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Soil Microbial Diversity Impacts Plant Microbiota More than Herbivory

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ABSTRACT

Interactions between plants and microbiomes play a key role in ecosystem functioning and are of broad interest due to their influence on nutrient cycling and plant protection. However, we do not yet have a complete understanding of how plant microbiomes are assembled. Here, we tested and quantified the effect of different factors driving the diversity and composition of plant-associated microbial communities. We manipulated soil microbial diversity (high or low diversity), plant species (*Solanum tuberosum* or *S. vernei*), and herbivory (presence or absence of a phloem-feeding insect, *Macrosiphum euphorbiae*) and found that soil microbial diversity influenced the herbivore-associated microbiome composition but also plant species and herbivory influenced the soil microbiome composition. We quantified the relative strength of these effects and demonstrated that the initial soil microbiome diversity explained the most variation in plant- and herbivore-associated microbial communities. Our findings strongly suggest that soil microbial community diversity is a driver of the composition of multiple associated microbiomes (plant and insect), and this has implications for the importance of management of soil microbiomes in multiple systems.

Keywords: bacteria, fungi, metabarcoding, microbiome, phyllosphere, potato aphid, rhizosphere, *Solanum*

Microbiomes can be considered an extension of the plant genome (Berg et al. 2014; Levy et al. 2018; Rosenberg and Zilber-Rosenberg 2016; Schlaeppi and Bulgarelli 2015). Although their functional importance has been widely dissected in the last two decades of microbiome research, how plant microbial communities assemble, respond to environmental stimuli, and interact with their host remains to be determined (Cordovez et al. 2019; Saikkonen et al. 2020). In addition, research has rarely examined or compared multiple drivers and, to the best of our knowledge, no study has tested the relative strength of different drivers of microbial community composition.

There have been a number of studies identifying individual factors that drive the microbiome composition of plants and their associated organisms and environments. It is well established that plant microbiota are structured mainly by plant compartment (e.g., different plant organs, and diverse between endosphere and ectosphere) (Trivedi et al. 2020). Plant genotype and developmental stage have been shown to influence the composition of both plant and soil microbiomes (Wagner et al. 2016). Soil microbiome composition has also been shown to shape plant microbiome composition (Cordovez et al. 2019), and plant pathogens and herbivory produce compositional shifts in plant-associated microbial communities (Lareen et al. 2016). However, we still know little about the relative strength of these factors in shaping plant microbiomes.

Soil provides microbial inoculum and sets the conditions for both plant and microbial growth (Schlaeppi et al. 2014). Although different plant tissues can develop distinct microbiomes, soil provides an important reservoir of microbial inoculum for both the phyllosphere and rhizosphere (Bai et al. 2015). The overlap of soil and plant microbiota has been found in a variety of plants; for example, *Saccharum officinarum* (de Souza et al. 2016), *Boechera stricta* (Wagner et al. 2016), *Vitis vinifera* (Mezzasalma et al. 2018; Zarraonaindia et al. 2015), and the biofuel crops *Panicum virgatum* and *Miscanthus* × *giganteus* (Grady et al. 2019). This suggests that soil microbial

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communities can represent a major factor shaping plant microbiomes in both above- and belowground plant compartments. Furthermore, it has been shown that soil microbial community can influence the feeding behavior of insect herbivores (Badri et al. 2013). However, only one study, to our knowledge, has reported that soil microbial community can directly influence both the aboveground microbiota of plants (*Taraxacum officinale*) and the microbiota of an insect herbivore (*Mamestra brassicae*), showing overlap between the microbial communities of the insect and soil (Hannula et al. 2019). However, it was unclear whether the influence of soil microbiome on the caterpillar's microbiome was due to passive transfer (e.g., microbe dispersal when watering) or an active colonization mechanism (Hannula et al. 2019). Thus, there is potential for the influence of soil microbial communities on plant-associated microbial communities to extend beyond their plant host.

Plant species and genotype also contribute to the composition of multiple plant-associated microbiota (Turner et al. 2013). Plant microbial communities assemble differently in different organs, and their composition varies according to plant phylogeny (Dastogeer et al. 2020). Plant traits (tissue morphology and physicochemical properties) and resources might drive these plant-genotype effects on plant microbiota (Dastogeer et al. 2020; Fitzpatrick et al. 2018). For example, an analysis of 30 species of angiosperms revealed differences in the diversity and composition of root microbiomes across plant species (Fitzpatrick et al. 2018). Similarly, the rhizosphere microbiota of wheat, maize, tomato, and cucumber each had unique microbial communities (Ofek et al. 2014), and leaf and root microbial communities of *Agave* spp. clustered according to the host plant species (Coleman-Derr et al. 2016). Furthermore, several studies reported that host plant identity is an important factor in the assembly of insect herbivore-associated microbial communities (Colman et al. 2012; Malacrinò 2018). For example, host plant species influenced the composition of the microbiome associated with the herbivores *Ceratitis capitata* (Malacrinò et al. 2018) and *Thaumetopoea pytiocampa* (Strano et al. 2018). Therefore, plant species identity has an effect on the communities of microorganisms in the rhizosphere, living in the different plant organs, and even within plant herbivores.

Only a few studies have tested the effects of herbivory on plant microbiomes. For example, whitefly infestation of pepper plants led to an increased proportion of Gram-positive bacteria in the rhizosphere (Yang et al. 2011), and aphid herbivory on pepper plants increased the abundance of *Bacillus subtilis* and decreased that of the pathogen *Ralstonia solanacearum* in roots (Lee et al. 2012). Although whitefly herbivory shifted the rhizosphere microbiome composition in pepper plants (Kong et al. 2016), there was no effect of aphid herbivory on the rhizosphere microbiota of *Brassica oleracea* var. *capitata* (O'Brien et al. 2018). Herbivore attack may alter plant root exudates which, in turn, promote the assembly of beneficial rhizosphere microbiota (Hu et al. 2018; Rolfe et al. 2019). Thus, herbivory can alter plant and rhizosphere microbiomes but the relative impact of herbivory versus plant species or initial soil microbial diversity on plant-associated microbiomes has not been investigated.

