Soil microbial diversity impacts plant microbiomes 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, for the first time, we show interactions between plant-associated microbial communities that drive their diversity and community composition. We manipulated soil microbial diversity, plant species, and herbivory, and found that soil microbial diversity influenced the herbivore-associated microbiome composition, but also plant species and herbivory influenced the soil microbiome composition. We used a novel approach, quantifying 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

Rhizosphere; phyllosphere; bacteria; fungi; metabarcoding

Background

Microbiomes can be considered an extension of the plant genome [1–4]. While 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 [5,6]. In addition, research has rarely examined or compared multiple drivers, and 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. Plant genotype and developmental stage have been shown to influence the composition of both plant and soil microbiomes [7]. Soil microbiome composition has also been shown to shape plant microbiome composition [5], and plant pathogens and herbivory produce compositional shifts in plant-associated microbial communities [8]. 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 [9]. While different plant tissues can develop distinct microbiomes, soil provides an important reservoir of microbial inoculum for both the phyllosphere and rhizosphere [10]. Work on *Arabidopsis* suggests soil type is a major driver structuring plant microbiomes [10,11]. The overlap of soil and plant microbiota has been found in a variety of plants, for example *Saccharum officinarum* [12], *Boechera stricta* [7], *Vitis vinifera* [13,14] and in the biofuel crops *Panicum virgatum* and *Miscanthus x giganteus* [15]. Thus, soil microbial communities can alter plant microbiomes in above and belowground plant compartments.

Only one study to our knowledge has reported that soil microbial community can directly influence both the aboveground microbiome of plants (*Taraxacum officinale*) and an insect herbivore (*Mamestra brassicae*), showing overlap between the microbial communities of the insect and soil [16]. 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 colonisation mechanism [16]. Thus, there is potential for the influence of soil microbial communities on plant-associated microbial communities to extend beyond their plant host.

Plant species identity also contributes to the composition of multiple plant-associated compartments including insect herbivores, plant organs, and rhizosphere microbiomes. Indeed, several studies reported that identity of the host plant is an important factor in the assembly of insect-associated microbial communities [17,18]. For example, host plant species influenced the composition of the microbiome associated with *Ceratitis capitata* [19] and *Thaumetopoea pytiocampa* [20]. The microbial community thriving on leaves and roots of different *Agave* species was found to cluster according to the host plant [21]. Also, the analysis of 30 species of angiosperm revealed differences in the diversity and composition of root microbiomes across plant species [22]. Similarly, the rhizosphere microbiota of wheat, maize, tomato and cucumber was characterized by different taxonomical compositions, unique to each plant species [23]. Therefore, plant species 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 [24], and aphid herbivory on pepper plants increased the abundance of *Bacillus subtilis* and decreased that of the pathogen *Ralstonia solanacearum* in roots [25]. While whitefly herbivory shifted the rhizosphere microbiome composition in pepper plants [26], there was no effect of aphid herbivory on the rhizosphere microbiota of *Brassica oleracea* var. *capitata* [27]. Clearly, herbivory

can alter plant and rhizosphere microbiomes, but the relative impact of herbivory versus plant species or initial soil 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 no study has tested the strength and directionality of multiple drivers expected to shape microbiomes *in vivo*. This represents a major gap in our understanding of the relative importance of factors determining microbiome assembly. Here we ask, for the first time, how plant microbiome composition is shaped by three different major drivers of plant-associated microbial communities — plant species, soil microbiome diversity and herbivory — and whether they have equal impact on plant-associated microbiomes. By manipulating insect herbivory (presence/absence), plant species identity and soil microbial diversity in a microcosm system, and quantifying their effects on both bacterial and fungal plant-associated microbiome composition, we generate novel data on the relative strength of these three factors in shaping rhizosphere, plant (root and shoot), and herbivore microbiomes. We hypothesize that soil microbial diversity drives changes in rhizosphere, plant and herbivore microbiota, but this effect is modulated by plant species and herbivory.

