Conditionality of Soil Microbial Mediation of Plant Phenotype

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Abstract

Purpose: While distinct soil microbiomes and individual soil microbial taxa can alter particular plant traits under highly controlled conditions, little is known about the role of particular microbial taxa and microbial functions within complex soil microbial communities for mediating plant phenotypes or if the strength of microbial mediation of plant phenotype varies among plant species or plant phenotypic traits. Examining how the plant phenotype spectrum is influenced by the taxonomic and functional composition of complex soil microbial communities allows for a more accurate understanding of the biotic environmental drivers of plant phenotype.

Methods: Using rhizosphere soil collected from field sites, we conducted a microbiome transfer glasshouse experiment to test the hypothesis that the taxonomic and functional composition of different soil microbiomes would differentially shift growth, physiological or reproductive phenotypes of three *Solidago* species.

Results: We found that soil microbiome inoculations influenced *Solidago* growth traits more than physiological and reproductive traits. We found that root growth of one of the *Solidago* species was negatively correlated with 77% of the indicator bacterial and fungal taxa from one of the soil microbiome treatments.

Conclusions: Soil microbial mediation of plant phenotype varies by plant traits, is not universal across plant species, and can be associated with a small number of microbial taxa. This study illustrates that specific microbial taxa within a soil microbiome are associated with shifts in plant phenotype by pinpointing important individual microbial taxa from complex field soil microbial communities.

Introduction

In the past 10-15 years, numerous and diverse relationships discovered between plants and the soil microbiome have shifted the long-established paradigm of plant phenotype as the sole product of interactions between a plant's genes (G) and the abiotic environment (E) (i.e., G x E interactions; Clausen et al., 1948; Conner & Hartl, 2004) to that of a 'holobiont' interpretation (G x G x E interactions), in which microbes at the root-soil interface serve as a reservoir of additional genes and functions for the host plant (Bordenstein & Theis, 2015; Theis et al., 2016; Vandenkoornhuyse et al., 2015; Zilber-Rosenberg & Rosenberg, 2008). Multiple avenues of research have informed this updated perspective. At a broad scale, the existence of plant-soil feedbacks, in which soil conditioned from one plant species can promote or inhibit the growth of other conspecific or heterospecific plants (Bever, 1994; Bever et al., 1997; Ehrenfeld et al., 2005; Mangan et al., 2010; Putten et al., 2013), has established that individual soil microbes collectively, referred to as a microbiome, can influence plant phenotype. The mechanisms that underlie plant-soil feedbacks are largely attributed to the fact that phenotypically distinct plants can differentially alter both the abiotic and microbial composition of the surrounding soil, in part due to differences in organic matter turnover, and root chemical exudates that in turn favor distinct communities of microbes (Hu et al., 2018; Jones et al., 2019). The identification of core rhizosphere microbiome members for a diversity of plant taxa (Colin et al., 2017; Fitzpatrick et al., 2018; Hugoni et al., 2018; Lasa et al., 2019; Lundberg et al., 2012; Schweitzer et al., 2008; Singer et al., 2019; Timm et al., 2018; Yeoh et al., 2017) provides further evidence for the ability of different plants to recruit particular soil microbes. At a fine scale, single inoculation studies—mostly targeted towards crop health and production—have determined that particular individual soil microbial taxa can modify plant traits, such as growth phenotypes or flowering phenology. In isolation, bacterial genera such as *Microbacterium*, *Pseudomonas*, and *Enterobacter*, for example, can increase desiccation tolerance in some crop varieties by stimulating trehalose production (Niu et al., 2018; Vílchez et al., 2016). Similarly, plant growth promoting rhizobacteria assist in nutrient acquisition of nitrogen, phosphate, potassium, and iron by producing various phytohormones (Ali et al., 2009; Mishra et al., 2009; Ofek-Lalzar et al., 2014). Moreover, whole microbiome soil inoculation studies have documented microbial mediation of a diversity of plant phenotypes including physiological traits such as photosynthesis (Friesen et al., 2011; Kannenberg & Phillips, 2017; Lau & Lennon, 2011; Zhu et al., 2016), phenological traits such as flowering time (Panke-Buisse et al., 2015;

Wagner et al., 2014) and reproductive traits such as fruit (Lau & Lennon, 2011, 2012) and flower production (Lau & Lennon, 2012).

While findings from these research fields show that both isolated individual microbial taxa and diverse soil microbial communities can influence plant function, pinpointing the important individual microbial taxa and functions within complex soil microbial communities remains a challenge. Identifying significant individuals or functions within complex microbial communities is crucial for advancing ecology of natural ecosystems because plants in natural landscapes interact simultaneously with a multitude of beneficial, benign, and pathogenic microbes (Morris et al., 2007; Putten et al., 2016; Zolla et al., 2013). Beneficial or deleterious effects from individual taxa may be enhanced or suppressed by interactions with other nearby microbial members. Examining how the taxonomic and functional composition of soil microbial communities affects plant phenotype will allow for a more accurate understanding of the surrounding biotic environmental drivers of plant phenotype.

The goal of this study was to identify the strength of soil microbial mediation for different plant phenotypes, the consistency of these relationships among plant species, and identify specific soil microbial taxa and/or functions in complex field soil communities that are associated with particular plant phenotypes. Using field soils associated with three phenotypically distinct *Solidago* species, we conducted a glasshouse experiment and inoculated three *Solidago* species in separate treatments of each field-collected soil and microbiome. We tested the following hypotheses: 1) Soil microbiome source inoculation will differentially alter phenotypes of three *Solidago* species; 2) Soil microbiome source is associated with distinct taxonomic and/or functional soil microbial communities; 3) Specific microbial taxa and/or microbial functions are associated with particular *Solidago* phenotypes. Shifts in *Solidago* phenotypes between microbiome source treatments would indicate that plant traits are influenced by variation in microbial taxonomic and/or functional composition. Variation in response to microbiome treatments among traits would indicate conditional effects of microbial mediation of plant phenotype. Variation in response to microbiome treatments among *Solidago* species would indicate conditional effects of microbial mediation of plant phylogeny. Correlations between specific microbial taxa and/or microbial functions and particular *Solidago* phenotypes would provide evidence for the importance of individual taxonomic or functional components within a microbiome for influencing plant phenotype.