To date, most studies have focused on one or two drivers of microbiome composition (e.g., soil microbiota, plant species, or herbivory) and, to the best of our knowledge, no study has tested the comparative strength of multiple biotic drivers expected to shape above- and belowground microbiomes in vivo. This represents a major gap in our understanding of the relative importance of factors determining microbiome assembly. Here, we ask how plant microbiome composition is shaped by three different major drivers of plant-associated microbial communities—soil microbial diversity, plant species, and herbivory—and whether they have equal impact on plant-associated microbiomes. By manipulating insect herbivory (presence or absence), plant species identity, and soil microbial diversity in a microcosm system, and by quantifying their effects on both bacterial and fungal plant-associated microbiome composition, we tested the relative strength of these three biotic factors in shaping rhizosphere, plant (root and shoot), and herbivore microbiomes. As outlined above, there is evidence of overlaps between soil and plant microbiota; thus, we hypothesize that soil microbial diversity is the major driver structuring plant microbiomes at different compartments. Previous studies reported that, within the same plant species, geographical location greatly contributed to assemble the plant microbiota (Christian et al. 2016; Lin et al. 2020). Similarly, there is evidence that soil microbial community can influence herbivores (Badri et al. 2013) and their microbiota (Hannula et al. 2019). Thus, we hypothesize that plant species and herbivory will impact plant-associated microbial communities but their magnitude would be lower than soil microbial diversity.

MATERIALS AND METHODS

Experimental design. In this study, we used a two-by-two-by-three factorial design to test our hypothesis. We grew two *Solanum* spp. (*Solanum tuberosum* and *S. vernei*) in soil with different microbial diversities: high diversity and low diversity (see below). To evaluate the effects of herbivory on plant and rhizosphere microbiota, we infested plants (within each soil by plant species combination) with two clonal lines of the polyphagous aphid species *Macrosiphum euphorbiae* (potato aphid); uninfested plants served as a control. Each treatment combination of plant species (n = 2), soil microbial diversity (n = 2), and aphid clonal line and presence or absence (n = 3) was replicated five times, involving 60 plants in total.

Study system. *S. tuberosum* (genotype TBR-5642) and *S. vernei* (genotype VRN-7630) seed were obtained from the Commonwealth Potato Collection at The James Hutton Institute (Dundee, Scotland, U.K.). Seed were germinated in steam-sterilized coir, then transplanted to the experimental pots after 3 weeks.

We used two aphid clones of *M. euphorbiae* (AK13/08 and AK13/18) previously collected in the field (James Hutton Institute, Dundee, U.K.; 56.457 N, 3.065 W) and reared for several generations on excised leaves of *S. tuberosum* 'Desirée' in ventilated cups at 20°C with a photoperiod of 16 h of light and 8 h of darkness.

All inoculum was prepared from soil collected from an uncultivated field at the James Hutton Institute (56.457 N, 3.065 W) (Bennett et al. 2016; Karley et al. 2017), sieved to 3 cm to remove rocks and large debris, and homogenized. The high-diversity inoculum consisted of whole soil, and half of the high-diversity inoculum was steam sterilized by autoclaving at 121°C for 3 h, allowing it to cool for 24 h, then autoclaving it again at 121°C for a further 3 h. The low-diversity inoculum was prepared by blending 50 ml of high-diversity inoculum with twice the volume of water, filtering the solution through a 38-μm sieve, and vacuum filtering the collected solution through a Whatman filter paper no. 1 (Bennett et al. 2011). Half of the filtrate was autoclaved at 121°C for 20 min. When preparing the experimental pots, those assigned to the high-diversity treatment were inoculated with high-diversity inoculum (whole soil) and autoclaved low-diversity inoculum (filtrate), while those assigned to the low-diversity treatment were inoculated with low-diversity inoculum and autoclaved high-diversity inoculum. This process allowed us to homogenize the abiotic inputs across our pots while varying the microbial community. For further details please see the section below and Supplementary Figure S2.

The filtration process eliminated larger soil microbes such as arbuscular mycorrhizal (AM) fungi from the low-diversity inoculum. We found significant differences in microbial phylogenetic diversity (see below) between high- and low-diversity inoculum (Faith's phylogenetic diversity index 38.31 ± 3.66 for high-diversity soil and 8.76 ± 5.94 for low-diversity soil; $F_{1,10} = 22.05$, $P < 0.001$), which

also translated into different microbial diversities in the rhizosphere (see Results below).

Microcosm setup. Experimental pots (1 liter) were assembled as depicted in Supplementary Figure S2. We added 100 ml of sterile background soil to the bottom and top of each pot to reduce the risk of microbial contamination between pots when watering. Sterile background soil was prepared by mixing sterilized loam (Keith Singleton) and sand (ratio 1:1), autoclaving this mixture at 121°C for 3 h, allowing it to cool for 24 h, and then autoclaving it again at 121°C for a further 3 h. Between the layers of sterile background soil, we added a mix of 100 ml of live or sterile high-diversity inoculum (10% of the pot volume) and 700 ml of sterile background soil. Pots assigned to the high-diversity treatment were filled with live high-diversity inoculum and received 1 ml of sterile low-diversity inoculum, while pots assigned to the low-diversity treatment were filled with sterile high-diversity inoculum and received 1 ml of live low-diversity inoculum. In this way, we controlled for physical and chemical differences between pots, which only differed in terms of their microbial community. One potato seedling was transplanted into each pot, and pots were randomized into two blocks and left to grow in an insect-screened greenhouse with an average temperature of 25°C and a photoperiod of 16 h of light and 8 h of darkness.

Five weeks after transplanting, two apterous adult aphids of *M. euphorbiae* clone AK13/08 were added to 20 plants, two apterous adult aphids of clone AK13/18 were added to 20 plants, and 20 plants were left uninfested. All plants were screened with a microperforated plastic bag (Sealed Air) that allowed transpiration while preventing aphid escape. Three weeks following infestation, we collected from each pot five aphids, leaves, roots, and rhizosphere soil (approximately 500 mg each) and stored them at −80°C. The five aphids were randomly collected from five different leaves on each plant. Leaf samples were also randomly collected from each plant, being careful to not sample from leaves infested by aphids. Rhizosphere soil was sampled from soil still adhering to the roots after removing loose soil, released by vigorously shaking the roots, and then sampled. Roots were carefully washed with tap water before collecting a random sample of root tissue. Aphid infestation was scored using a 0-to-5 scale of severity, where 0 = no aphids, 1 = between 1 and 250 aphids, 2 = between 251 and 500 aphids, 3 = between 501 and 750 aphids, 4 = between 751 and 1,000 aphids, and 5 = more than 1,000 aphids).

