Plant age and genotype explain variation in the microbiome of natural *Lotus corniculatus* populations

Frank Reis[1], Katrina Lutap[2], Walter Durka[34], Jun Hee Jung[1], Eric Kemen[2], Oliver Bossdorf[1,*]

[1]Plant Evolutionary Ecology, Institute of Evolution and Ecology, University of Tübingen, Germany
[2]Microbial Interactions in Plant Ecosystems, Center for Plant Molecular Biology, University of Tübingen, Germany
[3]Department of Community Ecology, Helmholtz Centre for Environmental Research-UFZ, Halle, Germany
[4]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Leipzig, Germany
* Corresponding author: oliver.bossdorf@uni-tuebingen.de

Abstract

In natural populations, plants are associated with a huge diversity of bacteria, fungi and other microbes. There is usually substantial microbiome variation between different plant individuals and populations, and the drivers of this variation are still poorly understood, particularly in wild plants. Here, we were interested in the potential for plant genotype and plant age to explain intraspecific variation in the plant microbiome. In seven natural populations, we genotyped a total of 168 plants over four years, determined their ages through herb chronology, and then sequenced their root, shoot, flower and seed microbiomes. We found that plant genotypes differed both in the diversity and composition of microbes, and that some microbial taxa were associated with particular plant genotypes - a result that we experimentally confirmed for one of the observed genotype-microbe associations. The genotype effects tended to be strongest and most consistent for plant-associated bacteria, with the largest plant genotype differences in the microbiome diversity of flowers and seeds. In contrast to the widespread plant genotype effects, we found less evidence for an effect of plant age on microbiome diversity: the age of plants explained variation in fungi diversity, and it was associated with the abundance of several microbial taxa. Our study indicates that the genotype of a plant and - to a lesser degree its age - influence the diversity and composition of plant-associated microbiota, even in complex natural environments.

Key words

bacteria, fungi, herb chronology, metagenomics, microbial ecology, plant-microbe interactions

Introduction

Plants are colonized by a wide range of bacterial, fungal and eukaryotic microorganisms collectively called the plant microbiome. The diversity and composition of this microbiome is strongly shaped by the plant species (Bonito et al., 2014; Knief et al., 2010) and the surrounding microbial community, especially the soil microbiome (Bulgarelli et al., 2012; Schlaeppi et al., 2014), but there are many other biotic and abiotic factors that are known to influence plant microbiome variation among and within species, including climate (Chen et al., 2019; Trivedi et al., 2022), soil characteristics (Tkacz et al., 2015), land use (Estendorfer et al., 2017; Gaube et al., 2021), herbivory (Malacrinò et al., 2021a, 2021b) or plant disease (Cui et al., 2021; Diskin et al., 2017; Kusstatscher et al., 2019; Solís-García et al., 2021; Wen et al., 2020). Besides this plethora of external factors, the plant microbiome can also be influenced by the plant itself. Variations in gene expression and plant traits can result in changing physical and chemical properties of the plants, and thus habitat conditions for the microbes, which in turn can alter microbiome diversity and composition. Previous studies have shown that the plant microbiome indeed varies across different plant tissue types such as roots, shoots or flowers (Bernard et al., 2021; Junker et al., 2011; Trivedi et al., 2020) and also between the inside (endophytic) and outside (epiphytic) microbiome (Agler et al., 2016; Trivedi et al., 2020). Two other potentially important factors creating microbiome variation among plants of the same species are their genetic differences, and their differences in age or developmental stage.

It is well established that the genetic variation in plants can change the pathogen resistance (Deng et al., 2020; Karasov et al., 2020; Thrall and Burdon, 2000), and there is also good evidence that it can influence the overall composition of the plant microbiome, e.g. in soybean (Liu et al., 2019), potato (Malacrinò et al., 2021a) and black cottonwood (Leopold and Busby, 2020). While the studies mentioned above were all under controlled lab conditions, there is also evidence from more natural common garden experiments, e.g. for plant genotype effects on the microbiome of *Medicago truncatula* (Brown et al., 2020) and *Boechera stricta* (Wagner et al., 2016). So far the research on these questions has been largely restricted to crops and model species, and to - usually short-term - experiments, whereas studies on wild plants and natural populations remain rare. Still, extrapolating lab results about plant-microbe interactions to natural environments is often challenging (Yu et al., 2016) because in natural populations plant microbiomes are influenced by a much larger complexity of biotic and abiotic factors. A true understanding of plant genotype effects, and their relative importance therefore, requires to also study plant genotype-microbiome relationships in natural populations.

Another potentially important but so far very little studied intrinsic factor creating intraspecific variation in plant microbiomes is the age or developmental stage of a plant. As a plant grows and develops, it undergoes physiological and biochemical changes that influence important functional traits, such as defense against herbivory (Boege et al., 2007; Yang et al., 2020) or immune responses to pathogens (Develey-Rivière and Galiana, 2007). These changes across different life stages will inevitably also influence plant-microbiome interactions (Bressan et al., 2009). For example, the root-associated microbiome of rice varies between the vegetative phase and later life stages (Edwards et al., 2018), and very similar results were found for soybean (Sugiyama et al., 2014) and sugarcane (Hamonts et al., 2018). For annual plants like rice or soybean such a comparison between vegetative and reproductive phase covers their entire life cycle. However, many plants, in particular wild ones, are perennial, which creates additional possibilities for age-related microbiome variation e.g. through slower successional or competitive replacement processes. So far, there are hardly any studies of perennial plants examining relationships between plant age and plant microbiome. An exception is the study by Wagner et al. (2016) who found substantial changes in root (and to a lesser extent leaf) bacterial communities when comparing two- and four-year-old *Boechera stricta* plants in the common garden, suggesting that significant microbiome changes can also occur at later life stages of perennials. Considering that most plants are perennial, we clearly need more studies relating plant age to plant microbiome in longer-lived plants.

Understanding the influences of plant age and genotype on microbiome composition may be important not only from a fundamental scientific perspective but also for agriculture and plant conservation, for instance if it helps to better manage beneficial crop microbiomes that increase yield production, stress tolerance or pathogen resistance. The last point is particularly important given that pathogens are a huge threat to global food security (Strange and Scott, 2005). More generally, understanding the drivers and consequences of plant microbiome diversity may support the management of plant populations in rapidly changing environments, and the effects of plant age and genotype on plant microbiomes - particularly under realistic conditions - are important elements of this.

Here, we combined amplicon-based metagenomics with herb chronology and plant genotyping to study relationships between plant age and genotype, and the diversity and composition of plant microbiomes within and among natural plant populations. Our study organism was *Lotus corniculatus*, a widely distributed perennial legume that grows naturally in a broad range of environments in temperate Eurasia and is an important food source for many pollinators, such as bumblebees, honeybees, and many wild bees as well as butterflies, flies and beetles (Hennig and

Ghazoul, 2011; van der Kooi et al., 2016; Weiner et al., 2014). As a nitrogen fixer it is part of a complex plant-microbe symbiosis influencing the nutrient dynamics of grassland ecosystems (van der Heijden et al., 2016), and it therefore plays an important role in agriculture. As *L. corniculatus* is known to have a broad life span range at least up to 15 years (Roeder et al., 2017; Anna Roeder & Christiane Roscher, unpublished data), and it is known to harbor significant intraspecific genetic diversity (Abraham et al., 2015; Durka et al., 2025), the species is a suitable system for asking questions about effects of plant age and genotype. Specifically, we wanted to answer the following questions:

1. Do plant age and genotype affect the diversity of the plant microbiome, and if yes how do these effects vary between different plant tissues and microbial groups?
2. Are plant age and genotype related to the composition (beta diversity) of the plant microbiome, and how are these relationships influenced by the plant tissue and microbial groups?
3. Which specific microbial taxa are most differentiated between plants of different ages and genotypes, and can we confirm some of these findings experimentally?

To answer these questions, we collected *L. corniculatus* plants from seven semi-natural grasslands in the Swabian Jura region in Germany over four consecutive years, and we then determined the ages and genotype of all plants, and analyzed their bacterial, fungal and eukaryotic microbes separately for roots, shoots, flowers and seeds.

Methods

Plant material

We collected *Lotus corniculatus* plants from seven extensively managed meadows in the Swabian Alb region of southern Germany (Fig. 1a, Tab. S1) for four consecutive years from 2018 to 2021. All collections were done in the late summer (August/September), when the plants were still flowering but already carried some ripe fruits, so that both flowers and seeds could be sampled. In each of the seven populations, we carefully excavated six entire plants, resulting in a total of 4 years x 7 populations x 6 plants = 168 plants. In the lab, we divided the plants into roots, shoots, flowers and seeds, and surface sterilized all plant parts. Therefore, we washed them by shaking in Falcon tubes (50 ml for roots and shoots; 25 ml for flowers) and 1.5 ml Eppendorf tubes for seeds for 30 s with autoclaved ultrapure water (UPW), for 60 s with epiphyte wash solution (0.1 % Triton X-100 in 1x TE

buffer), for 30 s with 80% EtOH and for 30 s with 2 % NaOCl. After that, we rinsed the samples three times (seeds: five times) for 10 s with UPW and froze them.

Determination of plant ages

To determine the ages of the sampled plants, we cut 3 cm of the root crown just below the hypocotyl (Fig. 1b), the oldest part of the plant root, from all plants and preserved them in 70% ethanol. In the lab, we placed these root samples into a microtome (Reichert, Vienna, Austria) and sliced them into transverse sections. Very small roots with a diameter smaller than 3 mm were clamped using a piece of wood. To harden the specimens and prevent them from drying out and cracking, all specimens and the blade were kept moist with 99% ethanol during the cutting process. The root crown slices were then fixed on a microscope slide in a 1:1 mixture of water and glycerol and covered with a cover glass. We used a microscope to count the number of annual rings, with ring boundaries defined by marginal parenchyma, semi-ring porosity or both (Schweingruber and Poschlod, 2005) (Fig. 1b). Where necessary we used polarized light to improve the visibility of the annual rings. Since the annual rings were not equally distinct in all specimens, we classified the distinctness of the rings into 1 = very good, 2 = intermediate, 3 = poor and 4 = no annual rings visible (Tab. S2) and used only classes 1 to 3 for our analyses. For the statistical analysis we decided to group the plants into 5 age groups since the low number of replicates for the very young (2–3 years) and very old plants (6–13 years). Therefore, we grouped the plants into age categories: younger than 4, 4, 5, 6, and older than 6 years (Fig. 1d).

Plant genotyping

To genotype the sampled plants, and assess their relatedness, we employed ddRAD Peterson et al. (2012), a reduced-representation sequencing approach. We extracted plant DNA from all shoot samples using the QIAGEN DNeasy Plant Mini Kit (QIAGEN, 2019). We then double-digested the DNA and followed the library preparation protocol of Peterson et al. (2012) with small modifications as given in Durka et al. (2025). Multiplexed samples were sequenced paired-end (PE 150) on an Illumina Novaseq 6000 platform. After sequencing, we demultiplexed the reads using process_radtags from the Stacks 2.0 pipeline (Rochette et al., 2019) resulting in an average of 6.31 million reads retained per sample. We assembled reads, including three outgroup samples of *L. tenuis* and *L. pedunculatus* from Durka et al. (2025) and called SNPs using dDocent 2.7.8 (O'Leary et al., 2018; Puritz et al., 2014) with default parameters, except of using 0.90 as ClusteringSimilarity, filtered according to O'Leary et al. 2018 introducing a mean depth threshold of 5, and a minor allele frequency threshold of 0.05 and keeping only 1 SNP per contig. The resulting VCF file was converted

into a fasta file (containing only the SNPs for each sample) with PGDSpider version 2.1.1.5 (Lischer and Excoffier, 2012). To construct a Maximum Likelihood tree, we used the program IQ-TREE release 2.0.3 (Minh et al., 2020). We used ModelFinder to select the best-fitting substitution model based on the Bayesian Information Criterion (BIC) (Kalyaanamoorthy et al., 2017) which identified the General Time Reversible (GTR) model with empirical base frequencies (F), a proportion of invariable sites (I), and gamma-distributed rate heterogeneity with four categories (G4) as the best-fit model (GTR+F+I+G4). To assess branch support, we also performed ultrafast bootstrap approximation (UFBoot) with 1,000 replicates (Hoang et al., 2018). Finally, we used TreeCluster version 1.0.4 (Balaban et al., 2019) to classify the SNP-based genotypes into a more restricted number of genetic groups (later used in our analyses), based on the total branch length of all leaves in the cluster (Sum Branch) with a threshold of 3.1. We visualized the phylogenetic tree using Interactive Tree of Life (iTOL) (Letunic and Bork, 2024) and colored the samples by their genetic group assignments. From here on we will refer to these nine genetic groups as genotypes.