Materials And Methods

Study system

Solidago species are a model system for this study because they commonly occur across North America, with 120 species native to the United States (Semple, 2016) that grow in variable habitats, with different morphologies and phenotypes. We chose to use *S. caesia*, *S. flexicaulis*, and *S. gigantea* in this study because they were the most abundant *Solidago* species found across our sampling range (northeastern TN) and vary in evolutionary history, leaf, stem, and flower morphology and habitat preference. *Solidago caesia* and *S. flexicaulis* grow in woodlands and belong to the *Glomerulifloraea* subgroup of *Solidago* (Semple, 2016). *Solidago gigantea* grows in meadows and fields and belongs to the *Triplinerviae* subgroup (Semple, 2016). Furthermore, previous work has found evidence for the influence of interspecific and genotypic diversity on above- and belowground biomass of *S. altissima* and *S. gigantea* (Genung et al., 2012, 2013), suggesting that some *Solidago* phenotypes may be mediated in part by modifications of soil biota from neighboring *Solidago* species.

Preliminary field surveys

To assess differences in plant phenotypes among the three *Solidago* species, we conducted field surveys of three geographically distinct populations of each species, all located throughout northeastern Tennessee, U.S.A. In May 2017, we measured stem height, stem base diameter, specific leaf area (SLA), and stomatal density of 15 randomly selected putative genotypes of each species (*S. caesia*, *S. flexicaulis*, and *S. gigantea*) in northeastern TN for a total of 45

individuals per species (Fig. 1a). The field survey confirmed that the three species vary in this suite of growth and physiological phenotypes (Table S1).

Soil collection and processing

To assess Hypothesis 2 that the soil microbiome sources have distinct taxonomic and/or functional microbial communities, we collected rhizosphere soil from each genotype in the field surveys by collecting soil attached to the roots of each plant (Fig. 1b). We pooled individual soil samples by field site to represent an average belowground microbiome of three soil sources (n = 3 sites per soil source). While we tried to collect soil microbes that were only associated with the rhizosphere soil of each plant species, it is likely that we also captured microbes that are representative of surrounding non-rhizosphere soil. Due to this fact and that some climatic and edaphic soil characteristics including mean annual temperature, soil organic matter content, and soil bulk density slightly varied among the three groups of *Solidago* species sites (Table S2), we refer to the three groups of sites as soil microbiome sources rather than soils associated with each *Solidago* species. Soil samples were transported to the laboratory on ice and stored at 0°C until analysis at the University of Tennessee, Knoxville, TN, U.S.A. A 2 g subsample of soil from each field site was stored at -80°C for molecular analysis. We assessed the taxonomic community composition of the soils using high-throughput amplicon sequencing of the V3-V4 region of the 16S rRNA gene (16S) and the ITS2 region of the internal transcribed spacer gene regions for bacteria and fungi, respectively. Detailed methods are described in Methods S1 of the Supplementary Materials.

We performed all amplicon sequence processing using the DADA2 platform (Callahan et al., 2016). For 16S sequences, primers were removed prior to the DADA2 pipeline using the cutadapt function in conda. Samples were normalized for sampling depth with a variance stabilizing transformation with the DESeq2 package (Love et al., 2014). We chose this method over the common practice of rarefaction because rarefaction results in loss of data by using the lowest sampling depth and it inflates variances across samples (McMurdie & Holmes, 2014). Taxonomy of ASVs was assigned using the RDP (Wang et al., 2007) and UNITE (Abarenkov et al., 2010) databases for bacteria and fungi, respectively. After processing, we had 16,245 bacterial and 2,565 fungal ASVs, respectively. Additionally, we assigned fungal ASVs to functional guilds using the FUNGuild database (Nguyen et al., 2016). For analyses, we assigned taxa to one of seven broad functional guilds: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, ericoid mycorrhizal fungi, endophytic fungi, plant pathogenic fungi, saprotrophic fungi, and “other.” We considered only FUNGuild assignments with a confidence ranking of “highly probable” or “probable.” Unassigned taxa were excluded from further guild-based analyses. Of the 2,565 fungal ASVs, 1,741 were assigned to a fungal guild. Of those assigned, we used the 1,328 ASVs that had a confidence ranking of “highly probable” or “probable.”

We assessed functional community composition with shotgun metagenomic sequencing, as detailed in Methods S1 of the Supplementary Materials. Sequences retrieved from shotgun metagenomic sequencing were assigned to KEGG (Kyoto Encyclopedia of Genes and Genomes) ortholog numbers using the MG-RAST online annotation tool. KEGG orthologs assign genes to microbial complexes, functional sets, and metabolic pathways and are a common tool used to describe functional attributes of microbes (Ortiz-Álvarez et al., 2018; Sorensen et al., 2019). KEGG ortholog numbers were matched to hierarchical KEGG pathways.

Glasshouse experiment

To assess Hypotheses 1 and 3 that plant phenotypes are in part mediated by the taxonomic and/or functional composition of soil microbial communities and that the strength of microbial mediation varies among plant species and phenotypic traits, we conducted a glasshouse experiment and grew *S. caesia*, *S. flexicaulis*, and *S. gigantea* in factorial soil inoculum treatments of each microbiome source. Seeds of each *Solidago* species were purchased from separate nurseries to account for intraspecific variation in plant response to soil microbes (*S. caesia*: Ernst Conservation Seeds, Meadville, PA; NorthCreek Nurseries, Landenburg, PA; Michigan Wildflower Farm, Portland, MI; *S. flexicaulis*: Ernst Conservation Seeds, Prairie Moon Nurseries, Winona, MI; Minnesota Native Landscapes, Ostego, MN; *S. gigantea*: Prairie

Moon Nurseries, Minnesota Native Landscapes). Seeds were refrigerated at 4°C prior to sowing, and then were sown by population into a commercial peat moss-based, non-mycorrhizal potting mix (Premier Promix BX, containing perlite, vermiculite, and limestone). A subset of *Solidago* seeds did not withstand surface sterilization trials, so we did not surface sterilize the seeds used in the experiment. While it is possible that any seed-borne microbes may have impacted plant phenotype, all plants were grown in all soil treatments and exposed to the same glasshouse conditions, such that any effect of seed-borne microbes on plant phenotype should be equally distributed across treatment categories.

After approximately three weeks of growth, 54 similar-sized seedlings of each population were individually transplanted into half-gallon circular pots into soil inoculum treatments which consisted of factorial combinations of microbiome source (Microbiome source 1 vs. Microbiome source 2 vs. Microbiome source 3) (Fig. 1c). Furthermore, since soils from each field site of each microbiome source were kept separate, seeds were planted into three sites of Microbiome source 1, three sites of Microbiome source 2, and three sites of Microbiome source 3. Each pot was inoculated with 2 teaspoons of field soil (< 1% of the total pot volume) to reduce effects of variation in soil nutrients on plant phenotypic responses (Troelstra et al., 2001). In total, 243 pots were established: 3 *Solidago* species x 3 seed populations x 3 microbiome sources (Microbiome source 1, Microbiome source 2, Microbiome source 3) x 3 field soil sites x 3 replicates = 243 total pots). Pots were randomly positioned in the glasshouse based on random number assignments. All plants were treated monthly for thrips and whiteflies throughout the experiment (0.5 tsp/gal Avid 0.15 EC insecticide, 0.5 tsp/gal AzaGuard insecticide). Plants were equally watered from above, as needed (approximately 4 days/week), and allowed to grow for 5 months in a glasshouse at the University of Tennessee.