DNA extraction, Illumina MiSeq library preparation and sequencing. Samples were crushed in an extraction buffer (10 mM Tris, 100 mM NaCl, 10 mM EDTA, and 0.5% sodium dodecyl sulfate) using three 1-mm-diameter stainless steel beads per tube, with the aid of a bead mill homogenizer set at 30 Hz for 5 min (TissueLyzer II; Qiagen). Total DNA was extracted using phenol/chloroform, and it was subsequently checked for quantity and quality with a Nanodrop 2000 (Thermo Fisher Scientific Inc.). We conducted a metabarcoding analysis for both bacterial and fungal communities of leaves, roots, and rhizosphere soil, and bacterial communities of aphids. Bacterial communities were characterized by targeting the 16S ribosomal RNA (rRNA) gene with primer pair 515f/806rB (Caporaso et al. 2012). Fungal communities were analyzed by amplifying the fungal internal transcribed spacer 2 (ITS2) region of the rRNA with primer pair ITS3-KYO/ITS4 (Toju et al. 2012). Amplifications were also carried out on DNA extracted from soil inoculum and nontemplate controls, where the sample was replaced with nuclease-free water in order to account for possible contamination of instruments, reagents, and consumables used for DNA extraction (see Supplementary Material).

PCR assays were performed in a total volume of 25 µl, containing approximately 50 ng of DNA, 0.5 µM each primer, 1× KAPA HiFi HotStart ReadyMix (KAPA Biosystems). and nuclease-free water. Amplifications were performed in a Mastercycler Ep Gradient S (Eppendorf) set at 95°C for 3 min; 98°C for 30 s, 55°C for 30 s, and 72°C for 30 s repeated 35 times; and ending with 10 min of extension at 72°C. Reactions were carried out in technical triplicate, in order to reduce the stochastic variability during amplification (Schmidt et al. 2013), and a no-template control in which nuclease-free water (replacing target DNA) was utilized in all PCR assays (Supplementary Material). We also PCR tested all root samples for the presence of AM fungi using specific primers (Lee et al. 2008), finding the presence of AM fungi only in plants grown on high-diversity treatment soil.

Libraries were checked on agarose gel for successful amplification and purified with an Agencourt AMPure XP kit (Beckman and Coulter) using the supplier's instructions. A second short-run PCR was performed in order to ligate the Illumina i7 and i5 barcodes and adaptors following the supplier's protocol, and amplicons were purified again with an Agencourt AMPure XP kit. Libraries were then quantified using a Qubit spectrophotometer (Thermo Fisher Scientific Inc.), normalized using nuclease-free water, pooled together, and sequenced on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 300PE chemistry following the supplier's protocol.

Raw reads processing. Demultiplexed forward and reverse reads were merged using the PEAR 0.9.1 algorithm with default parameters (Zhang et al. 2014). Data handling was carried out using QIIME 1.9 (Caporaso et al. 2012), quality-filtering reads using default parameters, binning operational taxonomic units (OTUs) with a 97% cutoff, and discarding chimeric sequences using VSEARCH (Rognes et al. 2016). Singletons and OTUs coming from amplification of chloroplast DNA were discarded from the downstream analyses. Within the ITS2 dataset, all nonfungal OTUs were discarded using ITSx (Bengtsson-Palme et al. 2013). Taxonomy was assigned to each OTU through the BLAST method by querying the SILVA database (v. 132) for 16S (Quast et al. 2012) and UNITE database (v. 8.0) for ITS2 (Nilsson et al. 2019).

Data analysis. Data analysis was performed using R statistical software 3.5 (R Core Team 2013) with the packages *phyloseq* (McMurdie and Holmes 2013), *vegan* (Dixon 2003), and *picante* (Kembel et al. 2010).

Core microbiota. The core microbiota were identified separately for each compartment using the package *ampvis2* (Andersen et al. 2018), considering an OTU as a member of the core microbiota if it was retrieved at a relative abundance of >0.1% in more than 50% of samples. We also tested whether the number of core OTUs shared by pairs of compartments was greater or less than an overlap generated by random chance. To do so, for each compartment, we draw a group of random OTUs from all those identified in that compartment, in a quantity equal to the number of observed OTUs for that compartment (i.e., if we observed 10 core OTUs for a compartment, we drew 10 random OTUs from the pool of OTUs identified in that compartment). Then, we calculated the number of overlapping random OTUs between all pairs of compartments. We did this separately for bacteria and fungi, and we repeated it 10,000 times. Then, for each pair of compartments, we selected the highest number of random overlapping OTUs among the 10,000 permutations and we tested it against the number of observed overlapping OTUs for that pair of compartments using a χ^2 test.

Phylogenetic diversity. We selected Faith's phylogenetic diversity index (Faith 1992) to estimate the diversity of microbiotas in our system because, in contrast to other indices, it takes into account the phylogenetic relationship between taxa within the community. Comparison of diversity indices among groups was performed by fitting a linear mixed-effects model, separately for bacterial and fungal community, specifying compartment (i.e., rhizosphere soil, root, leaf, and aphid), soil treatment, plant species, and herbivory (and their

interactions) as fixed factors and aphid clonal line and block as random effects (Table 1). We also ran separate analyses for plants (rhizosphere soil, root, and leaf) and aphids, obtaining comparable results (Supplementary Tables S1 and S2). The use of aphid clonal line as a random variable in the mixed-effects model allowed for the control of differences in the performance of aphid clonal lines. Models were fitted using the *lmer*() function under the *lme4* package (Bates et al. 2015) and the package *emmeans* was used to infer pairwise contrasts (corrected using false discovery rate [FDR]).