Microbiome sequencing

To prepare the plant samples for microbiome sequencing, we first homogenized all frozen samples of soil, roots, shoots, flowers, and seeds with a Precellys 24 Tissue Homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France), and then extracted the DNA using the FastDNA Spin Kit for Soil (MP Biomedicals, Irvine, CA, USA). We then performed a two-step PCR targeting the bacterial 16S rRNA V5-V7 region, the fungal ITS2 region, and the eukaryotic 18S rRNA V9 region, using the primers 799F/1192R, fITS7/ITS4, and F1422/R1797, respectively (Tab. S3) (Agler et al., 2016). As controls we used blank samples (UPW and blank DNA extraction). To reduce the amplification of mitochondrial and chloroplast rRNA sequences from *L. corniculatus*, we incorporated blocking oligos designed with the R package *AmpStop* (Tab. S3) (Mayer et al., 2021) (Lutap et al., 2025 / Chapter III). We pooled the amplified products in equal concentrations, purified them with magnetic bead clean-up, and randomly assigned each to one of eight sequencing batches. We sequenced all pools on a Illumina MiSeq platform with PhiX control, using the MiSeq Reagent Kit v3 (600-cycle), and then processed all microbial 16S rRNA, ITS2, and 18S rRNA amplicon sequences using Mothur (Almario et al., 2022; Schloss et al., 2009; Lutap et al., 2025 / Chapter III). To remove the primer sequences from 16S rRNA and 18S rRNA data we used Cutadapt, and to remove it from ITS2 data we used ITSx (Bengtsson-Palme et al., 2013; Martin, 2011). For the taxonomic classification of the bacterial 16S rRNA reads, we used the Greengenes database (13_8_99 release), for fungal ITS2 reads the UNITE database (02.02.2019 release), and for eukaryotic 18S rRNA the PR2 database

(version 4.12.0), all of which included the PhiX genome (DeSantis et al., 2006; Guillou et al., 2013; Nilsson et al., 2019).

Data analysis

We carried out all data analyses in R Studio 2024.12.1 (RStudio Team, 2024), and we used *dplyr* for structuring and manipulating data frames (Wickham et al., 2023) *ggplot2* package (Wickham, 2016) for creating figures. We used the *vegan* package (Oksanen et al., 2025) for calculating the alpha diversity (Shannon index) of bacterial, fungal or eukaryotic microbiomes of each tissue in each plant, and then linear models to test for the effects of plant age or genotype. To account for variation in other factors, all models included the plant organ, and its respective interaction with plant genotype or plant age as well as sample location and year.

In addition to variation in microbial alpha diversity, we also analyzed the compositional turnover (beta diversity) between different samples, using the R packages *vegan*, *phyloseq*, and *microbiome* (Liu et al., 2021; McMurdie and Holmes, 2013; Oksanen et al., 2025). We first used the OTU relative abundances for a Principal Coordinate Analysis (PCoA) of the Bray-Curtis dissimilarities between samples, and then a PERMANOVA to test how much of the variation in microbial community composition could be explained by plant age, plant genotype, or their interaction. We also visualized the relative abundance profiles of the ten most abundant classes of bacteria, fungi or eukaryotes for the different *L. corniculatus* genotypes or age groups, using the *microeco* package (Liu et al., 2021). To further understand the abundance patterns of the three different main groups of microbes, we also created graphs of the frequencies of different levels of commonness of OTUs, occurring in one to nine genotypes, or in one to five age groups.

To identify specific bacterial, fungal or eukaryotic taxa that were associated with particular plant genotypes or plant age groups, we used the R packages *microbiomeMarker* and *phyloseq* (Cao et al., 2022; McMurdie and Holmes, 2013). We used linear discriminant analysis effect size (LEfSe) (p-value < 0.05, LDA score ≥ 2) (Segata et al., 2011) to identify microbial genera that were significantly associated with particular *L. corniculatus* genotypes or age groups, either separately for each plant organ, or across organs.

Genotype-specificity experiment

While LEfSe analyses can suggest a role of host genotype for specific microbial taxa, formal proof of genotype specificity is only possible with infection experiments. Although a comprehensive test of our LEfSe results was beyond our means, we exemplarily tested one LEfSe result for the fungal

pathogen *Gibberella*/*Fusarium* that showed a strong genotype association. We isolated *Fusarium* strains from *L. corniculatus* shoots collected around Tübingen using standard surface sterilization and plating on *Fusarium*-selective medium (Jung et al., 2013). The detailed protocol can be found in the Supplementary Methods (S1). We used the isolated *Fusarium* strain to experimentally inoculate daughter plants of our genotype 1 - associated with high *Fusarium* abundances in LEfSe - versus plants of the (presumably) less susceptible genotype 8. To break seed dormancy, we submerged the seeds of the two genotypes in liquid nitrogen for five minutes and then germinated them on wet tissue paper at 21°C/18°C with a 16 h/8 h light/dark cycle in a growth chamber. After seven days we transplanted the germinated seeds into individual pots filled with standard potting soil. For each genotype, we used six plants for the infection treatment and six plants as controls. For spore production, we transferred five 1 cm^2 pieces of Potato Dextrose Agar media with the isolated *Fusarium* in 250 ml of liquid mung bean media (García-Bastidas et al., 2019) and incubated them for four days in the dark at 28°C at 150 RPM. For the plant infection, we filtered the suspension through autoclaved cheesecloth, counted the spores using a counting chamber and standardized the suspension to 10^6 spores/ml. To infect the plants, we dipped the entire shoots of the infection treatment plants in the spore suspension, whereas the shoots of the control plants were dipped in UPW. Two weeks after the infection we harvested all plants and quantified infection intensities through image analysis. For each plant, we disassembled all shoots and leaves and scanned them with a photo scanner (Epson Perfection V600) at high resolution (~ 35 MP). We used ImageJ (Schneider et al., 2012) to mask the plant stems and restrict analyses to the leaf area, and used the OpenCV2 package (Bradski, 2000) in Python v3.17 (Van Rossum, G., & Drake Jr, F. L., 1995) to filter all green (HSV thresholds of 5,70,70 and 75,250,215) and yellow pixels (HSV thresholds of 0,70,71 and 30,234,234) in these images. We used the number of yellow and green pixels as a proxy for the plant health, since leaf wilt and chlorosis are the most common symptoms of a *Fusarium* infection (Ekwomadu and Mwanza, 2023). We tested the genotype specificity of the *Fusarium* infection with a linear model that used the number of yellow pixels as a response variable and included infection treatment, plant genotype and their interaction as categorical explanatory variables, and the number of green pixels as a covariate. To improve the model fit, we log-transformed the yellow to green pixel ratio prior to the analysis.

Results

The ddRAD sequencing identified a total of 5739 SNPs across all plant individuals. The Maximum Likelihood tree with best score (LL score = -733814, not shown) and consensus tree (LL score = -733810; Fig. S1) agree in all major branches. Both trees are robust, with most bootstrap values

above 50%. Based on the branch lengths of the ML tree, the TreeCluster algorithm grouped the plants into nine genotype groups, each represented by 14 to 26 samples (Fig. 1b, Fig. S1). While six of these groups were significantly overrepresented in some sites (Fig. 1c, χ2 test, $P < 0.001$), all groups occurred across multiple sites (3-6 sites, average 4.4 sites), usually with multiple replicates per site, and the use of the genotype groups therefore allowed us to test for their microbiome associations at least partly independently from site variation. The estimated ages of the same plant individuals ranged from two to 13 years, with most plants four to six years old (Fig. 1d). We found no between plant age and sampling year (χ2 test, $P = 0.076$), but there was a statistical association between plant age and sampling site (χ2 test, $P < 0.001$), with an overrepresentation of the younger than four-year-old plants in one of the sites. Thus, plant age was only to a small extent confounded with site, allowing us to test for its (largely) independent association with microbiome.

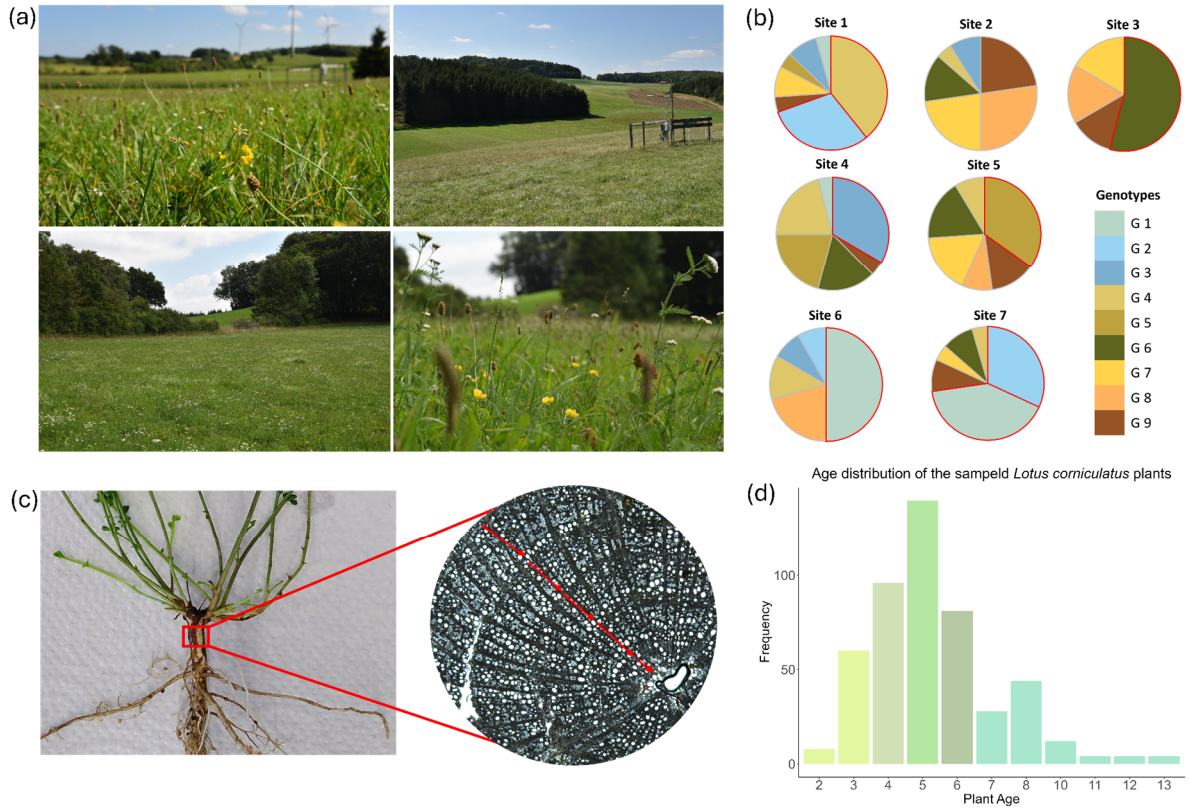

Figure 1. Sampling region, genotypic diversity, and determination of plant age. **a)** Photos of four of the sampling sites in southwest Germany. **b)** Frequencies of *Lotus* genotypes in the seven studied populations. The red borders indicate cases where genotypes are significantly overrepresented in specific populations. **c)** The root crown of a *Lotus* plant, and its cross-section, with the growth rings used to assess plant age. **d)** Age distribution of all sampled plants based on herb chronology. The colors indicate the five age groups we created for the statistical analysis.