A suite of plant phenotypes was measured during and post-experiment. Stem height and stem diameter were measured every two weeks for the first two months of growth, then at 13 weeks and at the termination of the experiment at 20 weeks. Relative growth rates were calculated from these data. For each individual plant, timing of flower bud formation (hereafter referred to as “flower bud break”) and flowering were monitored with daily surveys by recording the day of the appearance of the first distinguishable flower bud and first open flower, respectively. Prior to termination of the experiment, an average of four healthy and mature leaves were randomly selected per plant, scanned using WinFOLIA software (Regent Instruments Inc.), oven-dried at 70°C for 72 hours (Pérez-Harguindeguy et al., 2016), and weighed to calculate specific leaf area (cm^2/g) (SLA). After five months of growth and regular watering, each individual was harvested and separated into shoot and root biomass and inflorescence biomass. Shoot and root tissue was weighed after 48 hours of oven-drying at 60°C. Prior to drying, roots were carefully rinsed over 2 and 0.5 mm sieves to remove lingering soil and collect all fine roots.

Statistical Analyses

In the field survey, we analyzed differences in *Solidago* phenotypes using linear mixed-effects models with the lmer function in the lme4 package (Bates et al., 2014). We built separate mixed-effects models for each phenotype (stem height, stem diameter, SLA, and stomatal density) using *Solidago* species as the fixed effect and population as the random effect. When necessary, all data were transformed to conform to normality before analysis. To test Hypothesis 1 that phenotypes of each *Solidago* species differ when grown in soils inoculated with microbial communities associated with a different microbiome source, we built linear mixed effects models with the lmer function in the lme4 package. First, to identify traits most important to growth, physiology, and reproduction and to reduce Type I error, we tested for correlations between the ten phenotypes measured from the glasshouse experiment (relative growth rate in stem height, stem diameter at maturity, shoot biomass, root biomass, total biomass, root to shoot ratio, SLA, flower bud break, days to flower, inflorescence biomass) using the cor.test function. We chose to exclude stem diameter, total biomass, and root to shoot ratio from the analysis because they were all significantly correlated with two other growth phenotypes, shoot and root biomass (Table S5). We also chose to exclude days to flower and inflorescence biomass from the analysis because the experiment ended before the majority of *S. gigantea* individuals flowered. Relative growth rate, shoot biomass, root biomass, SLA, and flower bud break were included in the analysis.

Multiple models were used to assess Hypothesis 1. Separate models were built for the five phenotypes (relative growth rate, shoot biomass, root biomass, SLA, and timing of flower bud formation). When necessary, all data was transformed to conform to normality before analysis. First, to test that differences in soil microbial community composition have a general effect on plant phenotypes regardless of plant species, we built linear mixed effects models with microbiome source as a fixed effect and *Solidago* species, seed population, and field soil site as random effects. Then to identify interspecific variation in response to microbial community composition we separated the dataset by each *Solidago* species and built individual linear mixed effects models for each *Solidago* species with microbiome source as a fixed effect and seed population and field soil site as random effects. For all models, we used the Anova function to calculate ANOVA tables using Type II sums of squares, with significance assessed for each fixed effect using Wald X^2 statistics. If any of the fixed effects were significant, we conducted post hoc Tukey contrasts using the TukeyHSD function.

To test Hypothesis 2 that each microbiome source is associated with distinct taxonomic and/or functional soil microbial communities, we took multiple approaches. First, we assessed microbial diversity across microbiome source by calculating hill numbers based on ASV counts and unique KEGG identities using the hill_div function in the hilldiv package (Alberdi & Gilbert, 2019). Hill numbers serve as effective numbers of diversity that provide more intuitive estimates of diversity compared to traditional diversity indices based on entropy (Chao et al., 2014). We calculated hill numbers for all orders of diversity at q = 0, q = 1, and q = 2, and tested for significant differences in hill numbers between microbiome source at each order of diversity using the div_test function in the hilldiv package. A diversity order q = 0 provides raw richness by weighting rare taxa the same as abundant taxa and thus not accounting for species' abundances. A diversity order q = 1 weights ASVs by their abundance but without disproportionately favoring abundant taxa. A diversity order q = 2 overweighs abundant ASVs.

Second, we created Bray-Curtis distance matrices for microbial taxonomic and functional composition of the nine field soils. To assess variation in community composition of bacteria, fungi, and KEGGs across microbiome source, we conducted PERMANOVA analysis with 9,999 permutations using the adonis function in the vegan package (Oksanen et al., 2019). Prior to conducting PERMANOVA we confirmed homogeneity of dispersion across microbiome source with the betadisper function in the vegan package. We then performed a distance-based redundancy analysis (db-RDA) using the dbrda function in the vegan package to assign variation in composition of bacteria, fungi, and KEGGs to microbiome source and geographic location. We conducted three individual db-RDAs for bacteria, fungi, and KEGG composition. We used the anova.cca function in the vegan package to assess the cumulative significance of microbiome source and geographic location on community composition. We partitioned the variation in composition with respect to microbiome source and geographic location using the varpart function in the vegan package. To visualize composition of bacteria, fungi, and KEGGs among soil origin, we used principal coordinate analysis (PCoA) for ordination based on the Bray-Curtis distance matrices.

We then performed indicator species analysis with the multipatt function in the indicspecies package (Cáceres & Legendre, 2009) to identify particular bacteria, fungi, and KEGGs that are uniquely highly associated with each microbiome source. Because the FUNGuild data set contained a high amount of zero counts, we built individual zero-inflated models for each fungal guild using the glmmTMB function in the glmmTMB package (Brooks et al., 2017). We specified microbiome source as the fixed effect, count total per soil sample (i.e. site) as the random effect, zi formula as soil origin, and family as poisson. For all models, we used the Anova function in the car package (Fox et al., 2013) to calculate analysis of variance (ANOVA) tables using Type II sums of squares, with significance assessed for microbiome source using Wald X^2 statistics. If the effect of microbiome source was significant, we conducted post hoc Tukey contrasts using the emmeans function in the emmeans package (Lenth et al., 2020) and the cld function in the multcomp package (Hothorn et al., 2008).