Methods

Experimental design

In this study, we used a 2 × 2 × 3 factorial design to test our hypothesis. We grew two *Solanum* species (*Solanum tuberosum* and *Solanum 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' × '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/absence (n=3) was replicated five times, involving 60 plants in total.

Study System

Solanum tuberosum (genotype TBR-5642) and *Solanum vernei* (genotype VRN-7630) seeds were obtained from the Commonwealth Potato Collection at The James Hutton Institute (Dundee, Scotland, UK). Seeds were germinated in steam-sterilized coir, and 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, UK — 56.457 N, 3.065 W) and reared for several generations on excised leaves of *Solanum tuberosum* (cv. Desirée) in ventilated cups at 20 °C and 16:8 h (light:dark).

Sterile background soil was prepared by mixing Sterilized Loam (Keith Singleton, Cumbria, UK) 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.

All inoculum was prepared from soil collected from an uncultivated field at the James Hutton Institute (56.457 N, 3.065 W) [28,29], sieved to 3 cm to remove rocks and large debris, and homogenized. The high diversity inocula consisted of whole soil, and half of the high diversity inocula was steam sterilized in the same manner as the sterile background soil. The low diversity inoculum was prepared by blending 50 ml of high diversity inocula 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). 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 inocula

($F_{1, 10}$ = 22.05, $P < 0.001$; 38.31±3.66 for high diversity soil, 8.76±5.94 for low diversity soil) which also translated into different microbial diversities in the rhizosphere (see Results below). Half of the filtrate was autoclaved at 121°C for 20 min.

Microcosm setup

Experimental pots (1 L) were assembled as follows. 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. Between the layers of sterile background soil we added a mix of 100 ml live or sterile high diversity inocula (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 inocula and received 1 ml of sterile low diversity inoculum, while pots assigned to the low diversity treatment were filled with sterile high diversity inocula 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, pots were randomized into two blocks, and left to grow in an insect-screened greenhouse with an average temperature of 25 °C and 16:8 h (light:dark) photoperiod.

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, UK) that allowed transpiration while preventing aphid escape. Three weeks following infestation, we collected from each pot five aphids, leaves, roots and rhizosphere soil (≈500 mg each), and stored them at -80 °C. Aphid infestation was scored using a 0–5 scale of severity (scale 0 = no aphids, scale 1 = between 1 and 250 aphids, scale 2 = between 251 and 500 aphids, scale 3 = between 501 and 750 aphids, scale 4 = between 751 and 1,000 aphids, scale 5 = more than 1,000 aphids).

DNA extraction, Illumina Miseq libraries preparation and sequencing

Samples were crushed in an extraction buffer (10 mM Tris, 100 mM NaCl, 10 mM EDTA, 0.5% SDS) using three 1 mm Ø stainless steel beads per tube, with the aid of a bead mill homogenizer set at 30 Hz for 5 min (TissueLyzer II, Qiagen, UK). Total DNA was extracted using phenol/chloroform, and it was subsequently checked for quantity and quality with a Nanodrop 2000 (Thermo Fisher Scientific Inc., USA). 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 rRNA gene with primers 515f/806rB [30]. Fungal communities were analysed by amplifying the fungal ITS2 region of the rRNA with primers ITS3-KYO/ITS4 [31]. Amplifications were also carried out on DNA extracted from soil inoculum, and non-template 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 Results 1).

PCR reactions were performed in a total volume of 25 µl, containing about 50 ng of DNA, 0.5 µM of each primer, 1X KAPA HiFi HotStart ReadyMix (KAPA Biosystems, USA) and nuclease-free water. Amplifications were performed in a Mastercycler Ep Gradient S (Eppendorf, Germany) set at 95 °C for 3 minutes, 98 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, repeated 35 times, and ended with 10 minutes of extension at 72 °C. Reactions were carried out in technical triplicate, in order to reduce the stochastic variability during amplification [32], and a no-template control in which nuclease-free water replaced target DNA was utilized in all PCR reactions (Supplementary Results 1). We also PCR-tested all root samples for the presence of AM fungi using specific primers [33], finding presence of AM fungi just in plants grown on high-diversity treatment soil.