Microbiome diversity differs between plant genotypes and ages

Across all sampling sites, years, plant individuals and plant organs (672 samples), the metagenome sequencing identified a total of 4,225 bacterial (16S rRNA), 2,027 fungal (ITS2 rRNA), and 1,773 eukaryotic (18S rRNA) OTUs, clustered at 97% sequence similarity and classified into 113 phyla and 1,542 genera. The most abundant bacterial classes we detected in the *Lotus* plants were Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Actinobacteria, and Bacilli (Fig. 3a). The most abundant fungal classes are Dothideomycetes, Leotiomycetes, Eurotiomycetes, Sordariomycetes, and the most abundant eukaryotic classes Insecta, Dothideomycetes, Chromadorea_X, and Agaricomycetes (Fig. 3b,c).

Part of the observed microbial diversity differed systematically between plant organs, genotypes and age groups. Specifically, the variation in Shannon diversity (= within-sample) was highly significant between the different plant organs of all genotypes and age groups. In all cases, root diversity was substantially higher than that of other organs, followed by shoot and flower diversity. Notably, seed diversity was higher than that of flowers (Fig. 2a-f, Fig. S2). Both bacterial and fungal communities differed significantly among plant genotypes, with some genotypes harboring consistently higher bacterial or fungal diversity than others, across plant organs (Fig. 2a,b). We also found a significant interaction between genotype and plant organ for bacterial diversity: genotype differences were much more pronounced in *Lotus* flowers and seeds than in shoots and roots (Fig. 2a). A similar, albeit not statistically significant, pattern was visible for fungal diversity, where also the genotype differences were greatest in flowers (Fig. 2b). In contrast to the flower and seed microbiome, the microbial diversity of roots and shoots was much less variable between plant genotypes, and there were also no genotype differences in the diversity of eukaryotes (Fig. 2c). When comparing the microbiome diversity of different plant age groups, we found a significant age effect for fungal diversity, with younger plants harboring a higher diversity of fungal microbes than older ones (Fig. 2e). However, there were no plant age effects on bacterial or eukaryote diversity (Fig. 2d,f).

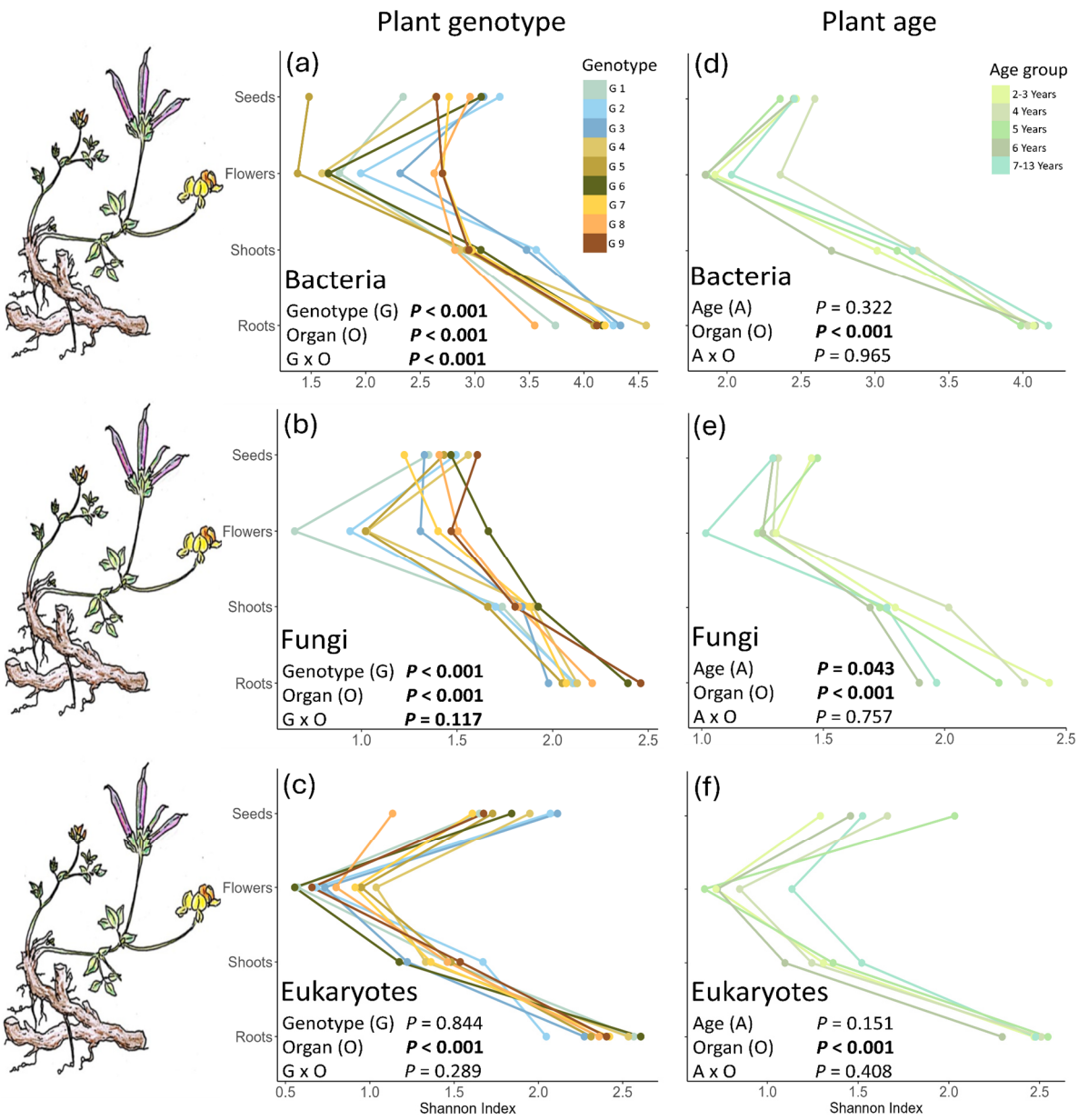

Figure 2: The average alpha diversity of bacterial, fungal and eukaryotic microbial communities associated with different genotypes (panels a - c) or different age groups (panels d - f) of *Lotus corniculatus* across seven semi-natural grasslands. Illustrations by S. Nicolai Rühl.

Plant genotype and age explain variation in microbiome composition

The age and genotypes of plants were not only associated with the diversity but also the composition of their microbiome. A large number of taxa associated with *L. corniculatus* were shared among all genotypes and age groups, but we also found taxa that were less widely distributed or even occurred in only one genotype or age group. Interestingly, the patterns of microbial commonness and rarity strongly differed between bacteria on the one side, and fungi and

eukaryotes on the other side. In bacteria the majority of OTUs (2572 out of 4225) occurred in all nine genotypes, while OTUs that occurred in only one or few genotypes were rare. In fungi and eukaryotes, such less widely distributed taxa were much more frequent (Fig. 3g). The results were similar when we examined OTU distribution across age groups: almost 80% of all bacterial OTUs occurred in all age groups, and such that occurred in only one or few age groups were rare, whereas the patterns were much more even in fungi and eukaryotes (Fig. 3h).

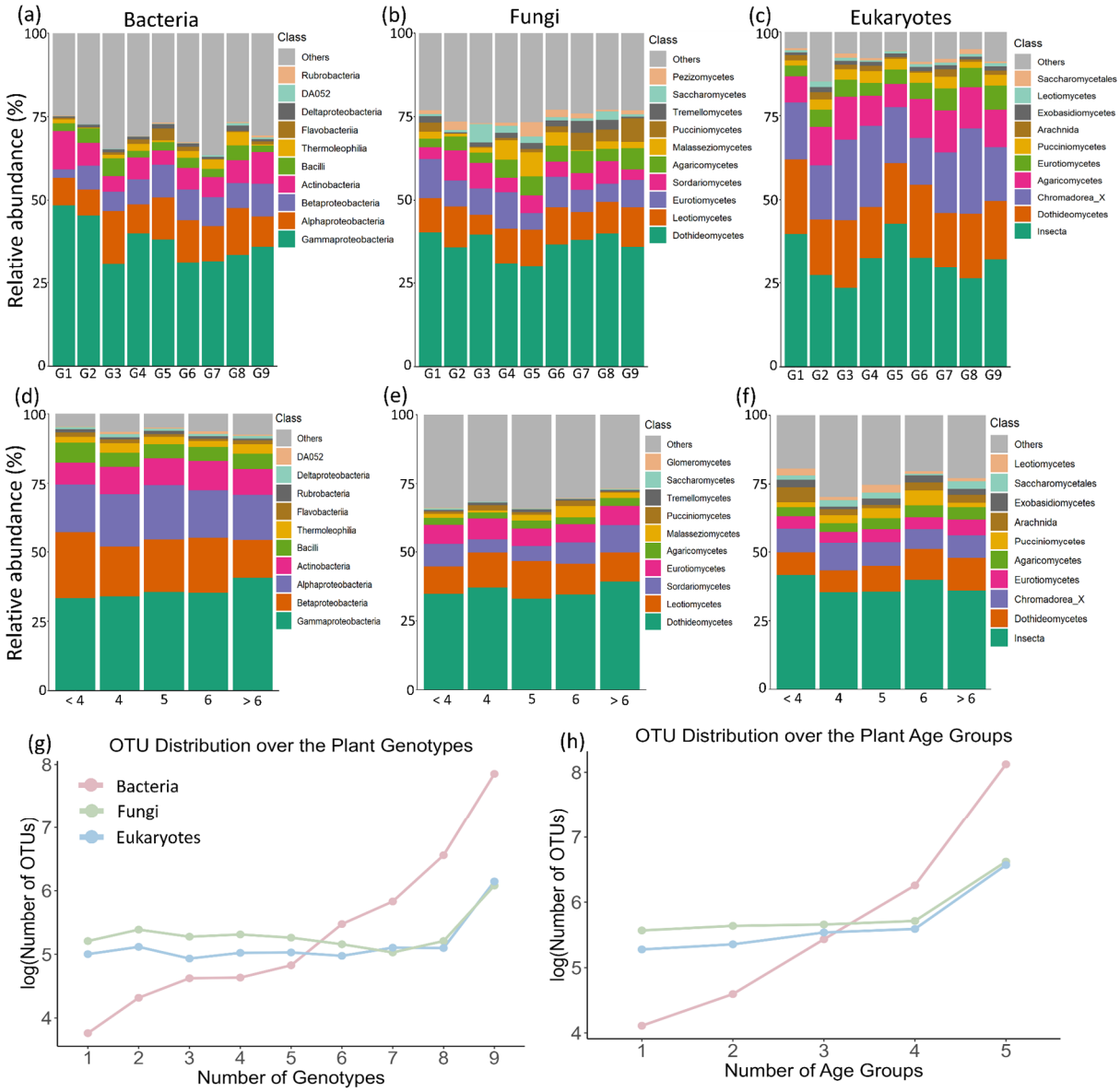

Figure 3: Relative abundance profiles of microbial communities associated with different genotypes (G1 - G9; panels a-c) or ages (panels d-f) of *Lotus corniculatus*, separately for bacteria, fungi and eukaryotes. We restricted the plots to the ten most abundant classes in each microbial group. Panels (g) and (h) summarize the frequencies of different levels of commonness across genotypes or ages in the three microbial groups.

The most consistent genotype effects were in roots and shoots, where the PERMANOVA identified significant plant genotype effects in the composition of all three microbial groups, with around 10% of the variation in community composition explained by plant genotype. In addition, there were significant genotype effects for bacterial communities in flowers and seeds, and for fungal communities in flowers (Tab. 1, Fig. S3). Interestingly, these genotype effects in flowers and seeds were even stronger, with over 15% variance explained.

We found no significant main effects of plant age on the composition of bacteria, fungal and eukaryotic communities in any of the four plant organs (Tab. 1). However, there were significant plant age by genotype interactions for fungal communities in roots and shoots and eukaryotic communities in roots, indicating a complex interplay between plant genotype and age in explaining microbial community composition in these cases.