To test Hypothesis 3 that specific microbes and/or microbial functions are associated with particular *Solidago* phenotypes, we assessed the effect of variation in microbial indicator taxa composition on *Solidago* phenotypes that responded to microbiome source treatment. Since no KEGG identities were identified as indicators across the three microbiome sources, subsequent analyses were conducted only with bacterial and fungal indicator taxa. Using a db-RDA, we assigned variation in composition of bacterial and fungal indicator taxa to the three microbiome sources and geographic location. We then extracted the axes scores from the db-RDA model. For each phenotype, we built a linear model that included the two axes (CAP1, CAP2) from the db-RDA model as fixed effects. A significant relationship between db-RDA axes and plant phenotypes would indicate that differences in the community of bacterial and fungal indicator taxa associated with each microbiome source are associated with shifts in plant phenotype. To pinpoint individual bacterial and fungal indicator taxa that may be associated with particular plant phenotypes, we built linear models to test for correlations between the relative abundance of each bacterial and fungal indicator taxon and each phenotype that showed significant responses to the axes of variation from the indicator species db-RDA model.

All analyses were performed in R (R Core, 2020). Boxplot, and linear regression figures were made with the ggplot2 package (Wickham, 2016). Ordination figures were made with the phyloseq package (McMurdie & Holmes, 2013). Heatmap figures were made with the Heatplus (Ploner, 2020) and gplots (Warnes et al., 2020) packages. Individuals figures were aggregated with the patchwork package (Pedersen, 2020).

Results

Plant phenotype responses to soil microbiome sources (glasshouse experiment)

While the three *Solidago* species overall varied significantly in relative growth rate, shoot and root biomass, and flower bud break, only root biomass differed by microbiome source (χ^2 = 6.14, p = 0.04) (Table 1). Among all three *Solidago* species, there was 29% greater root biomass production when plants were grown in inoculum from microbiome source 1 relative to microbiome source 3 (Tukey post hoc: p = 0.05) (Fig. 2a). In partial support of Hypothesis 1, the species-specific models showed that phenotypic responses to microbiome source inoculum varied by *Solidago* species and by phenotype. *Solidago caesia* shoot biomass differed among microbiome source treatments, whereas no *S. flexicaulis* or *S. gigantea* phenotypes differed among microbiome source treatments (Table 2). *Solidago caesia* produced 8.9% more shoot biomass when grown in inoculum from microbiome source 2 relative to microbiome source 3 (Tukey post hoc: p = 0.09) (Fig. 2b), indicating that different soil microbiomes can shift plant traits.

Table 1
Results of ANOVA showing the effect of microbiome source on the five *Solidago* phenotypes measured from the glasshouse experiment. *Solidago* species, seed population, and soil inoculum field site were included as random effects in the models. Statistically significant results are shown in bold.

		Plant growth						**Plant physiology**		**Plant phenology**	
		RGR		**Shoot**		**Root**		**SLA**		**flower bud break**	
Effect	**df**	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Microbiome source	2	1.65	0.44	4.38	0.11	**6.14**	**0.046**	3.05	0.22	0.37	0.83

Table 2
ANOVA table of the effect of microbiome source on the five phenotypes of each *Solidago* species measured from the glasshouse experiment. Seed population and soil inoculum field site were included as random effects in the models. Statistically significant results are shown in bold.

			Plant growth						Plant physiology		Plant phenology	
			RGR		Shoot		Root		SLA		flower bud break	
Solidago **species**	**Effect**	df	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
S. caesia	Microbiome source	2	0.99	0.61	**5.65**	**0.06**	3.34	0.19	1.53	0.46	1.88	0.39
S. flexicaulis	Microbiome source	2	1.35	0.51	0.95	0.62	2.74	0.25	1.6	0.45	1.16	0.56
S. gigantea	Microbiome source	2	2.51	0.28	0.65	0.72	1.14	0.56	4.52	0.1	3.37	0.180

Community composition among soil microbiome sources

Across the three soil microbiome sources, we identified over 16,000 bacterial and 2,500 fungal ASVs. Taxonomic and functional diversity of soil microbial communities did not vary by microbiome source at any order of diversity (Tables S3, S4). In partial support of Hypothesis 2, whole microbiomes did not differ in taxonomic or genetic pathway composition among the microbiome sources, but distinct indicator taxa were identified for each microbiome source. PERMANOVA analysis revealed that taxonomic and functional composition of soil microbial communities did not vary significantly by microbiome source (Bacteria: F = 1.04, p = 0.37; Fungi: F = 1.10, p = 0.18; KEGGs: F = 0.92, p = 0.55) (Fig. 3). The db-RDA revealed that microbiome source and geographic location cumulatively accounted for less than 10% of variation in bacteria, fungi, and KEGG composition (Bacteria: adj. R^2 = -0.013; Fungi: adj. R^2 = 0.053; KEGGs: adj. R^2 = -0.068). Microbiome source accounted for less than 5% of variation in bacteria, fungi, and KEGG composition (Bacteria: adj. R^2 = 0.011; Fungi: adj. R^2 = 0.025; KEGGs: adj. R^2 = -0.021). Similarly, geographic location also accounted for less than 5% of variation in bacteria, fungi, and KEGG composition (Bacteria: adj. R^2 = 0.026; Fungi: adj. R^2 = 0.033; KEGGs: adj. R^2 = -0.02).

Indicator species analysis identified significant bacteria and fungi indicator taxa for each microbiome source. In total, 77 bacterial ASVs (out of 16,245 detected; 0.5%) and eight fungal ASVs (out of 2,565 detected; 0.3%) were identified as indicator taxa among the three microbiome sources (Fig. 4, Tables S6, S7). Twenty-nine bacterial ASVs were uniquely shared among microbiome sources 1 and 2, whereas microbiome source 3 uniquely shared only six bacterial ASVs with either microbiome source 1 or 2. Fungal guilds were assigned to approximately 68% of the fungal ASVs. Of those assigned to a guild, approximately 76% had a confidence ranking of “probable” or “highly probable.” Out of the five fungal guilds (arbuscular mycorrhizal fungi, ectomycorrhizal fungi, ericoid mycorrhizal fungi, endophytic fungi, and plant pathogenic fungi), ericoid mycorrhizal fungi (χ^2 = 11.29, p = 0.004) and endophytic fungi (χ^2 = 20.14, p < 0.0001) differed significantly among the microbiome sources (Table S8). Both microbiome sources 1 and 2 had approximately 1.5- and 2-fold greater abundance of ericoid mycorrhizal fungi and endophytic fungi, respectively, than microbiome source 3 (Fig. 5d, e).

KEGG composition overall did not differ among the three microbiome sources, indicating functional redundancy among soil microbial communities. Of the 122 pathways identified, less than a quarter accounted for more than 1% of relative abundance of all KEGGs among the three origins (Table S9). Of this subset, 70% were pathways involved in metabolism of either energy (in the form of nitrogen, methane, sulfur, and oxidative phosphorylation), amino acids, carbohydrates, or

lipids. The most abundant pathways across the three soil origins were two pathways for ATP-binding cassette (ABC) transporters, which accounted for 20% of the relative abundance. No KEGG pathways were detected as indicators among the microbiome sources.

