strong selection filter.

Influence of spatial and temporal dynamics

Spatial and temporal dynamics are stronger discriminatory axes for plants microbiomes as our data shows. However, the influence and importance of one of these factors cannot be overstated as their interactions modulate community structure and composition. Evidence of microbial niche differentiation along these spatial and temporal axes is emerging for model plant systems (Ofek-Lalzar *et al.*, 2014, Niwa *et al.*, 2018, Toju *et al.*, 2018), and recent interest in the role and the use of microbes in agriculture has spurred research into microbiomes associated with crops (Montanari-Coelho *et al.*, 2018, Toju *et al.*, 2018, Merloti *et al.*, 2019). Most importantly, these studies have indicated that the assembled microbiome of greenhouse plants are compositionally similar to their wild and field counterparts (Bai *et al.*, 2015).

Developmental stage influenced the composition and structure of soybean phyllosphere (Copeland *et al.*, 2015) and the rhizosphere communities (Xu *et al.*, 2009, Sugiyama *et al.*, 2014). Here, by sampling all plant compartments in the same experiment, it was then possible to detect a seasonal trend in the data that influenced the abundance and diversity of microbial communities. The abundance and composition of the released plant exudates at each developmental stage influences microbial successional patterns within the rhizosphere and root compartment (White *et*

al., 2015, Lian *et al.*, 2017). This selection imposed by rhizodeposition was more pronounced for the fungal community than the bacterial/archaeal community. Consequently, plant microbiomes become compositionally nested from the ground up- where rhizosphere communities contain the highest diversity (Amend *et al.*, 2019). Developmental stage explained a more substantial proportion of the observed variation within the belowground compartments than in the aboveground. However, the large amount of residual variation within aboveground communities often arises in diversity deplete communities and implies a strong influence of ecological drift (Leibold & Chase, 2017). Here, the aboveground microbial communities had lower abundances, and developmental stage influenced overall diversity. Temporal variations in the size of ecological communities as a result of extrinsic factors, such as plant developmental stage amplifies the influenced of ecological drift (de Mazancourt *et al.*, 2013, Gilbert & Levine, 2017). Further, these temporal fluctuations in community abundance and diversity, act as a stabilising ecological filter and creates different temporal niches that are then occupied by various taxa (Adler & Drake, 2008, Gilbert & Levine, 2017). In ecological communities, drift causes stochastic fluctuations in abundances and lowers diversity and this further results in divergence in community structure (Gilbert & Levine, 2017). It is then possible that observed variations in the aboveground communities are a result of ecological drift and the interactions between developmental stage and sample site creates specialised microbial niches. For instance, various fungal taxa can exhibit similar levels of host colonisations, but there are clear distinctions in their degree of infection and developmental patterns that are mediated by plant metabolic response (Macia-Vicente *et al.*, 2009). As a result, plant-fungal communities tend to display discordant nestedness topology and community structure (Toju *et al.*, 2015). However, bacterial community composition and diversity are influenced primarily by specific localised host response, such as, production of organ-specific

metabolites (Horton *et al.*, 2014), secondary metabolites that regulate the biotic interactions (Cotton *et al.*, 2019), and plant-microbe communication (Lareen *et al.*, 2016).

In model systems, the successional pattern of leaf and root-associated microbiomes has tended to mirror one another with taxonomic and function overlap (Bai *et al.*, 2015). However, this study found spatial dynamics are durable discriminatory axes for belowground microbial communities. Plant organs and sample site strongly influences the abundance and distribution of available microbial niches in soybean (Miller & Roy, 1982). Plant samples site is a robust discriminatory axis for microbial diversity across different plant systems (Poudel *et al.*, 2019, Singer *et al.*, 2019). Microbial successional patterns tend to be strongly linked to sample site in part because only adapted taxa can inhabit these specialised niches within plant tissues (Dickie *et al.*, 2002, Duran *et al.*, 2018, Qian *et al.*, 2019). In soybean, these successional patterns are highly variable between the primary root and secondary lateral roots (Sakamoto & Kaji, 2017), whilst fungal colonisation is a result of niche competition within the rhizosphere (Niwa *et al.*, 2018). These findings indicate that in soybean root-soil interface niche differentiation is delimited along spatial and temporal axes, whereas it is their interaction that modulates communities. Equally, ecological processes that explain these patterns have been extensively reviewed (Turner *et al.*, 2013). Our findings further support the notion that assembled microbiomes from greenhouse plants recapitulate not only similar community structure (Bai *et al.*, 2015), but also internal community dynamics that are comparable to those of their field counterparts (Copeland *et al.*, 2015). Dispersal rates vary across all plants sample sites, and these often influence microbial abundance diversity (Amend *et al.*, 2019). It is then possible that in a closed dispersal-limited growth chamber, soil microbes that are not subsurface soil-bound could be airborne and colonise the aboveground