Table 1: Results of PERMANOVA analyses testing the effects of plant genotype, plant age, or their interaction, on the Bray-Curtis dissimilarities of microbial communities associated with the roots, shoots, flowers, and seeds of *Lotus corniculatus* across seven semi-natural grasslands in SW Germany.

	Bacteria											
	Roots			Shoots			Flowers			Seeds		
	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*
Plant genotype	0.102	1.53	**0.009**	0.103	1.57	**0.004**	0.174	2.67	**0.001**	0.160	2.59	**0.001**
Plant age	0.033	0.99	0.455	0.043	1.3	0.107	0.026	0.81	0.732	0.049	1.55	0.064
Genotpye x Age	0.234	1.04	0.309	0.231	1.04	0.277	0.180	0.82	0.956	0.204	0.98	0.549
Residual	0.632			0.623			0.620			0.588		

	Fungi											
	Roots			Shoots			Flowers			Seeds		
	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*
Plant genotype	0.092	1.41	**0.005**	0.108	1.61	**0.001**	0.173	3.02	**0.001**	0.070	1.04	0.385
Plant age	0.029	0.90	0.71	0.034	1.06	0.351	0.042	1.48	0.103	0.025	0.74	0.844
Genotpye x Age	0.264	1.21	**0.012**	0.248	1.15	**0.036**	0.239	1.23	0.079	0.258	1.12	0.385
Residual	0.615			0.615			0.545			0.646		

	Eukaryotes											
	Roots			Shoots			Flowers			Seeds		
	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*	R^2	*F*	*Pr(>F)*
Plant genotype	0.107	1.66	**0.001**	0.090	1.38	**0.008**	0.082	1.21	0.139	0.073	1.13	0.235
Plant age	0.035	1.07	0.264	0.037	1.13	0.257	0.029	0.85	0.641	0.046	1.41	0.076
Genotpye x Age	0.246	1.13	**0.021**	0.245	1.11	0.067	0.244	1.07	0.308	0.264	1.20	**0.043**
Residual	0.612			0.628			0.645			0.617		

Specific microbial taxa associated with plant genotypes and age groups

While a large number of taxa are shared among the plant genotypes and age groups, the LEfSe analyses revealed that some microbe taxa were significantly associated with particular individual plant genotypes or age groups (Fig. 4). Interestingly, these patterns of differential abundance were generally stronger in bacteria and fungi than in eukaryotic microbes, and they also tended to be more frequent with regard to plant genotypes (Fig. 4a) than for plant age groups (Fig. 4b),

corroborating the PERMANOVA results with more frequent plant genotype than plant age effects (Tab. 1). Moreover, the LEfSe analyses also found that some plant genotypes and age groups showed much larger numbers of specific microbial taxa associations (e.g. genotypes 2, 3, 7 and 8, and age groups 2/3 and 5) than others. When analyzed separately for each plant organ, the LEfSE results differed significantly between organs, both for plant genotypes and plant age groups (Tab. S4), indicating that the observed microbial taxa associations are to some degree organ-specific.

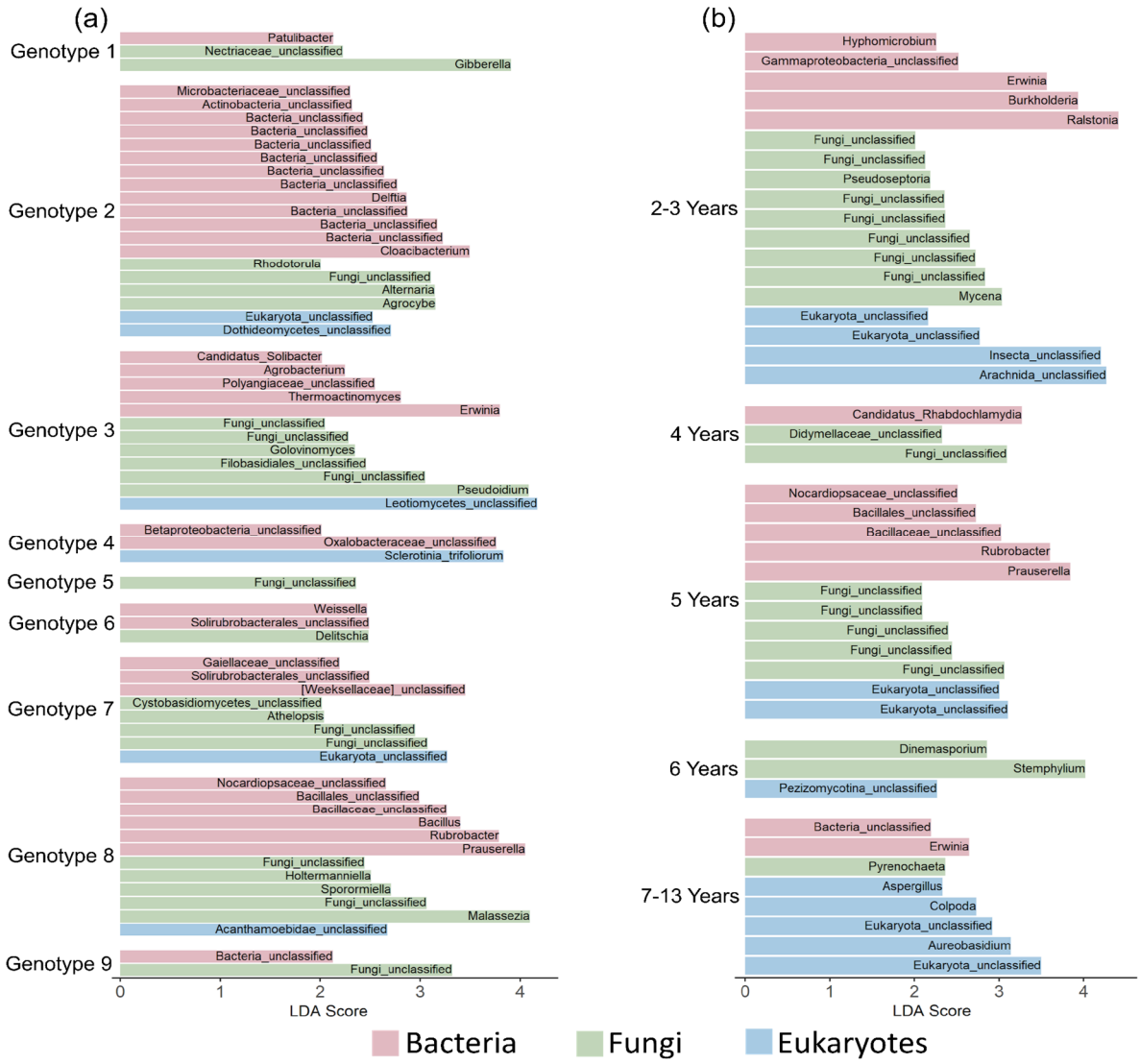

Figure 4: Microbial taxa significantly associated with specific genotypes (a) or age groups (b) of *Lotus corniculatus*, as identified through LefSe analyses. The three colors represent separate LefSe analyses conducted for bacteria, fungi, and eukaryotes, which were analyzed independently. These groups are presented together to provide a clearer overview of microbial variation across genotypes and age groups; however, the LDA score of taxa from different groups cannot be directly compared with each other. The raw results can be found in the supplementary material (Fig. S4).

Our experimental test of one of the observed LEfSe results, the overabundance of *Gibberella*/*Fusarium* associated with *Lotus* genotype 1 confirmed the results from the field. In both tested genotypes the chlorotic area (Fig. 5b) increased after infection with *Fusarium* (Fig. 5a) compared to the control treatment. However, the strength of this effect strongly varied between the genotypes. In the more susceptible genotype 1 the chlorotic area increased on average over 10-fold, whereas in genotype 8 the average chlorosis only doubled in infected plants compared to control plants (Fig. 5c). These differences in effect sizes were also confirmed by a significant genotype by treatment effect in the linear model (see Fig. 5 for details).

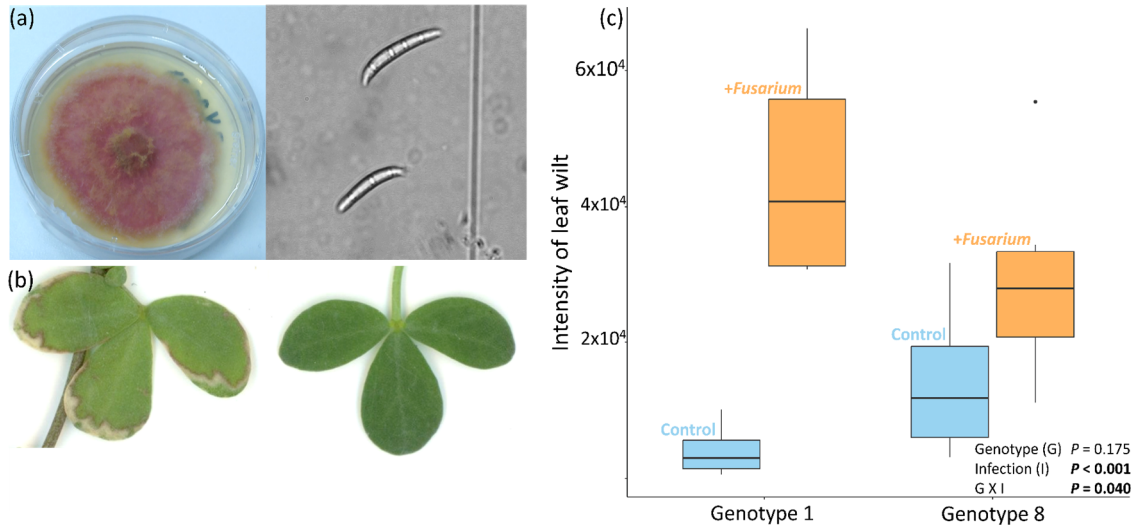

Figure 5: Experimental test of genotype-specificity of *Fusarium* infection of *Lotus corniculatus*. **a)** Isolated *Fusarium* in plate cultivation and microscopic image of *Fusarium* spores. **b)** Typical diseased and healthy leaves of *Lotus corniculatus*. **c)** Average intensities of leaf wilt of two *Lotus* genotypes, with or without experimental *Fusarium* infection.

Discussion

Plants harbor an astonishing diversity of microbes, but the factors shaping these complex communities throughout a plant's life, particularly in natural populations, remain poorly understood. Here, we show that in natural *Lotus corniculatus* populations both the age and genotype of plants is associated with changes in the diversity and composition of their associated microbiome. This is the case for the entire microbiome but also for individual microbial taxa that are significantly associated with specific genotypes or age groups.

Effect of plant age and genotype on microbiome diversity

We generally found large differences in the average microbiome diversity of different plant organs, a result that has already been described in detail elsewhere (Lutap et al., 2025 / Chapter III), and that

likely reflects the different properties of plant organs as habitats for microbes, and their different modes of connectedness with the environment (Huang et al., 2024). Because of this large variation, we included the plant organs as factors in our analysis or ran analyses separately for different organs.

The alpha diversity of bacterial and fungal microorganisms differed significantly among the studied *Lotus corniculatus* genotypes. Interestingly, this effect was particularly strong in the flowers where we observed the strongest among-genotype variation in bacteria and fungi, whereas the microbiome diversity of roots and shoots was much more stable across genotypes. A possible explanation for this could be that flower traits are generally thought to be under stronger genetic control than vegetative plant traits (Campbell et al., 2022), and that these traits can influence the microbiome, particularly if they affect the abundance and diversity of flower visitors (Genung et al., 2010), which are known to influence the flower microbiome (Keller et al., 2021; Zemenick et al., 2021). For example, nectar secretion can vary among *L. corniculatus* genotypes (Murrell et al., 1982), potentially influencing the rate of flower visitation. In the study area, the flower visitors of *L. corniculatus* include various bee species, bumblebees, honeybees, and wild bees, but also butterflies (primarily Lycaenidae) and sometimes flies and beetles (Weiner et al., 2014). This broad range of pollinators may play a role in the observed flower microbiome variation between different plant genotypes. Moreover, the flower microbiome is closely linked to the seed microbiome, particularly for bacteria (Compant et al., 2011; Dutta et al., 2015; Lessl et al., 2007; Mitter et al., 2017), which may explain why we also found considerably among-genotype variation for bacteria in seeds.