Correlations between soil microbiome composition and plant phenotypes

In support of Hypothesis 3, individual microbial taxa were associated with specific *Solidago* phenotypes. We only examined shoot biomass of *S. caesia* as it was the only phenotype that responded to microbiome source treatments. Axes of variation in composition of the bacteria indicator taxa were significantly correlated with *S. caesia* shoot biomass (Axis CAP1: $F = 7.63$, $p = 0.03$). Relative abundance of 77% (20 out of 26) of the bacterial and fungal indicator taxa of microbiome source 3 were significantly negatively correlated with *S. caesia* shoot biomass when *S. caesia* was grown in inoculum of microbiome source 3 (Fig. 6, Table S10). Although *S. caesia* produced more shoot biomass when grown in inoculum of microbiome source 2 compared to that of microbiome source 3 (Fig. 2b), none of the eight bacterial indicator taxa or the one fungal indicator taxon of microbiome source 2 were significantly positively correlated with *S. caesia* shoot biomass.

Discussion

Identifying ways in which the taxonomic and functional composition of the soil microbiome influences plant phenotype is a central challenge for understanding the overall importance of complex soil microbial communities on plant function, as well as how changes to soil microbial communities may in turn affect plant function. While recent studies have explored the importance of both whole soil microbiomes and individual soil microbial taxa on particular plant phenotypes, it is also crucial to understand if and how particular taxa and functions within complex soil microbial communities influence a broad spectrum of plant phenotypes and if these relationships are consistent across multiple plant species. In this study we compared the taxonomic and functional composition of rhizosphere soil microbial communities from three phenotypically distinct and naturally occurring *Solidago* species and sites (referred to as soil microbiome source above) and conducted a microbiome transfer glasshouse experiment to test for plant phenotypic shifts in response to field soil inoculum. We found that soil microbiome taxonomic variation can shift some plant phenotypes and that this response varied by plant species, with some species more responsive to microbial taxonomic variation than others. Specifically, we identified indicator bacteria and fungi associated with each microbiome source, some of which were correlated with shifts in plant growth responses. We found that microbiome source altered growth traits for only one of the three *Solidago* species. Lastly, we found that plant growth traits were more likely to be influenced by variation in soil microbial communities than physiological or reproductive traits. Together, these findings show that soil microbial mediation of plant phenotype 1) varies by plant traits, 2) is not consistent across plant species, and 3) can be influenced, in part, by a small number of microbial taxa.

Soil microbial mediation of plant phenotype varies by plant phenotype and plant species

We found that soil microbial communities can shift some plant phenotypes, but that the strength of microbially-mediated phenotypic plasticity varies by plant phenotype and by plant species. Across all three Solidago species, plants grown in inocula from microbiome source 1 produced more root biomass compared to plants grown in inocula from microbiome source 3. While no *S. flexicaulis* or *S. gigantea* phenotypes responded to microbiome source treatments, *S. caesia* produced more shoot biomass in microbiome source 2 inocula relative to microbiome source 3 inocula. Although we found that the amount of soil plant pathogenic fungi was similar across all three soil origins, we did find that microbiome sources 1 and 2 had significantly greater amounts of endophytic and ericoid mycorrhizal fungi than microbiome source 3.

A difference in plant-beneficial fungi may in part account for the greater growth of *S. caesia* in microbial communities of microbiome source 2 relative to microbial communities of microbiome source 3.

Despite the breadth of research on plant-soil biota relationships, the role of soil microbial communities on plant phenotype is heavily limited by the scarcity of knowledge of these relationships on traits such as physiology and reproduction. Of over 70 recent studies that have examined plant phenotype in response to unmanipulated and sterilized soil microbial treatments, only four studies have examined traits related to physiology (Hahn et al., 2018; Mursinoff & Tack, 2017; Siefert et al., 2018; Xi et al., 2018) and two have examined reproductive traits (Bauer & Flory, 2011; Dudenhöffer et al., 2018). In this study we found that neither SLA nor timing of flower bud formation responded to soil microbiome origin treatments, indicating that not all plant phenotypes are in part mediated by soil microbes. However, further examination of soil microbial mediation across plant phenotypes is needed to reveal if the findings here represent a general trend that microbial mediation is stronger for growth traits than for physiological or reproductive traits.

Soil microbial mediation of plant phenotype can be influenced in part by small number of microbial taxa

Despite similarity in overall microbiome composition among the three soil microbiome sources, we identified specific bacterial and fungal indicator taxa of each microbiome source. In general, *Proteobacteria* taxa were more highly abundant in microbiome sources 1 and 2 than in microbiome source 3, whereas *Acidobacteria* taxa were more highly abundant in microbiome source 3 than in microbiome sources 1 and 2. Within the *Proteobacteria* phylum, indicator taxa of microbiome sources 1 and 2 spanned a larger diversity of taxonomic orders including *Rhizobiales*, *Rhodospirillales*, *Burkholderiales*, and *Xanthomonadales* relative to those of microbiome source 3 which comprised the orders of *Rhizobiales* and *Myxococcales*. These findings highlight the importance of taxonomic resolution when assessing the role of the soil microbiome on plant phenotype as root-associated soil is known to contain some of the highest microbial biodiversity on Earth (Berendsen et al., 2012; Buée et al., 2009; Curtis et al., 2002). In this study, we identified over 16,000 bacterial ASVs and over 2,500 fungal ASVs among the three microbiome sources. These findings suggest that identifying microbial differences among focal groups may require focusing on specific indicator taxa that have high affiliation with the plant species or field site rather than overall microbiome composition.

We found that differences in indicator taxa among separate soil microbial communities may contribute to shifts in plant phenotype even though this relationship is likely not consistent across plant species. We found that *Solidago caesia* produced significantly less vegetative biomass in inocula of microbiome source 3 compared to inocula of microbiome source 2, and that the relative abundance of 77% of the indicator taxa of microbiome source 3 were correlated with decreases in *S. caesia* shoot production. These indicator bacteria included mostly members of the *Acidobacteria* and *Actinobacteria* phyla in addition to members of the *Bacteriodetes*, *Firmicutes*, and *Proteobacteria* phyla. Indicator fungi included members of the *Ascomycota* and *Chytridiomycota* phyla. Despite greater shoot production in microbiome source 2 inocula, none of the microbiome source 2 indicator taxa were correlated with positive shifts of *S. caesia* shoot production.