organs. Altogether, these results highlight the nuanced ecological processes modulating plant microbiome succession.

Existence of temporal niche spaces

Ecological succession characterises the dominance and abundance of taxa and their influence on community dynamics over time. However, in the microbial ecosystem, the most abundant taxa may not be drivers of ecosystem change and functioning (Elshahed *et al.*, 2008, Lynch & Neufeld, 2015). This study set out to detect temporal niche spaces occupied in soybean by 1) highlighting the spatial and temporal interactions between and within plant microbiomes on composition and structure and 2) plant development stage on plant microbiome. In this attempt, the study succeeded in highlighting microbial niche spaces influenced by spatial and temporal dynamics. For instance, although there were no visible signs of nodulation at each harvest period, there was a significant increase in the relative abundance of the soybean beneficial partner *Bradyrhizobium* at the earliest developmental stage. *Bradyrhizobium* was the least abundant of other taxa that were influenced by developmental stage. Plant metabolic needs demands vary with developmental stages, and to compensate; the plant invests biomass in root production to increase nutrient acquisition (Hodge, 2004). *Bradyrhizobium* and *Pseudomonas* are soybean microbial partners that co-ordinate and modify root architecture to increase nutrient acquisition (Egamberdieva *et al.*, 2017, Kumawat *et al.*, 2019). Likewise, *Streptomyces* co-operates *Bradyrhizobium* to improve nutrient acquisition (Htwe *et al.*, 2018). This study found that the relative abundance of *Bradyrhizobium* and *Pseudomonas* to be influenced by where the microbes localise; additionally, the developmental stage influenced the relative abundance of *Pseudomonas*. It is then possible to speculate that although spatial and temporal dynamics

influence the relative abundance of *Bradyrhizobium* and both *Pseudomonas* and *Streptomyces* to varying extends, these taxa occupy complimenting spatial and temporal niches. For instance, the relative abundance of *Pseudomonas* was predicted and peaked at the flowering stage in the root endosphere, the same compartment the relative abundance of *Bradyrhizobium* was highest at the emergence stage. Bacterial/archaeal communities constrained to a single spatial niche tend to quickly diversify under adaptative radiation (Rocabert *et al.*, 2017), however, in the presence of temporal niches, communities are more likely to circumvent the overshooting dynamics of adaptive radiant and maintain high levels of biodiversity over time (Tan *et al.*, 2013). Thus, we suggest that the immense abundance and diversity of bacterial/archaeal taxa is maintained through these spatial and temporal niche partitioning. Moreover, taxa that were predicted by random-forest algorithms were shown in culture-dependent studies to be highly beneficial for soybean growth and development (Kuklinsky-Sobral *et al.*, 2004).

Plant host interactions strongly modulate the realised niche of fungal communities, and niche specialisations are not delimited along abiotic and biotic (plant host age) axes (Chaloner *et al.*, 2020). Our study highlighted both spatial and temporal dynamics modulated the relative abundance of dominant fungal genera. For instance, random-forest algorithms predicted that OTUs from one of these dominant genera to be highly associated with the flowering stage. The relative abundance of the genus *Mortierella* increased at the flowering stage at the root-soil interface. Members of the genus use the enzyme xylanase to metabolise plant-derived sugars and are chitinolytic (Brzezinska *et al.*, 2014), and antagonistic to plant fungal pathogens such as those in the *Fusarium* genus (Liu *et al.*, 2019). Our data show that both spatial and temporal dynamics influence the relative abundance of most fungal genera, but this contrasts with the predictions from random-forest algorithms. Overall, OTUs from genus *Fusarium* were spatially predicted to

dominate at the root-soil interface; however, OTUs from *Mortierella* dominated at the flowering stage. The sharp increase in the relative abundance of *Mortierella* and depletion of *Fusarium* at the root-soil interface can in part be explicable by the antagonist nature of *Mortierella.* Thus, this study provides support that:

1. within plant-fungal communities niche specialisations evolve independently along spatial and temporal axes (Chaloner et al., 2020),
2. Only adapted taxa can inhabit these specialised niches (Qian et al., 2019), and
3. Niche competitions at the root-soil interface drives fungal assembly and colonisation (Toju *et al.*, 2015).