Community structure. We analyzed the effects of treatment factors (compartment, soil treatment, plant species, herbivory, and their interactions) on the structure of the microbial communities using a multivariate approach. Distances between pairs of samples, in terms of community composition, were calculated using a unweighted Unifrac matrix, then visualized using a canonical analysis of principal coordinates procedure (Anderson and Willis 2003). Differences between sample groups were inferred through permutational multivariate analysis of variance (PERMANOVA) (999 permutations stratified at the level of block and aphid clonal line). The use of aphid clonal line for stratification in PERMANOVA allowed for the control of differences in the performance of aphid clonal lines. We also ran separate analyses for plant (rhizosphere soil, root, and leaf) and aphids, obtaining comparable results (Supplementary Tables S1 and S2).

Soil diversity versus plant species versus herbivory driven effects: Which is strongest? We assessed the impact of soil treatment, plant species, and herbivory for each OTU using the R package *DESeq2* (Love et al. 2014). Using this package, we calculated the effect of each factor (herbivory, plant species, and soil microbial diversity) on the abundance of OTU (expressed as absolute $\log_2$ fold changes) in each plant compartment. We first built a model using compartment (leaves, roots, and rhizosphere), soil treatment, herbivory, and plant species as factors. Then, we extracted the appropriate contrasts (low versus high diversity for soil treatment, *S. vernei* versus *S. tuberosum* for plant species, and herbivore versus no herbivore for herbivore treatment) for each compartment (leaves, roots, and rhizosphere). From each contrast, we used the absolute $\log_2$ fold-change values (*ashr* shrinked) (Stephens 2017) for each OTU to quantify the impact of soil, plant, and herbivore treatments on the microbiota in each compartment. Comparisons of absolute $\log_2$ fold-change values were performed by fitting a linear mixed-effects model, specifying compartment, treatment (herbivory, plant, or soil), and their interaction as fixed factors and OTU identity as a random effect, and using the package *emmeans* to infer contrasts (FDR corrected).

Aphid infestation. We tested whether the aphid infestation levels were influenced by soil microbial diversity by fitting a cumulative link mixed model using the *ordinal* R package (Christensen 2015), specifying soil treatment, plant species, and their interaction as fixed factors and block and aphid clonal line as a random effect.

RESULTS

Dataset summary and community composition. Overall, we identified 43,879 bacterial and 4,713 fungal OTUs. The analysis of the core microbiota resulted in identifying 150 core bacterial OTUs and 26 fungal OTUs (Fig. 1). We identified 81 bacterial and 19 fungal OTUs as the core microbiota of rhizosphere soil, where the bacterial community was largely dominated by uncultured taxa (50.6%), *Ramlibacter* (10.1%), *Pseudomonas* (9.1%), *Massilia* (8.6%), and *Chitinophaga* (6.1%), while the fungal community was mostly represented by *Peziza* (44.9%), unidentified fungi (18.1%), *Humicola* (9.1%), *Mortierella* (8.8%), *Penicillium* (8.6%), *Mucor* (5.5%), and *Trichoderma* (4.9%). The core microbiota of roots were represented by 74 bacterial and 16 fungal OTUs. Root tissues were mainly associated with *Flavobacterium* (39.4%) and uncultured bacterial taxa

TABLE 1
Models testing the effect of compartment (Cp) (aphids, leaves, roots, and rhizosphere soil), soil treatment (S) (high diversity and low diversity), plant species (P) (*Solanum tuberosum* and *S. vernei*), herbivory (H) (infested and control), and their interaction on the phylogenetic diversity (linear mixed-effect model) and taxonomical structure (permutational multivariate analysis of variance [PERMANOVA]) of bacterial and fungal communities[a]

	Bacterial community					Fungal community				
		Phylogenetic diversity		PERMANOVA			Phylogenetic diversity		PERMANOVA	
Factors	df	χ^2	*P*	*F*	*P*	df	χ^2	*P*	*F*	*P*
Cp	3	843.62	**<0.001**	23.39	**0.001**	2	895.91	**<0.001**	12.98	**0.001**
S	1	2.19	0.13	13.26	**0.001**	1	225.62	**<0.001**	14.91	**0.001**
P	1	18.11	**<0.001**	2.37	**0.001**	1	10.67	**<0.01**	1.96	**0.003**
H	1	34.66	**<0.001**	2.52	**0.001**	1	47.21	**<0.001**	3.79	**0.001**
Cp × S	3	37.23	**<0.001**	5.67	**0.001**	2	252.44	**<0.001**	5.72	**0.001**
Cp × P	3	8.18	**0.04**	1.51	**0.005**	2	1.66	0.43	1.31	**0.03**
S × P	1	23.6	**<0.001**	1.69	**0.025**	1	3.74	0.05	1.70	**0.014**
Cp × H	2	16.51	**<0.001**	1.61	**0.007**	2	20.7	**<0.001**	2.03	**0.001**
S × H	1	0.01	0.9	1.25	0.11	1	5.61	**0.01**	1.25	0.1
P × H	1	2.26	0.13	1.17	0.18	1	0.16	0.68	1.14	0.21
Cp × S × P	3	12.57	**<0.01**	1.42	**0.005**	2	0.28	0.86	1.34	**0.029**
Cp × S × H	2	6.86	**0.03**	1.28	0.07	2	12.38	**<0.01**	1.11	0.21
Cp × P × H	2	1.9	0.38	1.04	0.31	2	1.18	0.55	1.01	0.4
S × P × H	1	2.59	0.1	1.13	0.20	1	1.33	0.24	1.17	0.15
Cp × S × P × H	2	0.25	0.87	1.05	0.29	2	0.84	0.65	1.12	0.18

[a] Values in bold represent $P < 0.05$.

(23.7%), while *Peziza* (22.9%), uncultured taxa (18.1%), and *Fusarium* (11.1%) dominated the fungal community. Leaf core microbiota were represented by 20 bacterial and 6 fungal OTUs, with a higher abundance of *Stenotrophomonas* (24.1%), '*Candidatus* Hamiltonella' (20.9%), *Flavobacterium* (10.5%), and *Pedobacter* (9.4%), while fungi were mainly represented by *Cladosporium* (42.3%), *Penicillium* (34.3%), and *Peziza* (13.6%). Aphids were mainly associated with *Buchnera* (64.1%) and '*Candidatus* Hamiltonella' (35.8%). We identified five bacterial OTUs (two OTUs of *Flavobacterium* plus *Citrobacter*, *Pedobacter*, and *Terrimonas*) shared between all compartments, and four fungal OTUs (*Cladosporium*, *Humicola*, *Penicillium*, and *Peziza*) shared between leaves, roots, and the rhizosphere. For a more detailed analysis of the core microbiota, please see the Supplementary Material.