Among-genotype variation in microbiome was generally lower in plant roots and shoots than in flowers and seeds. It is well known that the root microbiome is strongly influenced, in part through affecting root traits, by different environmental factors such as soil type, structure and pH, and by the surrounding soil microbiome (Bonito et al., 2014; Bulgarelli et al., 2012; Ristova and Busch, 2014; Schlaeppi et al., 2014; Schreiter et al., 2014). Together, this may 'override' the influence of host genetics and reduce its relative impact on root microbiomes and, as a consequence, also the neighboring shoot microbiomes.

Compared to the variation among genotypes, there was less variation in microbiome diversity between plants of different ages. Only for the Shannon diversity of fungi there were significant differences between age groups. This is interesting, as many fungi play important roles as plant symbionts or pathogens (Fisher et al., 2020, 2012; Martin and van der Heijden, 2024), and their interactions with plants are strongly influenced by the plant immune system. The immune response of plants changes throughout their development (Develey-Rivière and Galiana, 2007; Hu and Yang,

2019), and this could explain plant age-related variation in fungal microbiome diversity. Our results show that in principle plant age can also influence plant microbiome diversity, although to a lesser degree than plant genotype, and our evidence is limited to fungal microorganisms.

We should point out that comparisons of plant ages and genotypes in natural populations are not without challenges. In a field study, variation in plant age and genotype is inevitably confounded with variation in environment. We tried to account for this by aggregating both genotypes and ages into broader groups, which allowed 'replication' across populations. However, there was still statistical overrepresentation of some genotypes and age groups in some populations. Thus, despite our efforts both factors have not been fully independent of the sampling population, and we therefore cannot rule out that some of the observed genotype or age effects are overestimates.

Effect of plant genotype and age on microbiome composition

We found that different *L. corniculatus* genotypes were also associated with variation in the composition of the microbiome, i.e. genetic variation in the host not only affected the alpha diversity but also the beta diversity of the microbes. Again, the extent of these effects strongly depended on the plant organ: in roots and shoots the composition of all three major microbial groups (bacteria, fungi and eukaryotes) was affected by plant genotype, whereas in flowers only the composition of bacteria and fungi, and in the seeds only that of bacteria, showed significant plant genotype associations. A plethora of genetically variable plant traits are known to affect the plant microbiome and could thus be underlying these observed composition changes. For example, root exudates are an important determinant of the rhizosphere microbiome (Hu et al., 2018; Zhalnina et al., 2018), and can vary across plant genotypes (Micallef et al., 2009; Pacheco-Moreno et al., 2024). Intraspecific variation in leaf morphology and leaf chemistry has been shown to influence microbial communities in the phyllosphere (Hunter et al., 2010; Kniskern et al., 2007), and variation in plant defense genes is strongly affecting plant colonization by specific pathogens (Glander et al., 2018; Ronald et al., 1992).

In contrast to plant genotype, we found little evidence for effects of plant age on the composition of microbial communities. Only in fungi and eukaryotes, and mainly in roots and shoots, we found a significant plant age by genotype interaction, i.e. age effects were inconsistent and differed among genotypes, or were restricted to particular genotypes. This was counter to our expectation of age-related changes in species composition because of gene expression and functional trait changes during plant development (Hu and Yang, 2019). One possible reason for our findings could be that most species composition changes happen at early plant ages, and that the main factors shaping the

microbiome such as root exudates (Chaparro et al., 2014) stabilize after this initial phase, so that the microbial communities reach an equilibrium fairly early. Unfortunately our youngest sampled plants were two years old, so we could not compare with one-year old plants, to understand the early-stage dynamics better. Clearly, a better understanding of these questions, and of the observed plant age by genotype interactions, requires controlled experiments with longer-term observation of the colonization dynamics of different plant genotypes under standardized environmental conditions.

When looking at the overall frequency patterns of microbes, we found that although many taxa were shared across all plant genotypes and ages, there were still significant numbers of taxa that occurred only in a subset of plant genotypes or age groups, and there was an intriguing difference between bacteria on the one hand, and fungi and eukaryotes on the other hand. While in bacteria the level of generalism was particularly high, i.e. a large fraction of taxa occurred everywhere, frequency distributions of taxa were much more even in fungi and eukaryotes, showing greater dispersal rates of bacteria than of non-bacteria microbes. Bacteria generally possess dispersal mechanisms unavailable to other microorganisms, allowing them to spread more efficiently. For instance, they can rapidly disperse through fungal hyphae in soil (Vieira et al., 2025) and, in general, exhibit much higher dispersal rates than fungal microbes (Larsen et al., 2023). In contrast, fungal dispersal is more strongly constrained by potential dispersal limitation (Zhang et al., 2021), which may further contribute to the broader distribution of bacterial taxa.

Associations of microbial taxa with specific plant genotypes and age groups

The differential abundance analysis identified a number of significant associations of microbial taxa with specific plant genotypes. Interestingly, these associations were not randomly distributed, but some plant genotypes harbored many more specific microbial taxa than others. Plant genotypes can differ in their production of secondary metabolites, which influence the recruitment or inhibition of specific microbes (Su et al., 2023). Additionally, modifications in root morphology can limit microbial attachment and interaction, reducing the presence of specialized microbial associations in the root (Robertson-Albertyn et al., 2017). Changes in these chemical and morphological traits can require greater levels of specialization of associated microbes.

If we look at the genotype associations in more detail, we find that several mutualistic bacteria, such as *Bacillus* (Idriss et al., 2002; Lozo et al., 2022; Tiwari et al., 2010), primarily soil-associated bacteria like *Cloacibacterium*, *Prauserella* and *Rubrobacter*, and some growth-promoting fungi like *Agrocybe* (Albrechtova et al., 2012; Wu and Kawagishi, 2020) were overrepresented in some plant genotypes. Similar host genotype effects on *Bacillus* have been observed in other studies, e.g. in maize cultivars

resistant to corn stalk rot versus non-resistant cultivars (Xia et al., 2024), or in sweet pepper cultivars (Lozo et al., 2022). While plant interactions with the soil-associated bacteria are not yet fully understood, previous studies have shown that the plant genotype can influence the abundance of specific soil microbes in the rhizosphere (Yang et al., 2022), and that plants actively recruit genotype-specific beneficial soil microbes (Dilla-Ermita et al., 2021). We also found several pathogens with differential abundance among plant genotypes, e.g. *Erwinia* bacteria responsible for soft rot (Toth et al., 2003) and the fungal pathogens *Sclerotinia*, *Alternaria*, *Pseudoidium*, *Golovinomyces* and *Gibberella*. Our results corroborate previous studies with different plant species that also found genetic variation in plant resistance to these pathogens (Abe et al., 2010; Sobiczewski et al., 2015; Wegulo et al., 1998; Yang et al., 2010). In general, plant genetic effects on pathogen resistance are a well-known phenomenon, and a key topic in agriculture and crop management (Crute and Pink, 1996). They also play an important role in natural populations, and are a cornerstones of the geographic mosaic of coevolution observed in many wild species (Laine, 2009; Thompson, 2005; Thrall et al., 2012).

We also found that some microbial taxa were significantly overrepresented in particular plant age groups, e.g. bacteria from the genera *Burkholderia, Erwinia and Ralstonia* and fungi from the genus *Mycena* in the youngest plant group. *Burkholderia* bacteria are known for their growth-promoting effects (Dilla-Ermita et al., 2021) and have been shown to benefit maize plants during germination and seedling development (dos Santos et al., 2022). Although the studied *Lotus* plants were already beyond these initial stages of development, the observed higher abundances of *Burkholderia* in young plants suggest that these bacteria also play a role in early *Lotus* growth and development. *Mycena* is a saprotrophic fungus opportunistically invading plant roots (Harder et al., 2023), and *Erwinia* and *Ralstonia* are bacterial pathogens causing wilt in various plant species. The increased abundances of these taxa in young *Lotus* plants suggests these may have a less well developed immune system (Lemessa and Zeller, 2007; Liu et al., 2018) or other, e.g. root physiological, differences that make them more susceptible to invasion (Ando et al., 2015). There were also microbial taxa that were particularly abundant in older plants, e.g. the pathogenic fungus *Pyrenochaeta* which is known to cause root lesions (Hilton et al., 2013), and which we found overrepresented in plants older than six years. Our results confirm a previous study with oilseed rape that also observed a higher abundance of *Pyrenochaeta* in later plant developmental stages (Bennett et al., 2020). In general, our data show that while plant age does not have strong effects on the overall composition of microbes, there are some significant influences at the level of individual microbial taxa, even in a long-lived plant such as *Lotus corniculatus*.

It is important to keep in mind that all of the results discussed above are statistical associations between microbial taxa and plant genotypes/age groups in the field, but no formal proof of causal relationships. The latter require manipulative experiments, and to obtain such formal proof at least for one example of an observed significant genotype-microbe association, we conducted a small lab experiment testing the susceptibility of two of the sampled *Lotus corniculatus* genotypes to the fungal pathogen *Fusarium* (teleomorph *Gibberella*) under controlled conditions. As expected, the genotype with overabundance of *Fusarium* in the LEfSe analysis developed significantly greater leaf wilt when infected with *Fusarium* in the lab than another plant genotype that had not shown the same abundance of *Fusarium* in the field. This strongly indicates that the observed plant genotype association of *Fusarium* is indeed true and reflects genetically-based plant variation.

Conclusion

Our findings show the host genotype is an important driver of natural microbiome diversity of *L. corniculatus*. This influence is evident not only in overall microbial diversity and composition but also in the abundance of specific microbial genera across different genotypes. We also found evidence of plant age influencing the fungal diversity. Additionally, the abundance of specific microbial genera varies between the different host age groups. With varied genetics or age, the physiology and requirements of the plants change, and due to the close interaction between the microbiome and the host, this can result in an altered optimal microbiome composition. Therefore, it is valuable to combine insights from controlled laboratory experiments with data from natural environments to gain a more comprehensive understanding of the impact of genotype and age on the microbiome. Overall, our results show that genotype, and to a lesser extent age, play important roles in shaping the microbiome diversity and composition of natural *L. corniculatus* populations. This knowledge can also help optimize plant health, improve crop yields, and enhance ecosystem resilience which is crucial for effective ecosystem management and plant breeding practices.

Acknowledgements

We thank the Kemen and Bossdorf labs for help with the field sampling, in particular Elke Klenk for her invaluable assistance in collecting and processing the samples, as well Sabine Silberhorn and Julia Rafalski for their help in the lab. We are also indebted to Anna Röder and Christiane Roscher who taught us the method of herb chronology, and to Ralf Lauterbach and Jörg Hailer from the Schwäbische Alb local management team of the Biodiversity Exploratories for instructions and support during the field work. We have been lucky to be granted access to the Schwäbische Alb plots of the Biodiversity Exploratories (DFG Priority Program 1374) through a cooperation agreement with this project. We thank the managers of the Schwäbische Alb exploratory, Kirsten Reichel-Jung and Julia Bass and all former managers for their work in maintaining these plots and the project infrastructure, Victoria Grießmeier for her support through the central office, Andreas Ostrowski for managing the central database, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. We thank the administration of the UNESCO Biosphere Reserve Swabian Alb as well as all landowners for the excellent collaboration. Field work permits were issued by the responsible state environmental office of Baden-Württemberg.

Author contributions

OB, EK, FR and KL designed the study; FR and KL planned and conducted the field sampling, lab work and data analyses; WD helped with the library preparation and bioinformatics of the ddRAD sequencing; FR performed the *Fusarium* experiment and analyzed the data with the support of JHJ; FR, KL and OB wrote the manuscript with input from all authors. All authors read and approved the final manuscript.