Lastly, we argue that innate physiological (generation time, colonisation capacity, and cell size) characteristics contribute to the divergent response in bacterial and fungal communities.

Conclusion

In conclusion, our results highlight the complexity of the soybean microbiome and call for more spatially- and temporally resolved studies to capture this complexity, with the view of harnessing the crop microbiome to optimise microbial services in agriculture. We highlight the influence of spatial and temporal dynamics on the occupied niche within soybean microbiome, by focusing on microbes that seem to be consistently influenced by the interaction between spatial and temporal dynamics as potential candidates for microbiome engineering efforts.

Acknowledgements

The authors would like to thank the Benjamin Mimee from Agriculture and Agri-Food Canada for providing the seeds used in the study. This work was supported by a Discovery grant from the Natural Sciences and Engineering Research Council (NSERC) grant RGPIN 2014-05274 to EY. IM was supported by the Innovation and Scarce Skills scholarship from South African National Research Foundation (NRF), Fonds de Recherche du Québec (FRQNT), and partly by Foundation Armand-Frappier. We also wish to acknowledge Compute Canada for access to the University of Waterloo's High Performance Computing (HPC) infrastructure (Graham system) through a resources allocation granted to EY.

Table and Figures

Table 1 Permutation analysis of variance (PERMANOVA) results indicating the influence of temporal and spatial interaction on microbial community structure *(p* value *<0.05, ** <0.01, ***<0.001)

Fig.1 Belowground alpha diversity patterns. Top panel indicating fungal diversity and bottom panel indicating bacteria/archaeal diversity. Total OTU abundance influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction *(p* value *<0.05, ** <0.01, ***<0.001). Pairwise comparisons were not reported for control and pod samples.

Fig.2 Principle Coordinate Analysis (PCoA) of bacterial/archaeal community based on Bray-Curtis dissimilarity across different plant compartment and developmental stages (Compartment= Control Soil, Control Seed, Rhizosphere, Root, Stem, Leaf, and Pod). Samples that are closer to each other have similar community composition, whereas samples that are further apart are distinct to each other in composition

Fig.3 Principle Coordinate Analysis (PCoA) of fungal community based on Bray-Curtis dissimilarity across different plant compartment and developmental stages (Compartment= Control Soil, Control Seed, Rhizosphere, Root, Stem, Leaf, and Pods). Samples that are closer to each other have similar community composition, whereas samples that are further apart are distinct to each other in composition

Fig.4 Belowground bacteria/archaeal relative abundance of most abundant genera that were significantly influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction *(p* value *<0.05, ** <0.01, ***<0.001)

Fig.5 Belowground fungal relative abundance of most abundant genera that were significantly influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction *(p* value *<0.05, ** <0.01, ***<0.001)

Fig.6 Random forest analysis based on the overall taxonomic profile and could distinguish amongst different developmental stages with increased predictive accuracy. The more the accuracy of the random forest decreases due to the exclusion (or permutation) of a single taxa, the more important that taxa is deemed, and therefore taxa with a large mean decrease in accuracy are more important for classification of the data. The figure shows taxa with the highest discriminatory power and their classification (panel: OTU identity) based on their importance for the identification each developmental stage and sample site

Fig.7 Random forest analysis based on the overall taxonomic profile and could distinguish amongst different developmental stages with increased predictive accuracy. The more the accuracy of the random forest decreases due to the exclusion (or permutation) of a single taxa, the more important that taxa is deemed, and therefore taxa with a large mean decrease in accuracy are more important for classification of the data. The figure shows taxa with

the highest discriminatory power and their classification (panel: OTU identity) based on their importance for the identification each developmental stage and sample site

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Table 1 Permutation analysis of variance (PERMANOVA) results indicating the influence of temporal and spatial interaction on microbial community structure *(p* value *<0.05, ** <0.01, ***<0.001)