To gain understanding of the overlap of core OTUs between compartments, we tested the number of observed core OTUs overlapping between two compartments against the number of OTUs overlapping between two compartments due to random sampling. We found that the overlap of bacterial core OTUs between pairs of plant compartments was always greater than random chance ($P < 0.05$). Aphids also showed a higher number of shared bacterial core OTUs with leaves than random chance (eight OTUs, $\chi^2 = 5.4$; $P = 0.01$) but not with roots (seven OTUs, $\chi^2 = 2.7$; $P = 0.09$) and rhizosphere soil (five OTUs, $\chi^2 = 2$; $P = 0.15$). In fungi, we saw that the OTUs shared between leaves and roots ($\chi^2 = 1.3$; $P = 0.25$) or rhizosphere soil ($\chi^2 = 0.5$; $P = 0.47$) were not different than those shared by chance, while OTUs shared between roots and rhizosphere soil were greater than those shared by chance ($\chi^2 = 6.2$; $P = 0.01$).

Phylogenetic diversity. For bacterial communities, we found a significant compartment–soil treatment–plant species interaction (Table 1). In all plant compartments (leaves, roots, and rhizosphere), we found a higher phylogenetic diversity in *S. vernei* than in *S. tuberosum* when plants were grown on a low-diversity soil treatment, and no differences between the two plant species were found when plants were grown on a high-diversity soil treatment (Supplementary Table S3). Plant species did not influence aphid bacterial diversity when they were exposed to plants grown on high- or low-diversity soil treatment (Supplementary Table S3). In fungal communities, we found a significant effect of the factor plant species, reporting a higher diversity in *S. vernei* than *S. tuberosum* plants ($P = 0.001$) (Table 1), although we did not find any significant interaction with other factors.

We found a significant compartment–soil treatment–herbivory interaction in both bacterial and fungal communities. Posthoc contrasts show a higher leaf bacterial diversity in aphid-infested plants compared with uninfested control plants when they were grown on a low-diversity soil treatment (Supplementary Table S4). Root bacterial and fungal communities, in both soil treatments, had higher diversity values in infested plants compared with uninfested control plants (Supplementary Table S4). In the rhizosphere, we observed differences between infested and uninfested plants in both bacterial and fungal community diversity of plants grown on a high-diversity soil treatment, whereas this difference was found only in the fungal community of plants grown on a low-diversity soil treatment (Supplementary Table S4).

We found phylogenetic diversity of the aphid microbiota was highest in the low-diversity treatment (Supplementary Fig. S3), which mirrored differences in aphid infestation levels ($\chi^2 = 8.19$, df = 1, $P = 0.004$; mean infestation scores = 3.10 ± 0.23 for high-diversity soil and 2.45 ± 0.28 for low-diversity soil).

Microbial community composition. The multivariate analysis (i.e., the PERMANOVA) reported a significant compartment–soil treatment–plant species interaction (Table 1). Posthoc contrasts showed differences between *S. vernei* and *S. tuberosum* in the structure of leaf, root, and rhizosphere bacterial and fungal communities when plants were grown on low-diversity soil treatments (Fig. 2A to F; Supplementary Table S5). In the high-diversity soil treatment, only root fungal communities differed between *S. vernei* and *S. tuberosum* (Fig. 2E; Supplementary Table S5). On the other hand, differences between low-diversity and high-diversity treatments were found in both *S. vernei* and *S. tuberosum* in root and rhizosphere communities, both bacterial (Fig. 2B and C) and fungal (Fig. 2E and F) (Supplementary Table S6). In leaves, differences between soil treatments were found only in the bacterial community of *S. vernei* (Fig. 2A; Supplementary Table S6). We also found a significant compartment–herbivory interaction (Table 1), with herbivory influencing bacterial communities in all compartments but fungal community only in leaves and roots (Table 2). Our multivariate

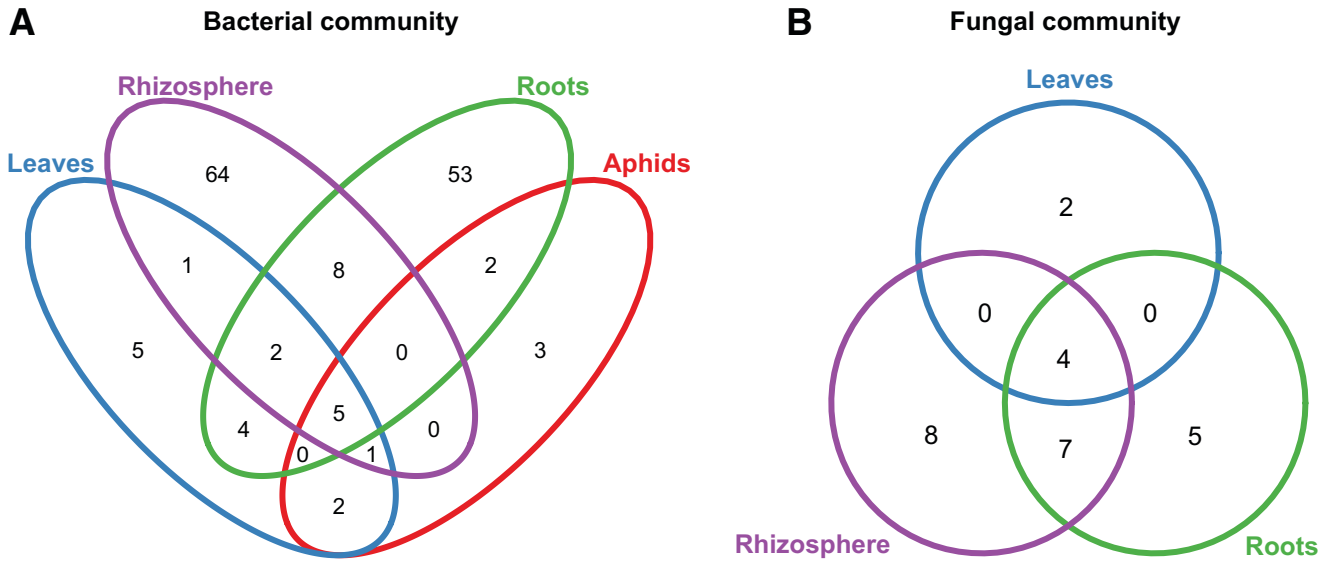

Fig. 1. Core **A,** bacterial and **B,** fungal operational taxonomic units (OTUs) shared between compartments. Core OTUs were identified among those with relative abundance of >0.1% in more than 50% of samples.