Competing interests

We declare that there are no conflicts of interest related to this study

Funding

This project has been funded by the Deutsche Forschungsgemeinschaft (DFG; projects BO 3241/11-1 to OB) as part of the priority program 2125 Deconstruction and Reconstruction of the Plant Microbiota “DECRyPT”.

References

Abe, K., Iwanami, H., Kotoda, N., Moriya, S., Takahashi (Sumiyoshi), S., 2010. Evaluation of apple genotypes and Malus species for resistance to Alternaria blotch caused by Alternaria alternata apple pathotype using detached-leaf method. Plant Breed. 129, 208–218. https://doi.org/10.1111/j.1439-0523.2009.01672.x

Abraham, E.M., Ganopoulos, I., Giagourta, P., Osathanunkul, M., Bosmali, I., Tsaftaris, A., Papaioannou, A., Madesis, P., 2015. Genetic diversity of *Lotus corniculatus* in relation to habitat type, species composition and species diversity. Biochem. Syst. Ecol. 63, 59–67. https://doi.org/10.1016/j.bse.2015.09.026

Agler, M.T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.-T., Weigel, D., Kemen, E.M., 2016. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. PLOS Biol. 14, e1002352. https://doi.org/10.1371/journal.pbio.1002352

Albrechtova, J., Latr, A., Nedorost, L., Pokluda, R., Posta, K., Vosatka, M., 2012. Dual Inoculation with Mycorrhizal and Saprotrophic Fungi Applicable in Sustainable Cultivation Improves the Yield and Nutritive Value of Onion. Sci. World J. 2012, 374091. https://doi.org/10.1100/2012/374091

Almario, J., Mahmoudi, M., Kroll, S., Agler, M., Placzek, A., Mari, A., Kemen, E., 2022. The Leaf Microbiome of Arabidopsis Displays Reproducible Dynamics and Patterns throughout the Growing Season. mBio 13, e02825-21. https://doi.org/10.1128/mbio.02825-21

Ando, K., Carr, K.M., Colle, M., Mansfeld, B.N., Grumet, R., 2015. Exocarp Properties and Transcriptomic Analysis of Cucumber (Cucumis sativus) Fruit Expressing Age-Related Resistance to Phytophthora capsici. PLOS ONE 10, e0142133. https://doi.org/10.1371/journal.pone.0142133

Balaban, M., Moshiri, N., Mai, U., Jia, X., Mirarab, S., 2019. TreeCluster: Clustering biological sequences using phylogenetic trees. PLOS ONE 14, e0221068. https://doi.org/10.1371/journal.pone.0221068

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4, 914–919. https://doi.org/10.1111/2041-210X.12073

Bennett, A.J., Hilton, S., Chandler, D., Mills, P., Bending, G.D., 2020. Relationships between yield, rotation length, and abundance of *Olpidium brassicae* and *Pyrenochaeta* sp. in the rhizosphere of oilseed rape. Appl. Soil Ecol. 147, 103433. https://doi.org/10.1016/j.apsoil.2019.103433

Bernard, J., Wall, C.B., Costantini, M.S., Rollins, R.L., Atkins, M.L., Cabrera, F.P., Cetraro, N.D., Feliciano, C.K.J., Greene, A.L., Kitamura, P.K., Olmedo-Velarde, A., Sirimalwatta, V.N.S., Sung, H.W., Thompson, L.P.M., Vu, H.T., Wilhite, C.J., Amend, A.S., 2021. Plant part and a steep environmental gradient predict plant microbial composition in a tropical watershed. ISME J. 15, 999–1009. https://doi.org/10.1038/s41396-020-00826-5

Boege, K., Dirzo, R., Siemens, D., Brown, P., 2007. Ontogenetic switches from plant resistance to tolerance: minimizing costs with age? Ecol. Lett. 10, 177–187. https://doi.org/10.1111/j.1461-0248.2006.01012.x

Bonito, G., Reynolds, H., Robeson II, M.S., Nelson, J., Hodkinson, B.P., Tuskan, G., Schadt, C.W., Vilgalys, R., 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. Mol. Ecol. 23, 3356–3370. https://doi.org/10.1111/mec.12821

Bradski, G., 2000. The OpenCV Library. Dr Dobbs J. Softw. Tools.

Bressan, M., Roncato, M.-A., Bellvert, F., Comte, G., Haichar, F. el Z., Achouak, W., Berge, O., 2009. Exogenous glucosinolate produced by Arabidopsis thaliana has an impact on microbes in the rhizosphere and plant roots. ISME J. 3, 1243–1257. https://doi.org/10.1038/ismej.2009.68

Brown, S.P., Grillo, M.A., Podowski, J.C., Heath, K.D., 2020. Soil origin and plant genotype structure distinct microbiome compartments in the model legume Medicago truncatula. Microbiome 8, 139. https://doi.org/10.1186/s40168-020-00915-9

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O., Amann, R., Eickhorst, T., Schulze-Lefert, P., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488, 91–95. https://doi.org/10.1038/nature11336

Campbell, D.R., Raguso, R.A., Midzik, M., Bischoff, M., Broadhead, G.T., 2022. Genetic and spatial variation in vegetative and floral traits across a hybrid zone. Am. J. Bot. 109, 1780–1793. https://doi.org/10.1002/ajb2.16067

Cao, Y., Dong, Q., Wang, D., Zhang, P., Liu, Y., Niu, C., 2022. microbiomeMarker: an R/Bioconductor package for microbiome marker identification and visualization. Bioinformatics 38, 4027–4029. https://doi.org/10.1093/bioinformatics/btac438

Chaparro, J.M., Badri, D.V., Vivanco, J.M., 2014. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 8, 790–803. https://doi.org/10.1038/ismej.2013.196

Chen, P., Zhao, M., Tang, F., Hu, Y., Peng, X., Shen, S., 2019. The effect of environment on the microbiome associated with the roots of a native woody plant under different climate types in China. Appl. Microbiol. Biotechnol. 103, 3899–3913. https://doi.org/10.1007/s00253-019-09747-6

Compant, S., Mitter, B., Colli-Mull, J.G., Gangl, H., Sessitsch, A., 2011. Endophytes of Grapevine Flowers, Berries, and Seeds: Identification of Cultivable Bacteria, Comparison with Other Plant Parts, and Visualization of Niches of Colonization. Microb. Ecol. 62, 188–197. https://doi.org/10.1007/s00248-011-9883-y

Crute, I., Pink, D., 1996. Genetics and Utilization of Pathogen Resistance in Plants. Plant Cell 8, 1747–1755.

Cui, Z., Huntley, R.B., Zeng, Q., Steven, B., 2021. Temporal and spatial dynamics in the apple flower microbiome in the presence of the phytopathogen Erwinia amylovora. ISME J. 15, 318–329. https://doi.org/10.1038/s41396-020-00784-y

Deng, Y., Ning, Y., Yang, D.-L., Zhai, K., Wang, G.-L., He, Z., 2020. Molecular Basis of Disease Resistance and Perspectives on Breeding Strategies for Resistance Improvement in Crops. Mol. Plant 13, 1402–1419. https://doi.org/10.1016/j.molp.2020.09.018

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072. https://doi.org/10.1128/AEM.03006-05

Develey-Rivière, M.-P., Galiana, E., 2007. Resistance to pathogens and host developmental stage: a multifaceted relationship within the plant kingdom. New Phytol. 175, 405–416. https://doi.org/10.1111/j.1469-8137.2007.02130.x

Dilla-Ermita, C.J., Lewis, R.W., Sullivan, T.S., Hulbert, S.H., 2021. Wheat Genotype-Specific Recruitment of Rhizosphere Bacterial Microbiota Under Controlled Environments. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.718264

Diskin, S., Feygenberg, O., Maurer, D., Droby, S., Prusky, D., Alkan, N., 2017. Microbiome Alterations Are Correlated with Occurrence of Postharvest Stem-End Rot in Mango Fruit. Phytobiomes J. 1, 117–127. https://doi.org/10.1094/PBIOMES-05-17-0022-R

dos Santos, I.B., Pereira, A.P. de A., de Souza, A.J., Cardoso, E.J.B.N., da Silva, F.G., Oliveira, J.T.C., Verdi, M.C.Q., Sobral, J.K., 2022. Selection and Characterization of Burkholderia spp. for Their Plant-Growth Promoting Effects and Influence on Maize Seed Germination. Front. Soil Sci. 1. https://doi.org/10.3389/fsoil.2021.805094

Durka, W., Michalski, S.G., Höfner, J., Bucharova, A., Kolář, F., Müller, C.M., Oberprieler, C., Šemberová, K., Bauer, M., Bernt, M., Bleeker, W., Brändel, S., Bucher, S.F., Eibes, P.M., Ewald, M., Goldberg, R., Grant, K., Haider, S., Harpke, A., Haun, F., Kaufmann, R., Korell, L., Kunzmann, D., Lauterbach, D., Leib, S., Lenzewski, N., Loritz, H., Madaj, A.-M., Mainz, A.K., Meinecke, P., Mertens, H., Meyer, M.H., Musche, M., Ristow, M., Rosche, C., Roscher, C., Rutte, D., Schacherer, A., Schmidt, W., Schmoldt, J., Schneider, S., Schwarz, J.-H., Skowronek, S., Socher, S.A., Stanik, N., Twerski, A., Weiß, K., Weiß, M., Wille, A., Zehm, A., Zidorn, C., 2025. Assessment of genetic diversity among seed transfer zones for multiple grassland plant species across Germany. Basic Appl. Ecol. 84, 50–60. https://doi.org/10.1016/j.baae.2024.11.004

Dutta, B., Ha, Y., Lessl, J.T., Avci, U., Sparks, A.C., Johnson, K.L., Walcott, R.R., 2015. Pathways of bacterial invasion and watermelon seed infection by Acidovorax citrulli. Plant Pathol. 64, 537–544. https://doi.org/10.1111/ppa.12307

Edwards, J.A., Santos-Medellín, C.M., Liechty, Z.S., Nguyen, B., Lurie, E., Eason, S., Phillips, G., Sundaresan, V., 2018. Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice. PLOS Biol. 16, e2003862. https://doi.org/10.1371/journal.pbio.2003862

Ekwomadu, T.I., Mwanza, M., 2023. Fusarium Fungi Pathogens, Identification, Adverse Effects, Disease Management, and Global Food Security: A Review of the Latest Research. Agriculture 13, 1810. https://doi.org/10.3390/agriculture13091810

Estendorfer, J., Stempfhuber, B., Haury, P., Vestergaard, G., Rillig, M.C., Joshi, J., Schröder, P., Schloter, M., 2017. The Influence of Land Use Intensity on the Plant-Associated Microbiome of Dactylis glomerata L. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.00930

Fisher, M.C., Gurr, S.J., Cuomo, C.A., Blehert, D.S., Jin, H., Stukenbrock, E.H., Stajich, J.E., Kahmann, R., Boone, C., Denning, D.W., Gow, N.A.R., Klein, B.S., Kronstad, J.W., Sheppard, D.C., Taylor, J.W., Wright, G.D., Heitman, J., Casadevall, A., Cowen, L.E., 2020. Threats Posed by the Fungal Kingdom to Humans, Wildlife, and Agriculture. mBio 11, e00449-20. https://doi.org/10.1128/mBio.00449-20

Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J., 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484, 186–194. https://doi.org/10.1038/nature10947

García-Bastidas, F.A., Van der Veen, A.J.T., Nakasato-Tagami, G., Meijer, H.J.G., Arango-Isaza, R.E., Kema, G.H.J., 2019. An Improved Phenotyping Protocol for Panama Disease in Banana. Front. Plant Sci. 10.