Bacterial/Archaeal Community	**Belowground**			**Aboveground**		
Source of variation	***MS***	***F value***	***R^2***	***MS***	***F value***	***R^2***
Developmental stage	1.00	5.91	**0.21*** **	0.90	4.52	**0.08*** **
Sample site	3.01	17.76	**0.25*** **	1.48	7.46	**0.19*** **
Developmental stage × Sample site	0.52	5.58	**0.13*** **	0.75	3.73	**0.24*** **
Residuals	0.16		0.38	0.19		0.46
Fungal Community						
Source of variation	***MS***	***F value***	***R^2***	***MS***	***F value***	***R^2***
Developmental stage	1.11	11.67	**0.30*** **	0.62	1.78	**0.06*** **
Sample site	1.23	76.48	**0.13*** **	0.71	2.06	**0.10*** **
Developmental stage × Sample site	0.23	7.30	**0.07** **	0.41	1.20	**0.14***
Residuals	0.15		0.47	0.34		0.62

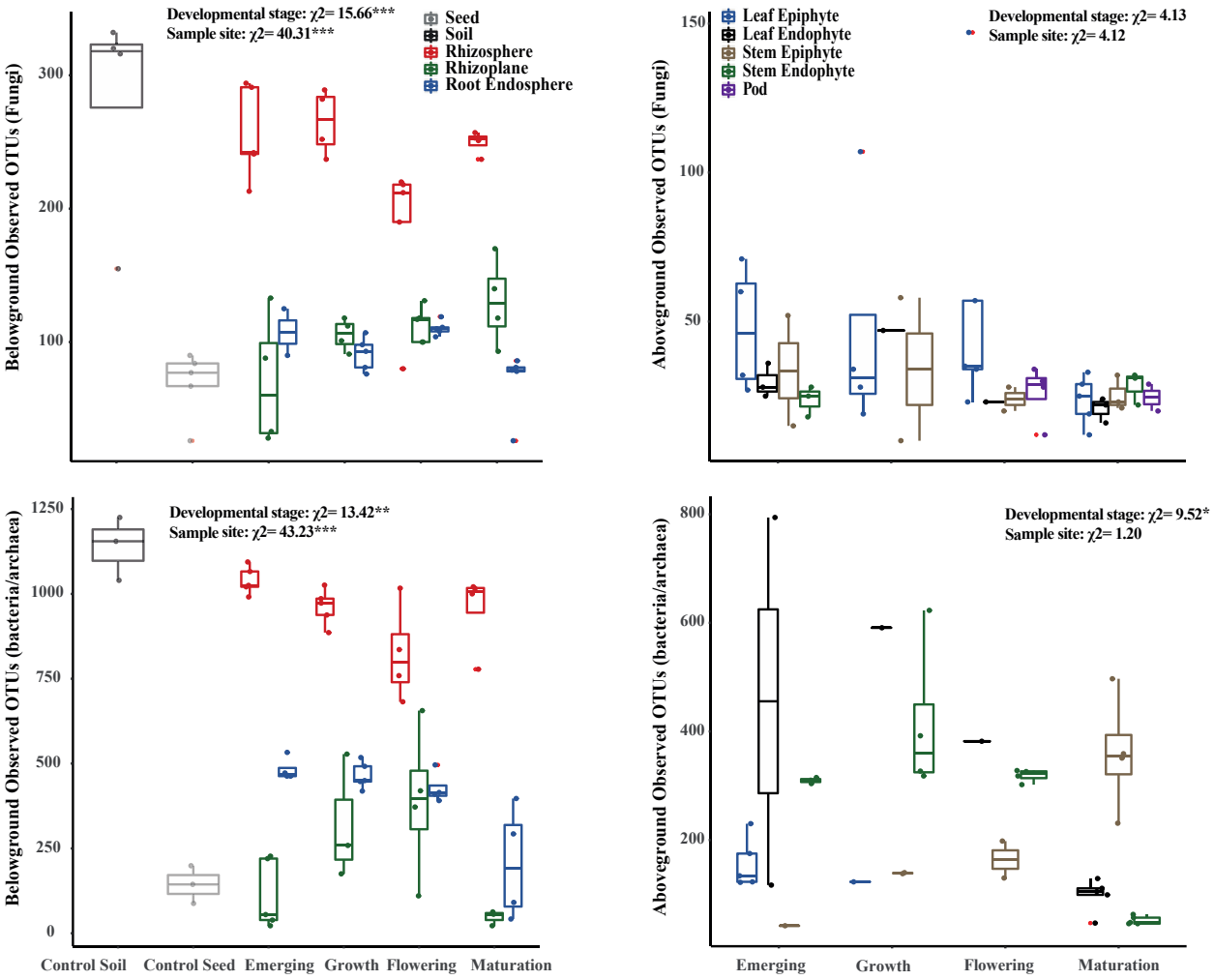
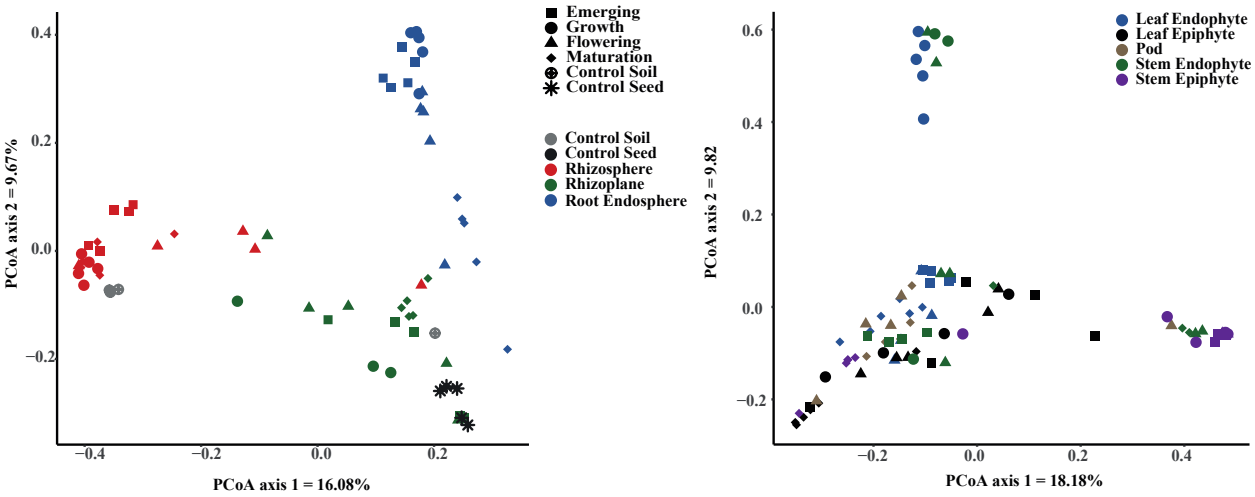