analysis demonstrated that the strongest driver of rhizosphere, root, and leaf microbial community structure was soil diversity treatment (Table 2) for both bacterial and fungal communities. Indeed, bacterial and fungal communities responded to soil diversity treatment and plant species across all compartments (roots, rhizosphere, and leaves) (Table 2; Fig. 2A to F). Based on the variation explained by each factor included in the model, soil microbial diversity was the most important factor shaping the microcosm's microbiota in all compartments (Table 2). The variation explained by soil microbial diversity tended to decrease when moving across compartments from rhizosphere to leaves and aphids (Table 2).

Soil diversity versus plant species versus herbivory driven effects. Which is strongest? We answered this question in two ways, focusing on the single factors included in our design (soil treatment, plant species, and herbivory). First, as discussed above, using the variation explained by each of our predictor variables in our PERMANOVA model, we determined that the predictor that explained the most variation was the initial soil community diversity. Soil diversity, plant species, and herbivory influenced bacterial and fungal assemblies in our system. Soil treatment explained approximately 30% (rhizosphere), approximately 20% (root), and approximately 7% (leaf) of variation in microbiome community composition (Table 2). However, the variance in community composition explained by both plant species and herbivory (3 to 5%) was always lower than the variance explained by the soil treatment. Furthermore, soil treatment explained approximately 8% of variation in aphid microbiota (Table 2). This suggests that the soil-driven effect is stronger than the other effects in our system.

Second, to investigate in more detail which factor (soil, plant, or herbivore) had a stronger influence on plant microbiome composition, we examined the magnitude of change in abundance for each OTU (absolute $\log_2$ fold changes) in relation to soil treatment, plant species,

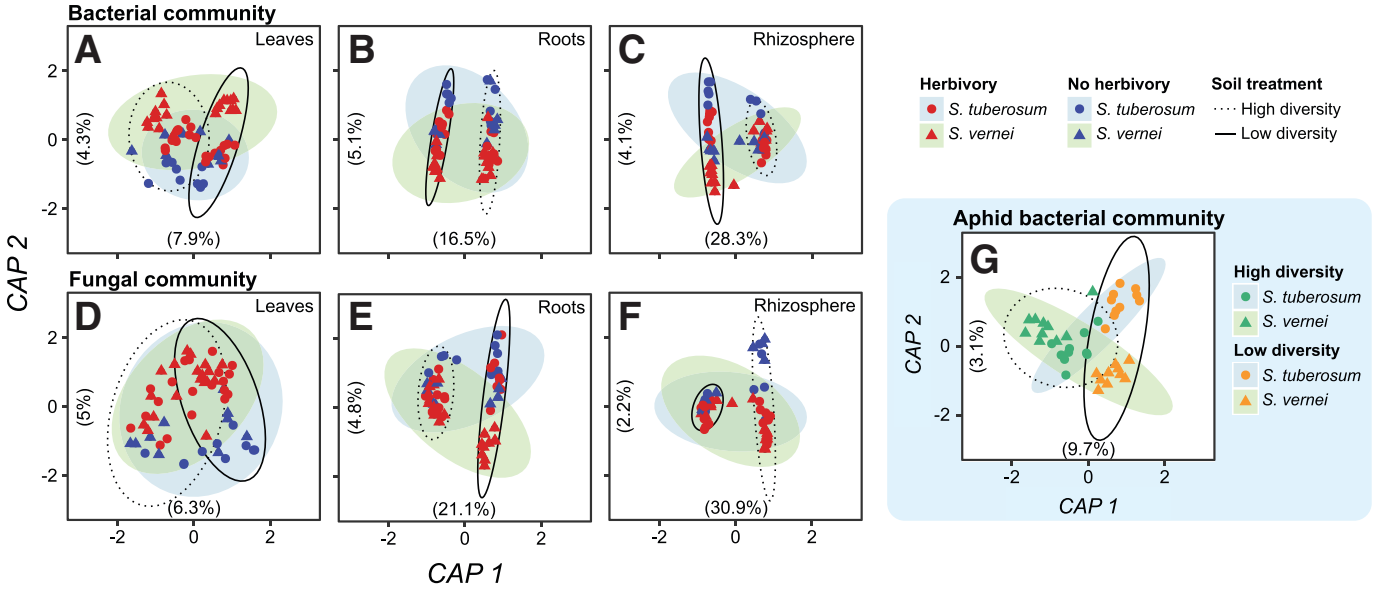

Fig. 2. Canonical analysis of principal coordinates (CAP) analysis of bacterial and fungal communities Unifrac distance matrix for each compartment. We report the response of **A to C,** bacterial and **D to F,** fungal communities to soil microbial diversity, plant species, and herbivory in leaves (A and D), roots (B and E), and rhizosphere soil (F and F). **G,** The aphid bacterial community responded to both plant species and soil microbial diversity. For each graph, percentages in parentheses inside each graph along the axes report the variance explained by the respective axis.