Although these indicator taxa account for a very small proportion of the total microbial communities identified in this study, it is notable that out of the thousands of ASVs identified in microbiome source 2, twenty bacterial and fungal ASVs explained on average 7% of the variation in root biomass when *S. caesia* was grown in inocula of microbiome source 2. This suggests that individual soil microbial taxa may be involved, in part, with mediating some plant traits. This study demonstrates the utility of this approach of examining correlations between individual microbes and plant traits. Identifying individual microbial taxa that are associated with shifts in plant traits can pinpoint particular microbial taxa to target for further experiments that test causative mechanisms of plant trait variation. While the correlative relationships we identified in this study are context-specific to these particular microbial taxa and plant species, the approach used here can be applied to any plant-microbial system.

While we did not assess microbial dormancy in this study, it likely plays a significant role in soil microbial mediation of plant phenotype. Dormancy, in which individuals undergo a temporary reduced state of metabolic activity, has long been hypothesized to be widespread among microorganisms because it allows them to cope with environmental variability, particularly when conditions are unfavorable (Lau & Lennon, 2011; Stevenson, 1977). Differentiating between active and dormant microbial taxa requires examining the active ribosomal RNA in addition to the total ribosomal DNA. Since we only used rDNA-based techniques in this study, our inferences are limited to microbial taxa that are potentially active. However, despite the fact that the indicator taxa accounted for a very small amount of the diversity of each microbiome source, evidence suggests that rare taxa may confer particular importance within a microbiome. A previous study examining proportions of rRNA to rDNA in temperate lakes found that rare taxa had a higher probability of being metabolically active than common taxa (Jones & Lennon, 2010). Combined with the observation that soil microbiome diversity is primarily comprised of rare taxa (Elshahed et al., 2008), our findings and those from Jones and Lennon (2010) highlight the importance of examining how less abundant rare taxa within soil microbial communities may influence plant phenotype. Other microbial interactions within the soil environment also likely influenced the plant trait variation we observed, such as differences in microbial growth rates, differences in decomposition via extracellular enzymes, and changes to the microbial communities due to conditioning from the plants. However, testing these mechanisms was outside of the scope of this study.

In this study, we found high similarity in soil microbial function (i.e. KEGG pathway composition) among the three microbiome sources, suggesting functional redundancy in which the absence of one or more microbial species does not greatly affect the functioning of the whole microbial community because the same functions are fulfilled by many different taxa (Fernández et al., 1999; Louca et al., 2016, 2018). Functional redundancy is widespread in microbial systems (Allison & Martiny, 2008; Bezemer et al., 2010; Martiny et al., 2013; Nelson et al., 2016; Tringe et al., 2005). The relatively small number of known functions associated with soil microbial communities indicates that much is still unknown about microbial functional genes that may translate to physiological differences among microbial taxa. While the metagenomic methods we employed in this study can be used to infer potential microbial functions, other molecular approaches such as metatranscriptomics can identify particular microbial genes that are being actively transcribed (Carvalhais et al., 2012; Damon et al., 2012; Moran, 2009), and in doing so provide a more accurate representation of the microbial functions that characterize a particular soil microbiome.

Conclusions

Soil microbes represent a largely overlooked but often important biotic factor for influencing plant phenotype. This is the first study, to our knowledge, to examine how taxonomic and functional gene composition of complex soil microbial communities influence a suite of multiple plant phenotypes across multiple plant species. Our study shows that soil microbiomes and specific taxa within complex soil microbial communities can alter some plant phenotypes, but that not all plant species, even those belonging to the same genus, will respond to soil microbial communities in the same manner. Thus, the belowground biotic environment is just one of a host of important biotic factors that can mediate plant phenotype, in addition to plant genetic background and abiotic environmental variation. While the findings from this study are founded in ecology theory, identifying the nuances of relationships between soil microbes and plant phenotype has wide scale applications. Substantial efforts to engineer core rhizosphere microbiomes to optimize plant production signify the need to identify functional linkages between soil microbial communities and plants (Bakker et al., 2012; Busby et al., 2017; Wallenstein, 2017; Qiu et al., 2019). While soil biota may not be a universal solution to enhance some plant phenotypes, specific microbial taxa may be harnessed to improve plant growth and plant tolerance to adverse environmental conditions. This study illustrates that it is possible to identify specific microbial taxa within a complex soil microbial community that are associated with shifts in some plant phenotypes.

Declarations

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Author Contributions

K.K.B. and J.A.S. conceived the ideas and designed methodology. K.K.B. conducted fieldwork, performed experiments, and collected data. S.L.L. provided guidance with molecular work. K.K.B. analyzed the data. K.K.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data Availability

All data and accompanying analysis code are available on GitHub at https://github.com/kbeals2/Trait_Linkages_new

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

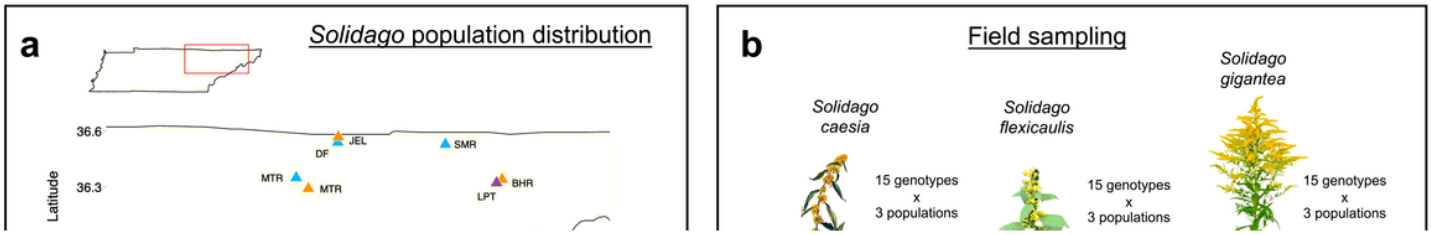

Figure 1

Solidago population locations, field sampling design, and experiment design for soil inoculation experiment. (a) Field sampling was conducted for three distinct natural field populations of *Solidago gigantea*, *S. caesia*, and *S. flexicaulis* in northeastern TN, U.S.A. (b) Growth and physiology phenotypes were measured from 15 putative genotypes at each population for each *Solidago* species. Rhizosphere soil (*S. caesia*-associated, *S. flexicaulis*-associated, and *S. gigantea*-associated) was collected from beneath each genotype and pooled at the site level. (c) Nursery-sourced seeds of *S. caesia*, *S. flexicaulis,* and *S. gigantea* and field soil were used in a 5-month glasshouse inoculation experiment. Seedlings of each species x nursery population were grown in separate treatments of soil containing inoculum from microbiome sources 1, 2, and 3 collected from the corresponding field sites. (N = 3 *Solidago* species x 3 seed populations x 3 microbiome sources x 3 field soil sites x 3 replicates = 243 total pots).