Fig.1 Belwground alpha diversity patterns. Top panel indicating fungal diversity and bottom panel indicating bacteria/archaeal diversity. Total OTU abundance influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction (*p* value *<0.05, ** <0.01, *<0.001). Pairwise comparisons were not reported for control and pod samples. The lines inside the box indicate the mean of samples where n=3~5.**



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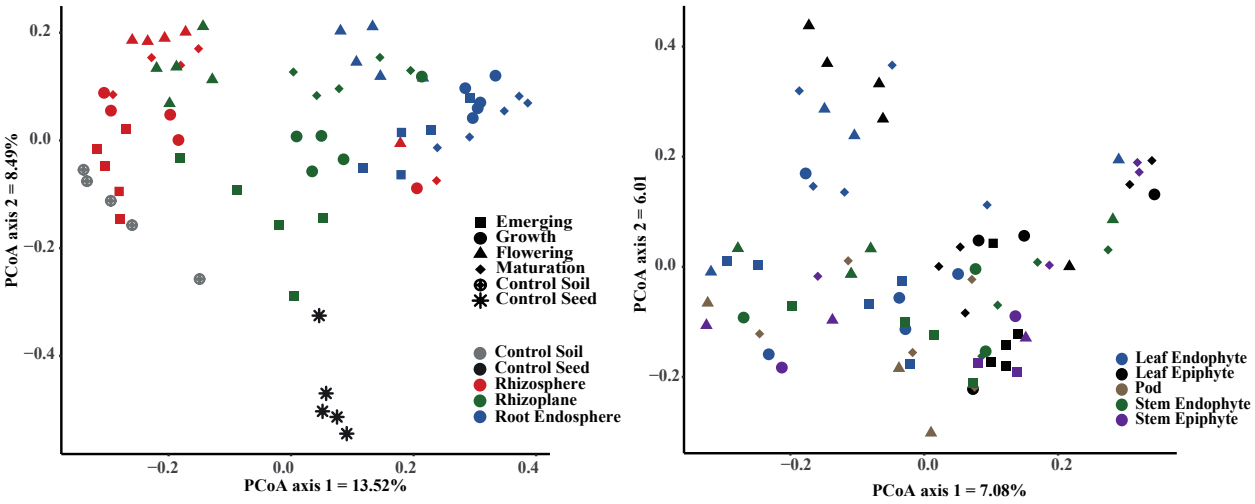