Gaube, P., Junker, R.R., Keller, A., 2021. Changes amid constancy: Flower and leaf microbiomes along land use gradients and between bioregions. Basic Appl. Ecol. 50, 1–15. https://doi.org/10.1016/j.baae.2020.10.003

Genung, M.A., Lessard, J.-P., Brown, C.B., Bunn, W.A., Cregger, M.A., Reynolds, W.N., Felker-Quinn, E., Stevenson, M.L., Hartley, A.S., Crutsinger, G.M., Schweitzer, J.A., Bailey, J.K., 2010. Non-Additive Effects of Genotypic Diversity Increase Floral Abundance and Abundance of Floral Visitors. PLOS ONE 5, e8711. https://doi.org/10.1371/journal.pone.0008711

Glander, S., He, F., Schmitz, G., Witten, A., Telschow, A., de Meaux, J., 2018. Assortment of Flowering Time and Immunity Alleles in Natural Arabidopsis thaliana Populations Suggests Immunity and Vegetative Lifespan Strategies Coevolve. Genome Biol. Evol. 10, 2278–2291. https://doi.org/10.1093/gbe/evy124

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.-L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a

catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41, D597–D604. https://doi.org/10.1093/nar/gks1160
Hamonts, K., Trivedi, P., Garg, A., Janitz, C., Grinyer, J., Holford, P., Botha, F.C., Anderson, I.C., Singh, B.K., 2018. Field study reveals core plant microbiota and relative importance of their drivers. Environ. Microbiol. 20, 124–140. https://doi.org/10.1111/1462-2920.14031
Harder, C.B., Hesling, E., Botnen, S.S., Lorberau, K.E., Dima, B., von Bonsdorff-Salminen, T., Niskanen, T., Jarvis, S.G., Ouimette, A., Hester, A., Hobbie, E.A., Taylor, A.F.S., Kauserud, H., 2023. Mycena species can be opportunist-generalist plant root invaders. Environ. Microbiol. 25, 1875–1893. https://doi.org/10.1111/1462-2920.16398
Hennig, E.I., Ghazoul, J., 2011. Plant–pollinator interactions within the urban environment. Perspect. Plant Ecol. Evol. Syst. 13, 137–150. https://doi.org/10.1016/j.ppees.2011.03.003
Hilton, S., Bennett, A.J., Keane, G., Bending, G.D., Chandler, D., Stobart, R., Mills, P., 2013. Impact of Shortened Crop Rotation of Oilseed Rape on Soil and Rhizosphere Microbial Diversity in Relation to Yield Decline. PLOS ONE 8, e59859. https://doi.org/10.1371/journal.pone.0059859
Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 35, 518–522. https://doi.org/10.1093/molbev/msx281
Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M.G.A., Schlaeppi, K., Erb, M., 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat. Commun. 9, 2738. https://doi.org/10.1038/s41467-018-05122-7
Hu, L., Yang, L., 2019. Time to Fight: Molecular Mechanisms of Age-Related Resistance. Phytopathology® 109, 1500–1508. https://doi.org/10.1094/PHYTO-11-18-0443-RVW
Huang, L., Fu, Y., Liu, Y., Chen, Y., Wang, T., Wang, M., Lin, X., Feng, Y., 2024. Global insights into endophytic bacterial communities of terrestrial plants: Exploring the potential applications of endophytic microbiota in sustainable agriculture. Sci. Total Environ. 927, 172231. https://doi.org/10.1016/j.scitotenv.2024.172231
Hunter, P.J., Hand, P., Pink, D., Whipps, J.M., Bending, G.D., 2010. Both Leaf Properties and Microbe-Microbe Interactions Influence Within-Species Variation in Bacterial Population Diversity and Structure in the Lettuce (Lactuca Species) Phyllosphere. Appl. Environ. Microbiol. 76, 8117–8125. https://doi.org/10.1128/AEM.01321-10
Idriss, E.E., Makarewicz, O., Farouk, A., Rosner, K., Greiner, R., Bochow, H., Richter, T., Borriss, R., 2002. Extracellular phytase activity of Bacillus amyloliquefaciens FZB45 contributes to its plant-growth-promoting effectaaThe GenBank accession numbers for the sequences determined in this work are AY055219 to AY055226. Microbiology 148, 2097–2109. https://doi.org/10.1099/00221287-148-7-2097
Jung, B., Lee, S., Ha, J., Park, J.-C., Han, S.-S., Hwang, I., Lee, Y.-W., Lee, J., 2013. Development of a Selective Medium for the Fungal Pathogen Fusarium graminearum Using Toxoflavin Produced by the Bacterial Pathogen Burkholderia glumae. Plant Pathol. J. 29, 446–450. https://doi.org/10.5423/PPJ.NT.07.2013.0068
Junker, R.R., Loewel, C., Gross, R., Dötterl, S., Keller, A., Blüthgen, N., 2011. Composition of epiphytic bacterial communities differs on petals and leaves. Plant Biol. 13, 918–924. https://doi.org/10.1111/j.1438-8677.2011.00454.x
Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285
Karasov, T.L., Shirsekar, G., Schwab, R., Weigel, D., 2020. What natural variation can teach us about resistance durability. Curr. Opin. Plant Biol., Biotic interactions ● AGRI 2019 56, 89–98. https://doi.org/10.1016/j.pbi.2020.04.010

Keller, A., McFrederick, Q.S., Dharampal, P., Steffan, S., Danforth, B.N., Leonhardt, S.D., 2021. (More than) Hitchhikers through the network: the shared microbiome of bees and flowers. Curr. Opin. Insect Sci., Ecology * Parasites/Parasitoids/Biological control 44, 8–15. https://doi.org/10.1016/j.cois.2020.09.007

Knief, C., Ramette, A., Frances, L., Alonso-Blanco, C., Vorholt, J.A., 2010. Site and plant species are important determinants of the Methylobacterium community composition in the plant phyllosphere. ISME J. 4, 719–728. https://doi.org/10.1038/ismej.2010.9

Kniskern, J.M., Traw, M.B., Bergelson, J., 2007. Salicylic Acid and Jasmonic Acid Signaling Defense Pathways Reduce Natural Bacterial Diversity on Arabidopsis thaliana. Mol. Plant-Microbe Interactions® 20, 1512–1522. https://doi.org/10.1094/MPMI-20-12-1512

Kusstatscher, P., Cernava, T., Harms, K., Maier, J., Eigner, H., Berg, G., Zachow, C., 2019. Disease Incidence in Sugar Beet Fields Is Correlated with Microbial Diversity and Distinct Biological Markers. Phytobiomes J. 3, 22–30. https://doi.org/10.1094/PBIOMES-01-19-0008-R

Laine, A.-L., 2009. Role of coevolution in generating biological diversity: spatially divergent selection trajectories. J. Exp. Bot. 60, 2957–2970. https://doi.org/10.1093/jxb/erp168

Larsen, S., Albanese, D., Stegen, J., Franceschi, P., Coller, E., Zanzotti, R., Ioriatti, C., Stefani, E., Pindo, M., Cestaro, A., Donati, C., 2023. Distinct and Temporally Stable Assembly Mechanisms Shape Bacterial and Fungal Communities in Vineyard Soils. Microb. Ecol. 86, 337–349. https://doi.org/10.1007/s00248-022-02065-x

Lemessa, F., Zeller, W., 2007. Pathogenic characterisation of strains of Ralstonia solanacearum from Ethiopia and influence of plant age on susceptibility of hosts against R. solanacearum / Charakterisierung der Pathogenese verschiedener äthiopischer Stämme von Ralstonia solanacearum und Einfluss des Pflanzenalters auf die Anfälligkeit von Wirten gegenüber dem Bakterium. J. Plant Dis. Prot. 114, 241–249.

Leopold, D.R., Busby, P.E., 2020. Host Genotype and Colonist Arrival Order Jointly Govern Plant Microbiome Composition and Function. Curr. Biol. 30, 3260-3266.e5. https://doi.org/10.1016/j.cub.2020.06.011

Lessl, J.T., Fessehaie, A., Walcott, R.R., 2007. Colonization of Female Watermelon Blossoms by Acidovorax avenae ssp. citrulli and the Relationship between Blossom Inoculum Dosage and Seed Infestation. J. Phytopathol. 155, 114–121. https://doi.org/10.1111/j.1439-0434.2007.01204.x

Letunic, I., Bork, P., 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res. gkae268. https://doi.org/10.1093/nar/gkae268

Lischer, H.E.L., Excoffier, L., 2012. PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. Bioinformatics 28, 298–299. https://doi.org/10.1093/bioinformatics/btr642

Liu, C., Cui, Y., Li, X., Yao, M., 2021. microeco: an R package for data mining in microbial community ecology. FEMS Microbiol. Ecol. 97, fiaa255. https://doi.org/10.1093/femsec/fiaa255

Liu, F., Hewezi, T., Lebeis, S.L., Pantalone, V., Grewal, P.S., Staton, M.E., 2019. Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. BMC Microbiol. 19, 201. https://doi.org/10.1186/s12866-019-1572-x

Liu, Q., Beattie, G.A., Saalau Rojas, E., Gleason, M.L., 2018. Bacterial wilt symptoms are impacted by host age and involve net downward movement of Erwinia tracheiphila in muskmelon. Eur. J. Plant Pathol. 151, 803–810. https://doi.org/10.1007/s10658-018-1418-7

Lozo, J., Danojević, D., Jovanović, Ž., Nenadović, Ž., Fira, D., Stanković, S., Radović, S., 2022. Genotype-Dependent Antioxidative Response of Four Sweet Pepper Cultivars to Water Deficiency as Affected by Drought-Tolerant Bacillus safensis SS-2.7 and Bacillus thuringiensis SS-29.2 Strains. Horticulturae 8, 236. https://doi.org/10.3390/horticulturae8030236

Lutap, K., Almario, J., Mahmoudi, M., Reis, F., Bossdorf, O., Kemen, E., 2025. Organ-specific microbiomes in natural Lotus corniculatus populations: Metacommunity dynamics in the plant endosphere. bioRxiv. https://doi.org/10.1101/2025.04.15.649008

Malacrinò, A., Karley, A.J., Schena, L., Bennett, A.E., 2021a. Soil Microbial Diversity Impacts Plant Microbiota More than Herbivory. Phytobiomes J. 5, 408–417. https://doi.org/10.1094/PBIOMES-02-21-0011-R

Malacrinò, A., Wang, M., Caul, S., Karley, A.J., Bennett, A.E., 2021b. Herbivory shapes the rhizosphere bacterial microbiota in potato plants. Environ. Microbiol. Rep. 13, 805–811. https://doi.org/10.1111/1758-2229.12998

Martin, F.M., van der Heijden, M.G.A., 2024. The mycorrhizal symbiosis: research frontiers in genomics, ecology, and agricultural application. New Phytol. 242, 1486–1506. https://doi.org/10.1111/nph.19541

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12. https://doi.org/10.14806/ej.17.1.200

Mayer, T., Mari, A., Almario, J., Murillo-Roos, M., Syed M. Abdullah, H., Dombrowski, N., Hacquard, S., Kemen, E.M., Agler, M.T., 2021. Obtaining deeper insights into microbiome diversity using a simple method to block host and nontargets in amplicon sequencing. Mol. Ecol. Resour. 21, 1952–1965. https://doi.org/10.1111/1755-0998.13408

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217

Micallef, S.A., Shiaris, M.P., Colón-Carmona, A., 2009. Influence of Arabidopsis thaliana accessions on rhizobacterial communities and natural variation in root exudates. J. Exp. Bot. 60, 1729–1742. https://doi.org/10.1093/jxb/erp053

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol. Biol. Evol. 37, 1530–1534. https://doi.org/10.1093/molbev/msaa015

Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., Berninger, T., Naveed, M., Sheibani-Tezerji, R., von Maltzahn, G., Sessitsch, A., 2017. A New Approach to Modify Plant Microbiomes and Traits by Introducing Beneficial Bacteria at Flowering into Progeny Seeds. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00011

Murrell, D.C., SHUEL, R.W., TOMES, D.T., 1982. NECTAR PRODUCTION AND FLORAL CHARACTERISTICS IN BIRDSFOOT TREFOIL (Lotus corniculatus L.). Can. J. Plant Sci. 62, 361–371. https://doi.org/10.4141/cjps82-055

Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47, D259–D264. https://doi.org/10.1093/nar/gky1022

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Borman, T., Carvalho, G., Chirico, M., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Geoffrey Hannigan, G., Mark O., H., Lahti, L., McGlinn, D., Ouellette, M.-H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J., 2025. vegan: Community Ecology Package.