175 Libraries were checked on agarose gel for successful amplification and purified with
176 Agencourt AMPure XP kit (Beckman and Coulter, CA, USA) using the supplier's instructions. A second
177 short-run PCR was performed in order to ligate the Illumina i7 and i5 barcodes and adaptors
178 following the supplier's protocol, and amplicons were purified again with Agencourt AMPure XP kit.
179 Libraries were then quantified through Qubit spectrophotometer (Thermo Fisher Scientific Inc.,
180 USA), normalized using nuclease-free water, pooled together and sequenced on an Illumina MiSeq
181 platform using the MiSeq Reagent Kit v3 300PE chemistry following the supplier's protocol.
182
183 *Raw reads processing*
184 De-multiplexed forward and reverse reads were merged using PEAR 0.9.1 algorithm using
185 default parameters [34]. Data handling was carried out using QIIME 1.9 [30], quality-filtering reads
186 using default parameters, binning OTUs using open-reference OTU-picking through the UCLUST
187 algorithm, and discarding chimeric sequences discovered with USEARCH 6.1. Singletons and OTUs
188 coming from amplification of chloroplast DNA were discarded from the downstream analyses.
189 Within the ITS2 dataset, all non-fungal OTUs were discarded using ITSx [35]. Taxonomy was assigned
190 to each OTU through the BLAST method by querying the SILVA database (v. 132) for 16S [36], and
191 UNITE database (v. 8.0) for ITS2 [37].
192
193 *Data analysis*
194 Data analysis was performed using R statistical software 3.5 [38] with the packages *phyloseq*
195 [39], *vegan* [40] and *picante* [41]. To test our hypotheses, we performed two analyses on the
196 metabarcoding data: phylogenetic diversity and community structure.
197 *Phylogenetic diversity.* We calculated Faith's phylogenetic diversity [42] which determines
198 the diversity of the microbial community taking into account the phylogenetic relationship between
199 taxa within the community. Comparison of diversity indices among groups was performed by fitting
200 a linear mixed-effects model, separately for bacterial and fungal community, specifying
201 *compartment* (i.e. soil, root, leaf, aphid), *soil treatment*, *plant species*, *herbivory* (and their
202 interactions) as fixed factors and *aphid clonal line* and *block* as a random effect (Table 1). The use of
203 *aphid clonal line* as a random variable in the mixed-effects model allowed for the control of
204 differences in the performance of aphid clonal lines. Models were fitted using the *lmer*() function
205 under the *lme4* package [43] and the package *emmeans* was used to infer pairwise contrasts
206 (corrected using False Discovery Rate, FDR).
207 *Community structure.* We analyzed the effects of treatment factors (*compartment*, *soil*
208 *treatment*, *plant species*, *herbivory* and their interactions) on the structure of the microbial
209 communities using a multivariate approach. Distances between pairs of samples, in terms of
210 community composition, were calculated using a Unifrac matrix, and then visualized using a
211 Canonical Analysis of Principal Coordinates (CAP) procedure [44]. Differences between sample
212 groups were inferred through PERMANOVA multivariate analysis (999 permutations stratified at the
213 level of *block* and *aphid clonal line*). The use of *aphid clonal line* for stratification in PERMANOVA
214 allowed for the control of differences in the performance of aphid clonal lines.
215 *Soil diversity vs. plant species vs. herbivory driven effects. Which is strongest?* To assess the
216 relative impact of each driver (herbivory, plant species, and soil microbial diversity) we conducted
217 a novel analysis adapted from transcriptomic analyses. Specifically, we assessed the impact of soil
218 treatment, plant species and herbivory for each OTU using the R package *DESeq2* [45]. We first built
219 a model using *compartment* (leaves, roots and rhizosphere), *soil treatment*, *herbivory* and *plant*
220 *species* as factors. Then, we extracted the appropriate contrasts (*Low diversity*/*High diversity* for soil
221 treatment, *S. vernei*/*S. tuberosum* for plant species and *Herbivore*/*No herbivore* for herbivore
222 treatment) for each compartment (leaves, roots and rhizosphere). From each contrast, we used the