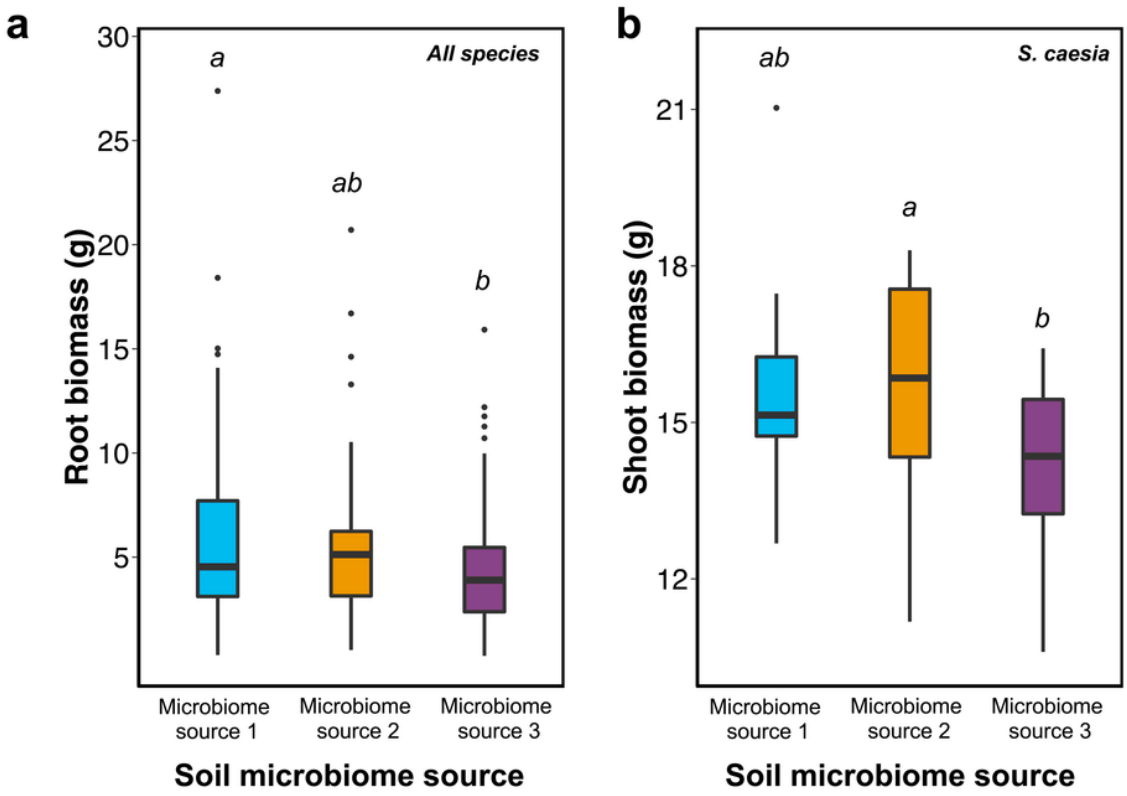

Figure 2

(a) Root biomass response of all *Solidago* species (*S. caesia*, *S. flexicaulis*, *S. gigantea*) and (b) shoot biomass response of *S. caesia* to treatments of microbiome source. Bars that do not share letters are significantly different from one other ($\alpha < 0.05$).

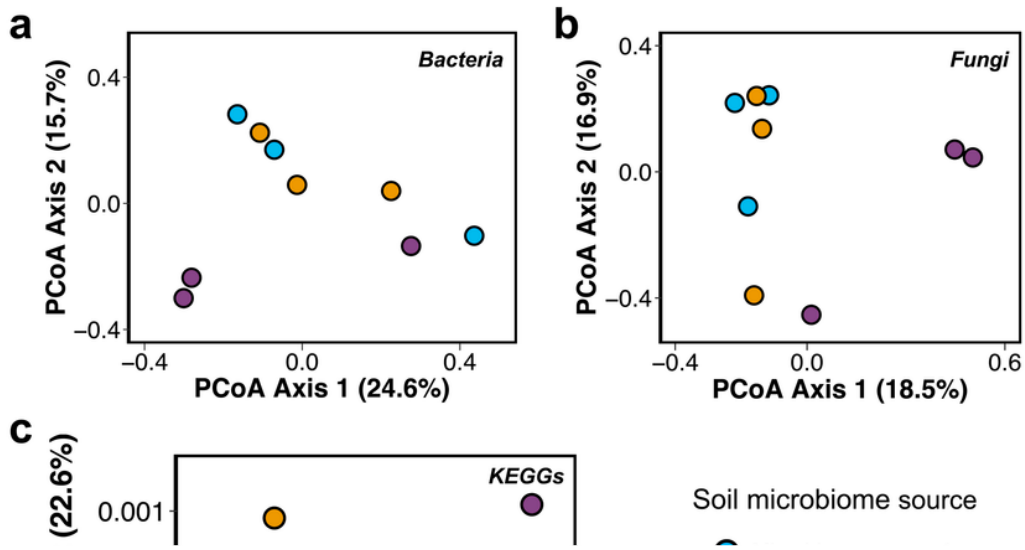

Figure 3

Composition of (a) bacteria, (b) fungi, and (c) KEGG pathways among the three microbiome sources. Each data point represents a field site.