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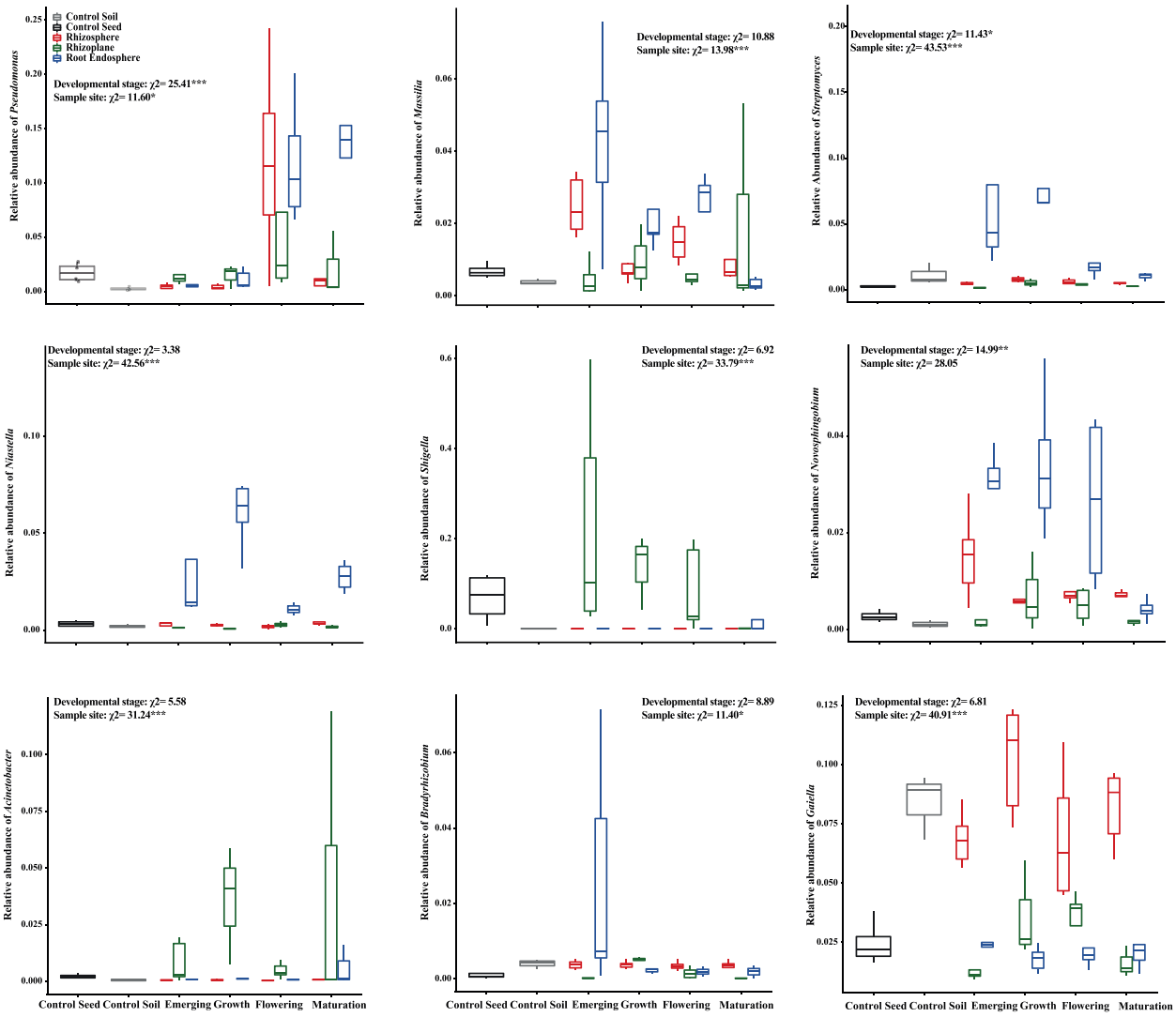