absolute log2 Fold Change values (*ashr* shrinked [46]) for each OTU to quantify the impact of soil, plant and herbivore treatments on the microbiota in each compartment. Comparisons of absolute log2 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 [47], specifying *soil treatment*, *plant species*, and their interaction as fixed factors and *block* and *aphid clonal line* as a random effect.

Results

Phylogenetic diversity

For bacterial communities, we found a significant compartment × soil treatment × plant species interaction (Tab. 1). In all plant compartments (leaves, roots, rhizosphere) we found a higher phylogenetic diversity in *S. vernei* than in *S. tuberosum* when plants were grown on low-diversity soil treatment, and no differences between the two plant species were found when plants were grown on high-diversity soil treatment (Tab. S1). Plant species did not influence aphid bacterial diversity when they were exposed to plants grown on high- or low-diversity soil treatment (Tab. S1). 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, Tab. 1), although we did not find any significant interaction with other factors.

Table 1. Models testing the effect of compartment (aphids, leaves, roots, rhizosphere), soil treatment (high diversity, low diversity), plant species (*S. tuberosum*, *S. vernei*), herbivory (infested, control) and their interaction on the phylogenetic diversity (linear mixed-effect model) and taxonomical structure (PERMANOVA) of bacterial and fungal communities.

	Bacterial community					**Fungal community**				
		Phylogenetic diversity		**PERMANOVA**			**Phylogenetic diversity**		**PERMANOVA**	
Factors	**df**	χ^2	**P**	**F**	**P**	**df**	χ^2	**P**	**F**	**P**
Compartment (Cp)	3	843.62	**<0.001**	23.39	**0.001**	2	895.91	**<0.001**	12.9894	**0.001**
Soil treatment (S)	1	2.19	0.13	13.26	**0.001**	1	225.62	**<0.001**	14.906	**0.001**
Plant species (P)	1	18.11	**<0.001**	2.37	**0.001**	1	10.67	**<0.01**	1.9625	**0.003**
Herbivory (H)	1	34.66	**<0.001**	2.52	**0.001**	1	47.21	**<0.001**	3.7912	**0.001**
Cp × S	3	37.23	**<0.001**	5.67	**0.001**	2	252.44	**<0.001**	5.7238	**0.001**
Cp × P	3	8.18	**0.04**	1.51	**0.005**	2	1.66	0.43	1.3138	**0.03**
S × P	1	23.6	**<0.001**	1.69	**0.025**	1	3.74	0.05	1.7088	**0.014**
Cp × H	2	16.51	**<0.001**	1.61	**0.007**	2	20.7	**<0.001**	2.0339	**0.001**
S × H	1	0.01	0.9	1.25	0.11	1	5.61	**0.01**	1.2592	0.107
P × H	1	2.26	0.13	1.17	0.18	1	0.16	0.68	1.1442	0.217
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**	1.107	0.211
Cp × P × H	2	1.9	0.38	1.04	0.31	2	1.18	0.55	1.0136	0.402
S × P × H	1	2.59	0.1	1.13	0.20	1	1.33	0.24	1.1739	0.152
Cp × S × P × H	2	0.25	0.87	1.05	0.29	2	0.84	0.65	1.1249	0.186

We found a significant compartment × soil treatment × herbivory interaction in both bacterial and fungal communities. Post-hoc contrasts show a higher leaf bacterial diversity in aphid-infested plants compared with uninfested control plants when they were grown on low-diversity soil treatment (Tab. S2). Root bacterial and fungal communities, in both soil treatments, had higher diversity values in infested plants compared with uninfested control plants (Tab. S2). In the rhizosphere we observed differences between infested and uninfested plants in both bacterial and fungal community diversity of plants grown on high-diversity soil treatment, while this difference was found just in the fungal community of plants grown on low-diversity soil treatment (Tab. S2).