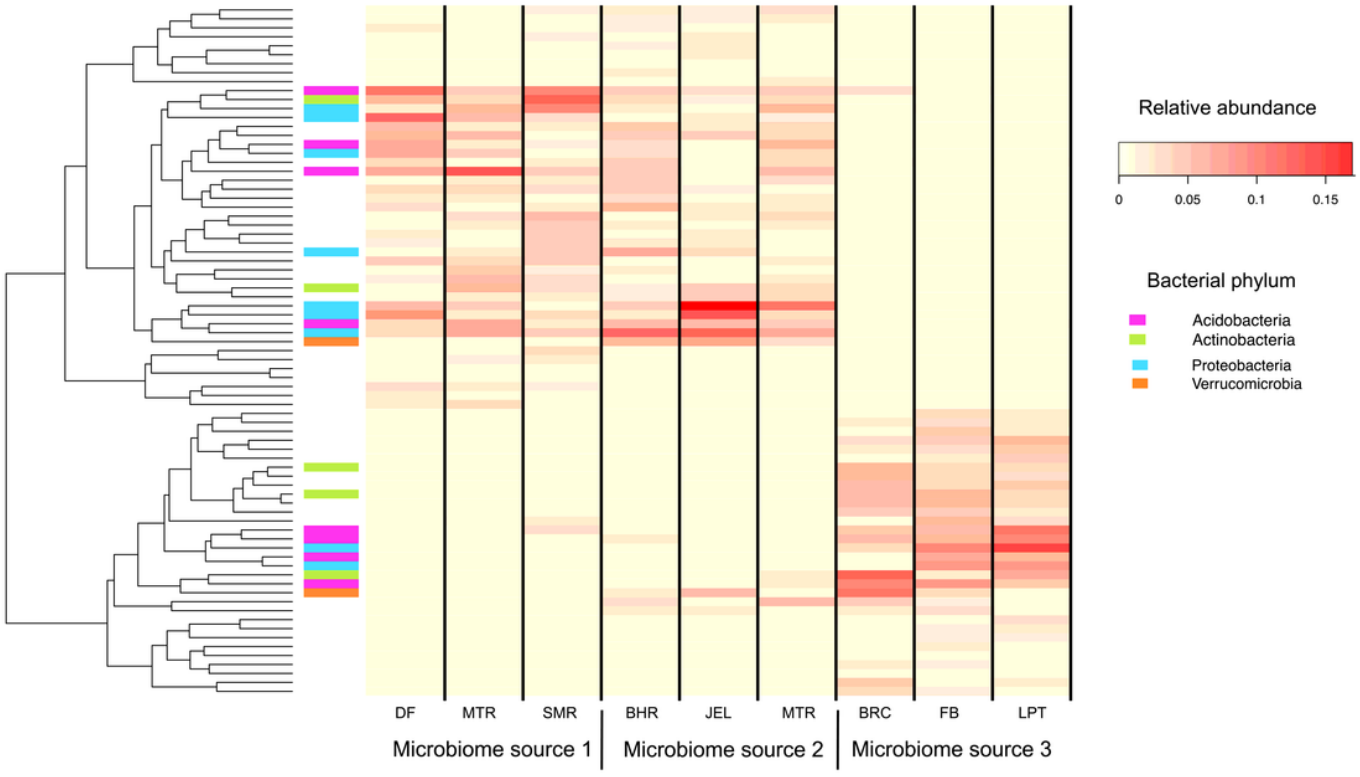

Figure 4

Heatmap of relative abundance of the 77 bacterial ASVs produced from Indicator Species analysis across the three microbiome sources. Rows represent individual ASVs. Columns represent soil from individual field sites. Taxa of each microbiome source with relative abundance 0.05 (5%) or greater are color coded by phylum.

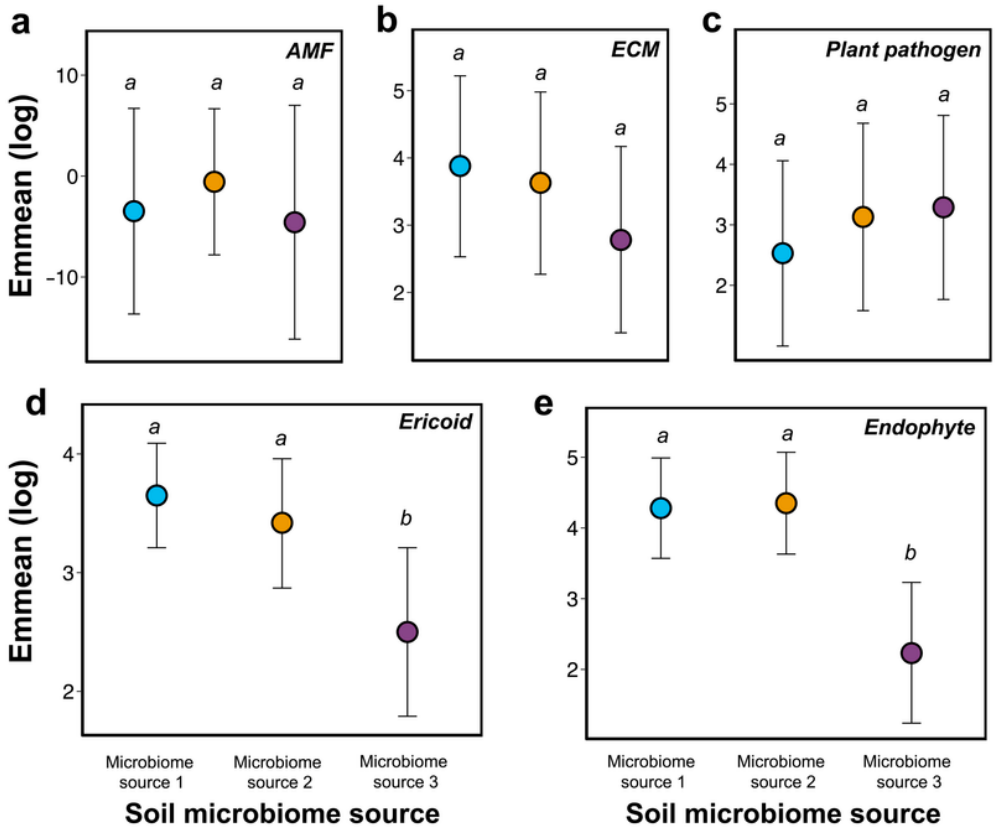

Figure 5

Mean abundance of (a) arbuscular mycorrhizal fungi (AMF), (b) ectomycorrhizal fungi (ECM), (c) plant pathogenic fungi, (d) ericoid mycorrhizal fungi, and (e) endophytic fungi. Emmeans are reported on the log scale. Data shown are pooled across samples (i.e. field sites). Data that do not share letters are significantly different from one other ($\alpha < 0.05$).

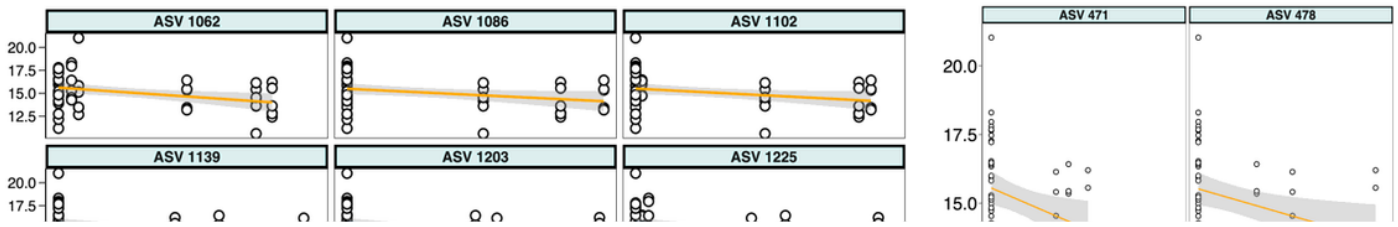

Figure 6

When grown in inoculum from microbiome origin 3, S. *caesia* shoot biomass is negatively correlated with relative abundance of 20 out of the 29 indicator bacterial and fungal taxa of microbiome source 3.

Supplementary Files

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- BealsetalPlantSoilSupplementary.docx