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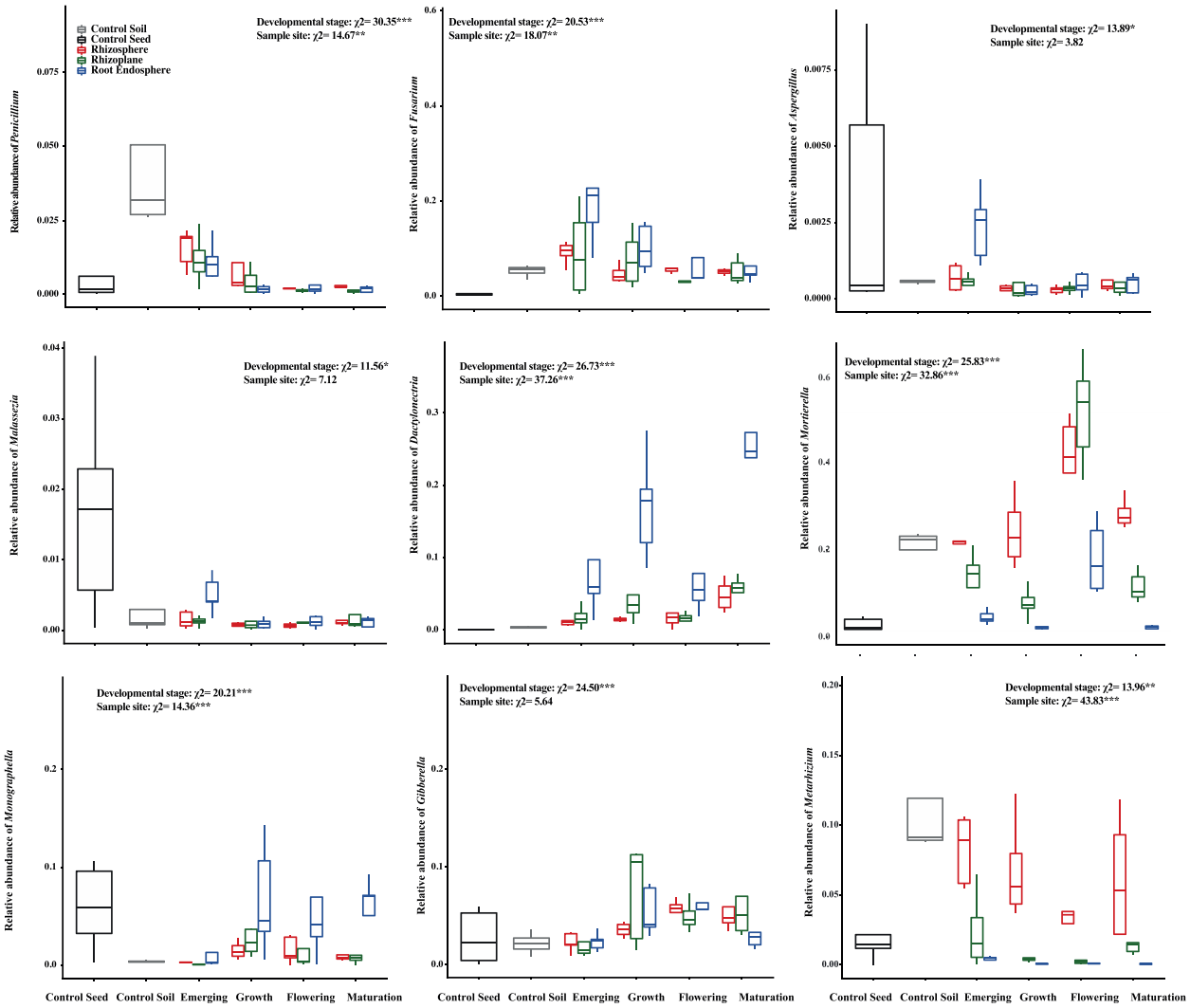