TABLE 2
Analysis of the effects of soil treatment (high diversity and low diversity), plant species (*Solanum tuberosum* and *S. vernei*), and herbivory (infested and control) on the bacterial and fungal community taxonomical structure for each compartment (aphids, leaves, roots, and rhizosphere) performed through permutational multivariate analysis of variance (Unifrac distance matrix)[a]

	Aphids		Leaves		Roots		Rhizosphere	
Communities	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
Bacterial community								
Soil treatment	0.08	**<0.01**	0.07	**<0.01**	0.16	**<0.01**	0.28	**<0.01**
Plant species	0.03	**0.01**	0.03	**<0.01**	0.03	**<0.01**	0.02	**0.04**
Herbivory	–	–	0.03	**<0.01**	0.04	**<0.01**	0.02	**0.04**
Fungal community								
Soil treatment	–	–	0.06	**<0.01**	0.21	**<0.01**	0.31	**<0.01**
Plant species	–	–	0.02	0.26	0.03	**<0.01**	0.02	0.17
Herbivory	–	–	0.05	**<0.01**	0.03	**0.01**	0.02	0.07

[a] Analyses were run separately for each compartment within each community. Values in bold represent $P < 0.05$.

and herbivory. For both bacterial and fungal communities, and in all compartments, the changes produced by soil treatment were greater than those produced by herbivory and plant species ($\chi^2_{bacteria}$ = 23,331.3 and χ^2_{fungi} = 1,055, df = 2, $P < 0.001$) (Fig. 3), with the only exception being the leaf fungal community, where no differences were found between the three factors (Fig. 3B). Also, in all cases, there was no difference between the changes produced by herbivory and those produced by plant species (Fig. 3). The analysis of changes in the abundance of OTUs in aphids revealed that soil diversity treatment had a greater influence than plant species in shaping aphid bacterial communities ($\chi^2 = 766.8$, df = 1, $P < 0.001$; absolute [$\log_2$ fold change]$_{soil}$ = 1.7 ± 0.1 and absolute [$\log_2$ fold change]$_{plant}$ = 0.37 ± 0.1). Collectively, these results demonstrate that the strongest effect on microbial taxa in the rhizosphere, roots, shoots, and aphid herbivores is driven by the initial soil community diversity.

DISCUSSION

Here, we test the influence of multiple drivers (and their interaction) on plant microbiome diversity and composition, and we show that, of all the drivers tested, soil microbial diversity had the greatest influence on the microbial community composition of rhizosphere, roots, leaves, and even aphid herbivores. Thus, we correctly hypothesized that soil microbial diversity drives changes in plant and herbivore microbiota and that this effect would be much stronger than plant species or herbivory. This influence of soil microbial diversity correlated with aphid abundance on infested plants. Furthermore, we showed that herbivory and plant species also affect the microbiome community composition of leaves, roots, and rhizosphere but their effects are weaker than those driven by soil diversity. We also observed that the response of plant microbiome to herbivory or plant species differs according to soil treatment.

Plant and insect microbial diversity was influenced by soil microbiota and interactions between soil microbiota and plant species and herbivory. When we quantified the relative contribution of the main effects (soil, plant species, and herbivory), soil treatment was the strongest driver of plant-related microbiota composition. Soil community composition is well known to influence plant microbial composition (Bai et al. 2015; Bulgarelli et al. 2012; de Souza et al. 2016; Grady et al. 2019; Mezzasalma et al. 2018; Schlaeppi et al. 2014; Wagner et al. 2016; Zarraonaindia et al. 2015) but, here, we reveal that soil produces a stronger effect when compared with other factors (i.e., plant species and herbivory). Soil represents a major reservoir of microbial inoculum for plants (Trivedi et al. 2020; Zarraonaindia et al. 2015), especially belowground. However, we also observed that the effect driven by soil is a function of plant species, likely because the intrinsic characteristics of each plant species can modulate specific changes in the diversity and composition of microbial communities at each compartment (Dastogeer et al. 2020; Turner et al. 2013).

Interestingly, we found that soil microbial diversity influences the phylogenetic diversity and structure of aphid microbiota. The influence of the soil microbial diversity on aphid bacterial communities and aphid infestation level could potentially be explained through two, nonmutually exclusive, mechanisms: (i) translocation of microbes from the rhizosphere through or on the plant and (ii) changes in plant physiology or metabolome. Although leaves are physically separated from roots, their microbiomes can still interact at interfaces such as the stem, and microbial translocation could occur due to active and passive mechanisms (Bai et al. 2015; Wagner et al. 2016). A recent study comparing the microbiota of caterpillars feeding on detached leaves and intact plants found the microbiota of caterpillars that fed on intact plants had a community composition similar to the soil microbiota (Hannula et al. 2019), suggesting direct (splashing of soil microbiota on leaves) or indirect (movement through the plant) microbial translocation. However, our data do not show this pattern, because the core microbiome belowground is different from shoots and herbivores, few OTUs are common to all compartments in the system, and the overlap of core OTUs between aphids and belowground compartments is not greater than random chance. The aphids in our system employ a different feeding strategy (sap feeding) compared with the caterpillars (chewing) in the previous study (Hannula et al. 2019), and chewing herbivores may have an increased likelihood of environmental uptake of microbes (Paniagua Voirol et al. 2018). Thus, we find it unlikely that microbes in our system were translocated through or on the plant to the herbivore.