We found phylogenetic diversity of the aphid microbiota was highest in the low diversity treatment (Fig. S1), which mirrored differences in aphid infestation levels (χ^2=8.19, df=1, *P*=0.004; mean infestation scores: 3.10±0.23 for high diversity soil, 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 (Tab. 1). Post-hoc 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 treatment (Fig. 1, Tab. S3). In high-diversity soil treatment, only root fungal communities differed between *S. vernei* and *S. tuberosum* (Fig. 1, Tab. S3). 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 and fungal, Fig. 1, Tab. S4). In leaves, differences between soil treatments were found just in the bacterial community of *S. vernei* (Fig. 1, Tab. S4). We also found a significant compartment × herbivory interaction (Fig. 1, Tab. 1), with herbivory influencing bacterial communities in all compartments, but fungal community just in leaves and roots (Tab. 2). Our multivariate analysis demonstrated that the strongest driver of rhizosphere, root and leaf microbial community structure was soil diversity treatment (Tab. 2, Fig. 1) 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. 1). 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).

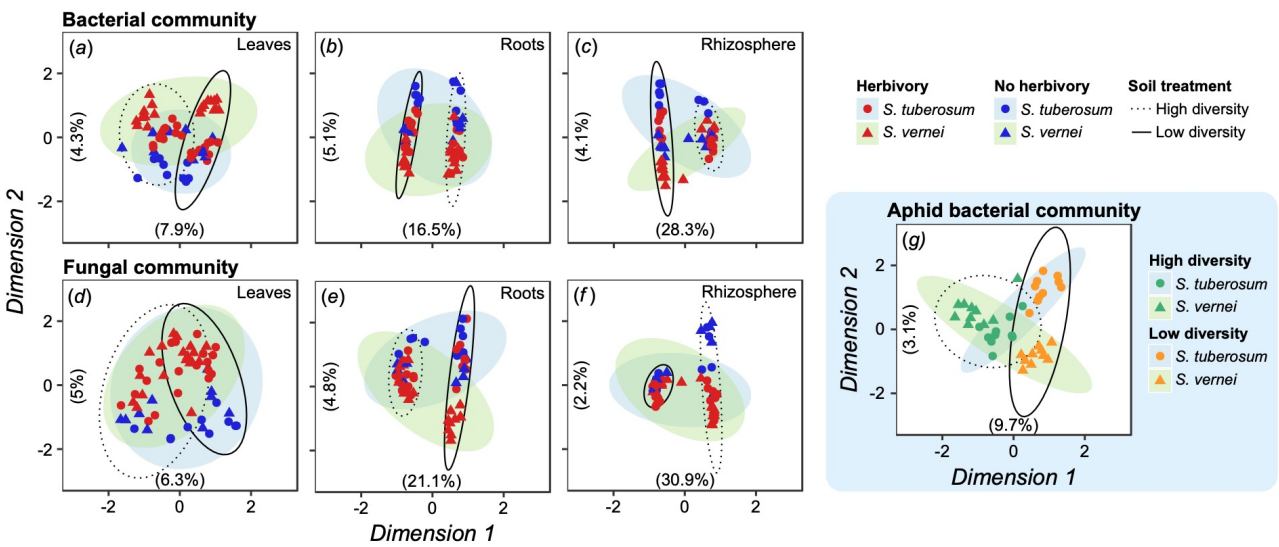

Figure 1. Structure of bacterial and fungal communities in each compartment. We report the response of bacterial (*a-c*) and fungal (*d-f*) communities to soil microbial diversity, plant species and herbivory in leaves (*a*, *d*), roots (*b*, *e*) and rhizosphere (*c*, *f*). Aphid bacterial community (*g*) 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 (Canonical Analysis of Principal Coordinates procedure).