O'Leary, S.J., Puritz, J.B., Willis, S.C., Hollenbeck, C.M., Portnoy, D.S., 2018. These aren't the loci you'e looking for: Principles of effective SNP filtering for molecular ecologists. Mol. Ecol. 27, 3193–3206. https://doi.org/10.1111/mec.14792

Pacheco-Moreno, A., Bollmann-Giolai, A., Chandra, G., Brett, P., Davies, J., Thornton, O., Poole, P., Ramachandran, V., Brown, J.K.M., Nicholson, P., Ridout, C., DeVos, S., Malone, J.G., 2024. The genotype of barley cultivars influences multiple aspects of their associated microbiota

via differential root exudate secretion. PLOS Biol. 22, e3002232. https://doi.org/10.1371/journal.pbio.3002232
Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLOS ONE 7, e37135. https://doi.org/10.1371/journal.pone.0037135
Puritz, J.B., Hollenbeck, C.M., Gold, J.R., 2014. dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ 2, e431. https://doi.org/10.7717/peerj.431
Ristova, D., Busch, W., 2014. Natural Variation of Root Traits: From Development to Nutrient Uptake1. Plant Physiol. 166, 518–527. https://doi.org/10.1104/pp.114.244749
Robertson-Albertyn, S., Alegria Terrazas, R., Balbirnie, K., Blank, M., Janiak, A., Szarejko, I., Chmielewska, B., Karcz, J., Morris, J., Hedley, P.E., George, T.S., Bulgarelli, D., 2017. Root Hair Mutations Displace the Barley Rhizosphere Microbiota. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.01094
Rochette, N.C., Rivera-Colón, A.G., Catchen, J.M., 2019. Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Mol. Ecol. 28, 4737–4754. https://doi.org/10.1111/mec.15253
Roeder, A., Schweingruber, F.H., Fischer, M., Roscher, C., 2017. Growth ring analysis of multiple dicotyledonous herb species—A novel community-wide approach. Basic Appl. Ecol. 21, 23–33. https://doi.org/10.1016/j.baae.2017.05.001
Ronald, P.C., Salmeron, J.M., Carland, F.M., Staskawicz, B.J., 1992. The cloned avirulence gene avrPto induces disease resistance in tomato cultivars containing the Pto resistance gene. J. Bacteriol. 174, 1604–1611. https://doi.org/10.1128/jb.174.5.1604-1611.1992
RStudio Team, 2024. RStudio: Integrated Development for R.
Schlaeppi, K., Dombrowski, N., Oter, R.G., Ver Loren van Themaat, E., Schulze-Lefert, P., 2014. Quantitative divergence of the bacterial root microbiota in Arabidopsis thaliana relatives. Proc. Natl. Acad. Sci. 111, 585–592. https://doi.org/10.1073/pnas.1321597111
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089
Schreiter, S., Ding, G.-C., Heuer, H., Neumann, G., Sandmann, M., Grosch, R., Kropf, S., Smalla, K., 2014. Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. Front. Microbiol. 5. https://doi.org/10.3389/fmicb.2014.00144
Schweingruber, F.H., Poschlod, P., 2005. Growth Rings in Herbs and Shrubs: life span, age determination and stem anatomy. For. Snow Landsc. Res. 79, 195–415.
Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60. https://doi.org/10.1186/gb-2011-12-6-r60
Sobiczewski, P., Peil, A., Mikiciński, A., Richter, K., Lewandowski, M., Żurawicz, E., Kellerhals, M., 2015. Susceptibility of apple genotypes from European genetic resources to fire blight (Erwinia amylovora). Eur. J. Plant Pathol. 141, 51–62. https://doi.org/10.1007/s10658-014-0521-7
Solís-García, I.A., Ceballos-Luna, O., Cortazar-Murillo, E.M., Desgarennes, D., Garay-Serrano, E., Patiño-Conde, V., Guevara-Avendaño, E., Méndez-Bravo, A., Reverchon, F., 2021. Phytophthora Root Rot Modifies the Composition of the Avocado Rhizosphere Microbiome and Increases the Abundance of Opportunistic Fungal Pathogens. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.574110

Strange, R.N., Scott, P.R., 2005. Plant Disease: A Threat to Global Food Security. Annu. Rev. Phytopathol. 43, 83–116. https://doi.org/10.1146/annurev.phyto.43.113004.133839
Su, Y., Wang, J., Gao, W., Wang, R., Yang, W., Zhang, H., Huang, L., Guo, L., 2023. Dynamic metabolites: A bridge between plants and microbes. Sci. Total Environ. 899, 165612. https://doi.org/10.1016/j.scitotenv.2023.165612
Sugiyama, A., Ueda, Y., Zushi, T., Takase, H., Yazaki, K., 2014. Changes in the Bacterial Community of Soybean Rhizospheres during Growth in the Field. PLOS ONE 9, e100709. https://doi.org/10.1371/journal.pone.0100709
Thompson, J.N., 2005. Coevolution: The Geographic Mosaic of Coevolutionary Arms Races. Curr. Biol. 15, R992–R994. https://doi.org/10.1016/j.cub.2005.11.046
Thrall, P.H., Burdon, J.J., 2000. Effect of resistance variation in a natural plant host–pathogen metapopulation on disease dynamics. Plant Pathol. 49, 767–773. https://doi.org/10.1046/j.1365-3059.2000.00523.x
Thrall, P.H., Laine, A.-L., Ravensdale, M., Nemri, A., Dodds, P.N., Barrett, L.G., Burdon, J.J., 2012. Rapid genetic change underpins antagonistic coevolution in a natural host-pathogen metapopulation. Ecol. Lett. 15, 425–435. https://doi.org/10.1111/j.1461-0248.2012.01749.x
Tiwari, R., Kalra, A., Darokar, M.P., Chandra, M., Aggarwal, N., Singh, A.K., Khanuja, S.P.S., 2010. Endophytic Bacteria from Ocimum sanctum and Their Yield Enhancing Capabilities. Curr. Microbiol. 60, 167–171. https://doi.org/10.1007/s00284-009-9520-x
Tkacz, A., Cheema, J., Chandra, G., Grant, A., Poole, P.S., 2015. Stability and succession of the rhizosphere microbiota depends upon plant type and soil composition. ISME J. 9, 2349–2359. https://doi.org/10.1038/ismej.2015.41
Toth, I.K., Bell, K.S., Holeva, M.C., Birch, P.R.J., 2003. Soft rot erwiniae: from genes to genomes. Mol. Plant Pathol. 4, 17–30. https://doi.org/10.1046/j.1364-3703.2003.00149.x
Trivedi, P., Batista, B.D., Bazany, K.E., Singh, B.K., 2022. Plant–microbiome interactions under a changing world: responses, consequences and perspectives. New Phytol. 234, 1951–1959. https://doi.org/10.1111/nph.18016
Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant–microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. 18, 607–621. https://doi.org/10.1038/s41579-020-0412-1
van der Heijden, M.G.A., de Bruin, S., Luckerhoff, L., van Logtestijn, R.S.P., Schlaeppi, K., 2016. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. ISME J. 10, 389–399. https://doi.org/10.1038/ismej.2015.120
van der Kooi, C.J., Pen, I., Staal, M., Stavenga, D.G., Elzenga, J.T.M., 2016. Competition for pollinators and intra-communal spectral dissimilarity of flowers. Plant Biol. 18, 56–62. https://doi.org/10.1111/plb.12328
Van Rossum, G., & Drake Jr, F. L., 1995. Python reference manual. Cent. Voor Wiskd. En Inform. Amst.
Vieira, C.K., Marascalchi, M.N., Rozmoš, M., Benada, O., Belova, V., Jansa, J., 2025. Arbuscular mycorrhizal fungal highways – What, how and why? Soil Biol. Biochem. 202, 109702. https://doi.org/10.1016/j.soilbio.2024.109702
Wagner, M.R., Lundberg, D.S., del Rio, T.G., Tringe, S.G., Dangl, J.L., Mitchell-Olds, T., 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat. Commun. 7, 12151. https://doi.org/10.1038/ncomms12151
Wegulo, S.N., Yang, X.B., Martinson, C.A., 1998. Soybean Cultivar Responses to Sclerotinia sclerotiorum in Field and Controlled Environment Studies. Plant Dis. 82, 1264–1270. https://doi.org/10.1094/PDIS.1998.82.11.1264
Weiner, C.N., Werner, M., Linsenmair, K.E., Blüthgen, N., 2014. Land-use impacts on plant–pollinator networks: interaction strength and specialization predict pollinator declines. Ecology 95, 466–474. https://doi.org/10.1890/13-0436.1

Wen, T., Zhao, M., Liu, T., Huang, Q., Yuan, J., Shen, Q., 2020. High abundance of Ralstonia solanacearum changed tomato rhizosphere microbiome and metabolome. BMC Plant Biol. 20, 166. https://doi.org/10.1186/s12870-020-02365-9

Wickham, H., 2016. ggplot2, Use R! Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-24277-4

Wickham, H., François, R., Henry, L., Müller, K., Vaughan, D., 2023. dplyr: A Grammar of Data Manipulation.

Wu, J., Kawagishi, H., 2020. Plant growth regulators from mushrooms. J. Antibiot. (Tokyo) 73, 657–665. https://doi.org/10.1038/s41429-020-0352-z

Xia, X., Wei, Q., Wu, H., Chen, X., Xiao, C., Ye, Y., Liu, C., Yu, H., Guo, Y., Sun, W., Liu, W., 2024. Bacillus species are core microbiota of resistant maize cultivars that induce host metabolic defense against corn stalk rot. Microbiome 12, 156. https://doi.org/10.1186/s40168-024-01887-w

Yang, C., Yue, H., Ma, Z., Feng, Z., Feng, H., Zhao, L., Zhang, Y., Deakin, G., Xu, X., Zhu, H., Wei, F., 2022. Influence of plant genotype and soil on the cotton rhizosphere microbiome. Front. Microbiol. 13. https://doi.org/10.3389/fmicb.2022.1021064

Yang, L.H., Cenzer, M.L., Morgan, L.J., Hall, G.W., 2020. Species-specific, age-varying plant traits affect herbivore growth and survival. Ecology 101, e03029. https://doi.org/10.1002/ecy.3029

Yang, Q., Yin, G., Guo, Y., Zhang, D., Chen, S., Xu, M., 2010. A major QTL for resistance to Gibberella stalk rot in maize. Theor. Appl. Genet. 121, 673–687. https://doi.org/10.1007/s00122-010-1339-0

Yu, Z., Krause, S.M.B., Beck, D.A.C., Chistoserdova, L., 2016. A Synthetic Ecology Perspective: How Well Does Behavior of Model Organisms in the Laboratory Predict Microbial Activities in Natural Habitats? Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00946

Zemenick, A.T., Vannette, R.L., Rosenheim, J.A., 2021. Linked networks reveal dual roles of insect dispersal and species sorting for bacterial communities in flowers. Oikos 130, 697–707. https://doi.org/10.1111/oik.06818

Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., da Rocha, U.N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B.P., Firestone, M.K., Northen, T.R., Brodie, E.L., 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat. Microbiol. 3, 470–480. https://doi.org/10.1038/s41564-018-0129-3

Zhang, G., Wei, G., Wei, F., Chen, Z., He, M., Jiao, S., Wang, Y., Dong, L., Chen, S., 2021. Dispersal Limitation Plays Stronger Role in the Community Assembly of Fungi Relative to Bacteria in Rhizosphere Across the Arable Area of Medicinal Plant. Front. Microbiol. 12. https://doi.org/10.3389/fmicb.2021.713523