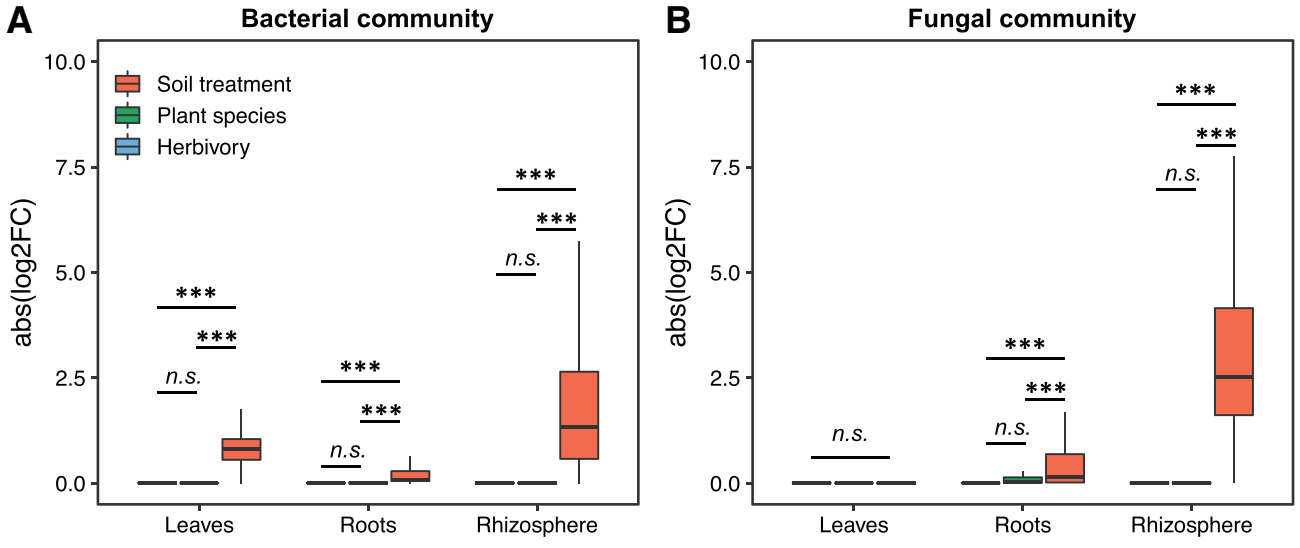

Fig. 3. Magnitude of changes in abundance for each operational taxonomic unit (OTU) (absolute $\log_2$ fold changes [FC]). For each compartment (leaves, roots, and rhizosphere), we investigated the response of single OTUs to soil microbial diversity (red), plant species (green), and herbivory (blue) for **A,** bacterial and **B,** fungal communities. Comparisons were tested using a linear mixed-effect model, and contrasts were extracted using the function *emmeans*. Asterisks (***) indicate $P < 0.001$ and n.s. = not significant.

The second potential mechanism is through changes in plant physiology. Many soil microbes are able to modulate plant nutrient intake or prime plant defenses (Martinez-Medina et al. 2016). The composition of belowground microbial communities can alter plant metabolism (Li et al. 2019) which, in turn, influences herbivore fitness (Mason et al. 2019). Our low-diversity treatment lacked large microbes, including AM fungi, a group well known to prime plant defenses (Bennett et al. 2018; Jung et al. 2012), although aphids are less susceptible to changes in defenses primed by AM fungi (Koricheva et al. 2009). Thus, the changes we observed in the aphid microbiome could be due to changes in host plant physiology and metabolome; for example, triggering of plant defenses, which has been shown to decrease the diversity of plant-associated microbial communities (Kniskern et al. 2007). The higher aphid abundance on plants grown in the high-diversity soil provides indirect evidence for such changes in plant biochemical composition, as does a previous study showing increased aphid suitability as a host for parasitoids when feeding on *Solanum* plants grown with AM fungi from the same site (Bennett et al. 2016). Although no previous study found a relationship between soil microbial diversity and aphid infestation, previous studies show that soil microbiota can influence insect feeding behavior through changes in the plant metabolome (Badri et al. 2013), which can represent the likely mechanism driving our observations.

Although a clear consumer-driven effect was observed on the plant microbiome in our study, it was a weaker effect than soil microbial diversity. Thus, herbivory plays a less significant role in determining plant microbiome composition. The herbivory-driven effect on the bacterial community composition of the roots and rhizosphere could be driven by changes in plant physiology (e.g., defense activation or carbon metabolism) and root exudation due to aphid feeding activity (Hoysted et al. 2018; Züst and Agrawal 2016). Herbivory has been shown to alter the types of organic compounds released at the root surface, leading to changes in the composition of rhizosphere microbial communities (Hu et al. 2018; Lareen et al. 2016). Previous research has shown that *Bemisia tabaci* (whitefly) herbivory can alter the rhizosphere microbiome of pepper plants (Kong et al. 2016), and artificial induction of plant defenses (Hein et al. 2008), or their deactivation (Kniskern et al. 2007), has been shown to shape rhizosphere microbial communities. In our study, we focused on a sap-feeding insect, which is known to trigger specific physiological responses in plants. Thus, further research is needed to test whether different herbivores with different feeding strategies are able to drive changes in the microbiota thriving in different plant compartments.

Plant species was also a predictor shaping the microbiome community composition of the rhizosphere, plants, and herbivores in our study. Plant species is known to be a strong driver of root and rhizosphere microbiota because plant species differ in morphology, chemistry, and relationship with microorganisms (Trivedi et al. 2020). *Solanum* plants are known to produce toxic glycoalkaloids as a chemical defense against herbivory (Altesor et al. 2014), and these compounds could also shape the microbiota associated with the plant. The strength of this effect might be context dependent due to the interaction with the soil microbial composition (Bulgarelli et al. 2013; Fierer 2017). This might explain why we observed a greater impact of soil treatment than plant species in the belowground microbiotas in our study. Also, it might explain why we found differences in the bacterial community between plant species in only one soil treatment. It has been previously shown that soil represents a reservoir for leaf microbial communities and that phyllosphere habitat selects for specific members (Grady et al. 2019), which partially explains our observation that the impact of soil microbial diversity was greater than plant species on the phyllosphere bacterial and fungal communities. The differences in the microbiome composition of aphids feeding and reproducing on the two different plant species is not surprising, because it is well known that the identity of the host plant is a major factor in shaping insect-associated microbial community composition (Colman et al. 2012; Malacrinò 2018). The fact that seed were not surface sterilized might be a caveat of our study, potentially influencing the structure of plant microbiome differently for each species. However, previous studies show that even surface-sterilized seed show differences in their endophytic microbiome according to the plant genotype (Liu et al. 2020; Raj et al. 2019; Walitang et al. 2018), and our results show a major effect driven by soil microbial community composition; thus, we are confident that our results were not biased by not surface sterilizing seed.

By quantifying and comparing the relative strength of multiple biotic drivers of microbial community composition, our work contributes to a more comprehensive understanding of the factors determining the outcome of plant–microbe–insect interactions, and how plant-associated microbiomes assemble and respond to resource- and consumer-driven effects. Thus, if understood and managed correctly, these interactions have the potential to be applied in natural and managed systems to improve food security and safety, or the success of ecological restoration efforts.

Availability of data. Raw data is available at the NCBI Sequence Read Archive database under accession number PRJNA557499.

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