Soil diversity vs. plant species vs. 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, 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. Both soil diversity, plant species and herbivory influenced bacterial and fungal assemblies in our system. Soil treatment explained ≈30% (rhizosphere), ≈20% (root), and ≈7% (leaf) of variation in microbiome community composition (Table 2). However, the variance in community composition explained by both plant species and herbivory (3–5%) was always lower than the variance explained by the soil treatment. Furthermore, soil treatment explained ≈8% of variation in aphid microbiota (Table 2). This suggests that the soil-driven effect is stronger than the other effects in our system.

Table 2. Analysis of the effects of soil treatment (high diversity, low diversity), plant species (*S. tuberosum*, *S. vernei*), herbivory (infested, control) on the bacterial and fungal community taxonomical structure for each compartment (aphids, leaves, roots and rhizosphere) performed through PERMANOVA. Values in bold represent *P*<0.05.

	Aphids		**Leaves**		**Roots**		**Rhizosphere**	
Bacterial community	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
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**
	Aphids		**Leaves**		**Roots**		**Rhizosphere**	
Fungal community	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
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

Second, to investigate in more detail which factor (soil, plant, 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 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 ($\chi^2_{bacteria}$=23331.3 and χ^2_{fungi}=1055, df=2, *P*<0.001, Fig. 2), with the only exception being the leaf fungal community, where no differences were found between the three factors (Fig. 2). Also, in all cases, there was no difference between the changes produced by herbivory and those produced by plant species (Fig. 2). 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 (χ^2=766.8, df=1, *P*<0.001; $abs(\log_2 FoldChange)_{soil}$ = 1.7±0.1 and $abs(\log_2 FoldChange)_{plant}$ = 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.

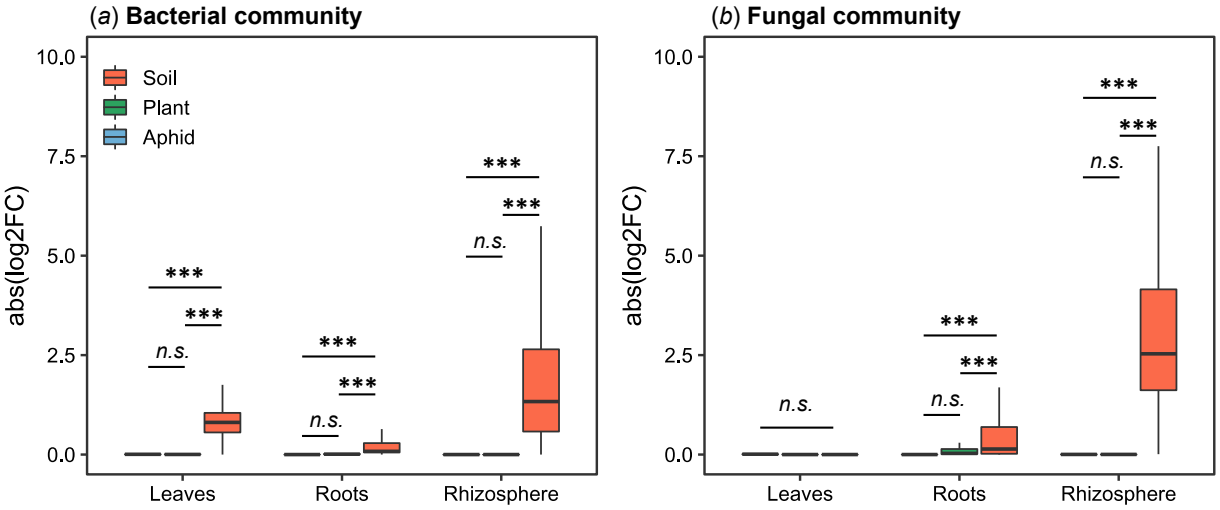

Figure 2. Magnitude of changes in abundance for each OTU (absolute log_2 Fold Changes). For each compartment (leaves, roots and rhizosphere) we investigated the response of single OTUs to soil microbial diversity (red), plant species (green) and herbivory (blue), for (*a*) bacterial and (*b*) fungal communities. *** $P<0.001$.