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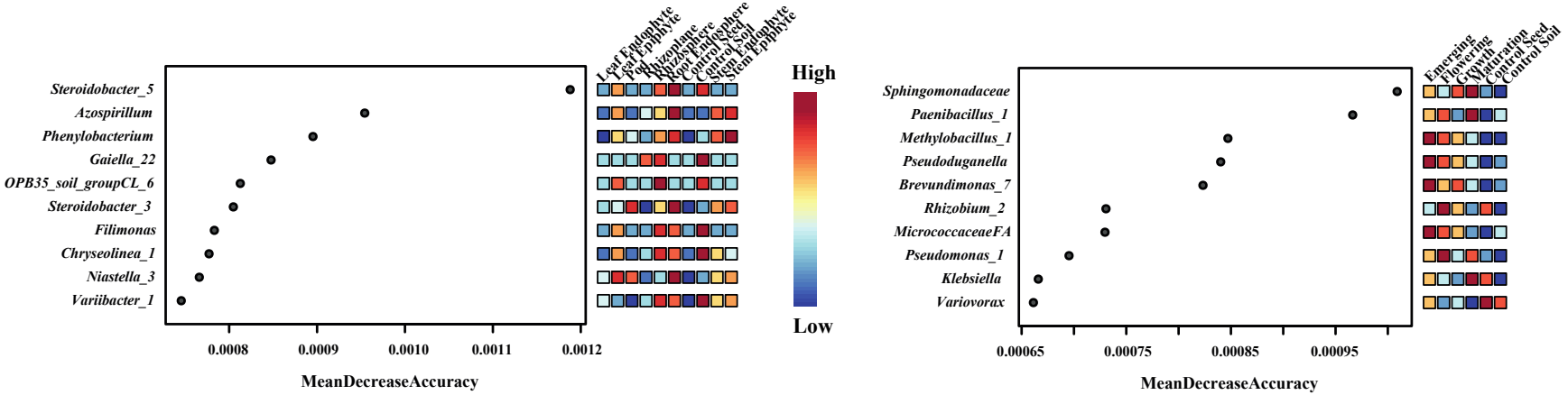

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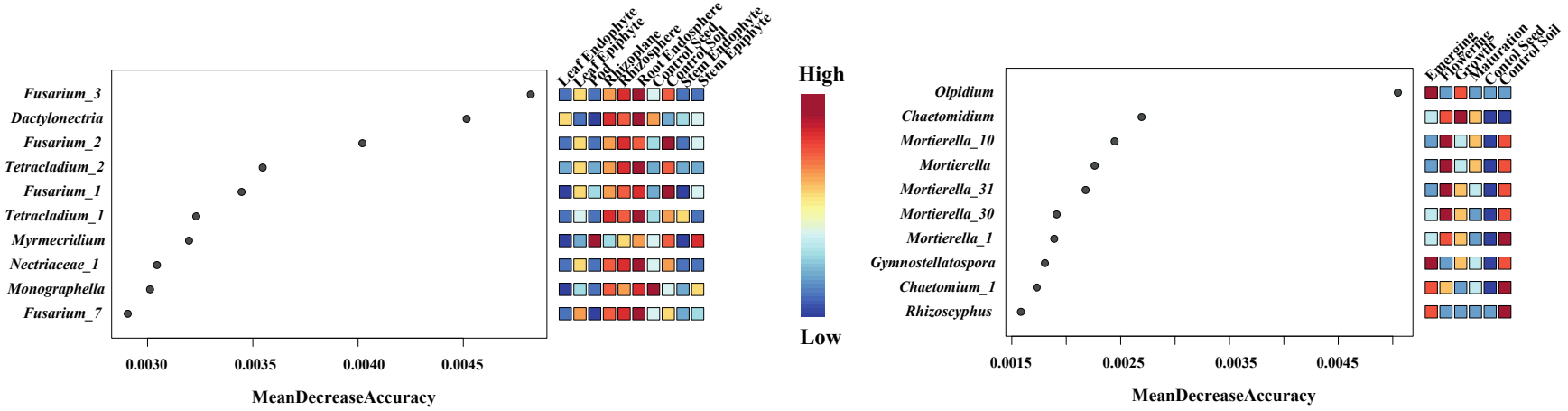

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1
2