Discussion

Here we test, for the first time, 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, but we did not predict that this effect would be so much stronger than plant species or herbivory. This influence of soil microbial diversity correlated with aphid abundance on infested plants. Furthermore, we showed 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.

The strong influence of the soil microbial diversity on aphid bacterial communities and aphid infestation level could potentially be explained through two, non-mutually exclusive, mechanisms: (i) changes in plant physiology and/or metabolome and (ii) translocation of microbes from the rhizosphere through or on the plant. Many soil microbes are indeed able to modulate plant nutrient intake, or prime plant defences [48]. The composition of belowground microbial communities can alter plant metabolism [49], which in turn influences herbivore fitness [50]. Our low diversity treatment lacked large microbes including arbuscular mycorrhizal fungi, a group well known to prime plant defences [51,52], although aphids are less susceptible to changes in defenses primed by arbuscular mycorrhizal fungi [53]. 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 defences which has been shown to decrease the diversity of plant-associated microbial communities [54]. 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 [29].

The second potential mechanism is the translocation of microbes between soil, plant, and aphid compartments. 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 [7,10]. 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 similar community composition to the soil microbiota [16] suggesting direct (splashing of soil microbiota on leaves) or indirect (movement through the plant) microbial translocation. However, our data does not show this pattern, as the core microbiome belowground is different from shoots and herbivores, and few OTUs are common to all compartments in the system (Supplementary Results 2). The aphids in our system employ a different feeding strategy (sap-feeding) compared to the caterpillars (chewing) in the previous study [16], and chewing herbivores may have an increased likelihood of environmental uptake of microbes [55]. We thus find it unlikely that microbes in our system were translocated through or on the plant to the herbivore.

While 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. defence activation, carbon metabolism) due to aphid feeding activity [56]. 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 [8,57]. Previous research has shown that *Bemisia tabaci* (whitefly) herbivory can alter the rhizosphere microbiome of pepper plants [26], and artificial induction of plant defences [58], or their deactivation [54], has been shown to shape rhizosphere microbial communities.

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 structure root and rhizosphere microbiota, although the strength of this effect might be context-dependent due to the interaction with the soil microbial composition [59,60]. 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 just in one soil treatment. It has been previously shown that soil represent a reservoir for leaf microbial communities and that phyllosphere habitat selects for specific members [15], 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, as it is well known that the identity of the host plant is a major factor in shaping insect-associated microbial community composition [17,18].

To our knowledge, this is the first examination comparing multiple drivers of microbial communities and quantifying the relative strength of, and interplay between, factors shaping rhizosphere, plant and herbivore microbiomes. Our work represents one of the first steps 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 potential to be applied in natural and managed systems to improve food security and safety, or the success of ecological restoration efforts.

Acknowledgements

We would like to acknowledge funding from EU COST Action FA1405 and the strategic research programme and the underpinning capacity programme funded by the Scottish Government's Rural and Environment Science and Analytical Services Division. We would also like to thank Davide Bulgarelli (University of Dundee, UK), Marta Jarzyna (Ohio State University, USA), Jeff Powell (Hawksbury Institute for the Environment, Western Sydney University, AU), and Philip Smith (Dundee, UK) for helpful comments on the manuscript.

Competing interests

We declare that none of the authors have competing financial or non-financial interests.

Statement of authorship

All authors designed the experiment. AM and AEB performed the greenhouse experiment. AM prepared library and performed data analysis. AM wrote the first draft of the manuscript and all authors contributed substantially to revisions.

Data accessibility

Raw data is available at the NCBI SRA database under accession number PRJNA557499.

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