Feedbacks of plant identity and diversity on the diversity and community composition of rhizosphere microbiomes from a long-term biodiversity experiment

Schmid, M. W., Hahl, T., van Moorsel, S. J., Wagg, C., De Deyn, G. B., & Schmid, B.

Schmid, M. W., Hahl, T., van Moorsel, S. J., Wagg, C., De Deyn, G. B., & Schmid, B. (2019). Feedbacks of plant identity and diversity on the diversity and community composition of rhizosphere microbiomes from a long-term biodiversity experiment. Molecular Ecology, 28(4), 863-878. https://doi.org/10.1111/mec.14987

Received: 23 March 2018 | Revised: 24 November 2018 | Accepted: 14 December 2018

DOI: 10.1111/mec.14987

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Feedbacks of plant identity and diversity on the diversity and community composition of rhizosphere microbiomes from a long-term biodiversity experiment

Marc W. Schmid[1,2*] | Terhi Hahl[1*] | Sofia J. van Moorsel[1*] | Cameron Wagg[1] | Gerlinde B. De Deyn[3] | Bernhard Schmid[1,4]

[1]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zürich, Switzerland

[2]MWSchmid GmbH, Zürich, Switzerland

[3]Department of Soil Quality, Wageningen University, Wageningen, The Netherlands

[4]Department of Geography, University of Zurich, Zürich, Switzerland

Correspondence
Marc W. Schmid, MWSchmid GmbH, Zürich, Switzerland.
Email: contact@mwschmid.ch

Present Address
Sofia J. van Moorsel, Department of Biology, McGill University, Montreal, Québec, Canada

Funding information
German Science Foundation, Grant/Award Number: FOR 1451 and SCHM 1628/5-2; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 147092 and 166457 ; NWO-ALW VIDI, Grant/Award Number: 864.11.003

Abstract

Soil microbes are known to be key drivers of several essential ecosystem processes such as nutrient cycling, plant productivity and the maintenance of plant species diversity. However, how plant species diversity and identity affect soil microbial diversity and community composition in the rhizosphere is largely unknown. We tested whether, over the course of 11 years, distinct soil bacterial communities developed under plant monocultures and mixtures, and if over this time frame plants with a monoculture or mixture history changed in the bacterial communities they associated with. For eight species, we grew offspring of plants that had been grown for 11 years in the same field monocultures or mixtures (plant history in monoculture vs. mixture) in pots inoculated with microbes extracted from the field monoculture and mixture soils attached to the roots of the host plants (soil legacy). After 5 months of growth in the glasshouse, we collected rhizosphere soil from each plant and used 16S rRNA gene sequencing to determine the community composition and diversity of the bacterial communities. Bacterial community structure in the plant rhizosphere was primarily determined by soil legacy and by plant species identity, but not by plant history. In seven of the eight plant species the number of individual operational taxonomic units with increased abundance was larger when inoculated with microbes from mixture soil. We conclude that plant species richness can affect below-ground community composition and diversity, feeding back to the assemblage of rhizosphere bacterial communities in newly establishing plants via the legacy in soil.

KEYWORDS

16S rRNA gene sequencing, legacy effects, plant diversity, rhizosphere microbiome, soil microbial diversity

*These authors contributed equally to this study.

1 | INTRODUCTION

Soil microbes play an essential role in several ecosystem processes including decomposition, nutrient cycling, plant productivity and the maintenance of plant species diversity (Bever, Mangan, & Alexander, 2015; Wagg, Bender, Widmer, & van der Heijden, 2014). Loss of soil biodiversity reduces ecosystem functioning as trophic networks collapse (Gosling, Hodge, Goodlass, & Bending, 2006; Wagg et al., 2014) and plants are increasingly exposed to specialized soil-borne pathogens from which they are normally protected by the large biodiversity of other soil organisms (Eisenhauer, Reich, & Scheu, 2012; van der Putten et al., 2013). However, the role of potential feedbacks of plant diversity on soil diversity and rhizosphere community assemblage is largely unexplored (Dassen et al., 2017).

Soil microbes form tight associations with plants (Bulgarelli, Schlaeppi, Spaepen, van Themaat, & Schulze-Lefert, 2013) and key mutualistic to antagonistic interactions between plants and soil biota take place in the rhizosphere, defined by a narrow zone of soil surrounding plant roots (Whipps, 2001). The composition of microbial communities in the rhizosphere is mostly determined by local biotic and abiotic conditions (van der Putten et al., 2013), which at least in part are shaped by the local plant community (Bardgett & Wardle, 2003). Plants can initiate large compositional changes in rhizosphere microbiomes (Dakora & Phillips, 2002; Grüter, Schmid, & Brandl, 2006; Latz, Eisenhauer, Rall, Scheu, & Jousset, 2016) and the conditions in the rhizosphere can vary strongly between plant species (Berg & Smalla, 2009; Eisenhauer et al., 2017; Latz et al., 2016). Given that plant species specifically influence the soil microbiome, the loss of plant species probably results in reduced soil microbial biodiversity (Broughton & Gross, 2000; Garbeva, Postma, van Veen, & van Elsas, 2006; Hooper et al., 2000; Schlatter, Bakker, Bradeen, & Kinkel, 2015; Stephan, Meyer, & Schmid, 2000). In turn, this may also negatively influence further plant growth (Bartelt-Ryser, Joshi, Schmid, Brandl, & Balser, 2005). However, it is unclear to what extent plant species diversity or the presence of specific plant species (i.e., their identity) affect soil microbial diversity and composition. A recent study suggests that differences between plant functional groups may be more important for the composition of the soil microbiome than plant species richness per se (Dassen et al., 2017). In addition, higher plant diversity in grassland ecosystems often increases plant below-ground (Balvanera et al., 2006) or total (Reich et al., 2012) biomass that can sustain a larger amount of soil bacteria (De Deyn, Quirk, & Bardgett, 2011).

The consequences of reduced plant diversity for soil biodiversity can be studied in long-term biodiversity experiments (Eisenhauer et al., 2011; Roscher et al., 2013; Tilman, Reich, & Knops, 2006; Zuppinger-Dingley, Flynn, De Deyn, Petermann, & Schmid, 2016). These experiments can also be used as (natural) selection experiments; that is, plants are being naturally selected in response to plant community diversity over a prolonged period of time (Cardinale et al., 2012; Eisenhauer et al., 2016). Over time, different selection pressures in experimental monocultures versus mixtures can result in genetic differentiation between populations grown in monocultures versus mixtures (van Moorsel, Schmid, Hahl, Zuppinger-Dingley, & Schmid, 2018; Zuppinger-Dingley, Flynn, Brandl, & Schmid, 2015; Zuppinger-Dingley et al., 2016, 2014). This may occur due to sorting from standing genetic variation (Fakheran et al., 2010). Plants with a history in monoculture versus mixtures have been shown to be distinguishable from each other after 8 or 11 years of selection based on plant performance and functional trait variation (van Moorsel, Schmid et al., 2018 and Zuppinger-Dingley et al., 2014, respectively). In addition, Zuppinger-Dingley et al. (2016) found that after 8 years of codevelopment of plant and soil communities, feedback of microbes from monoculture soil on plant performance was positive for plants with a history in monocultures, but negative for plants with a history in mixtures of the same species. The authors suggested that an accumulation of specialized pathogens in monocultures, and their dilution in mixtures, could create differential selection pressures on plants (Zuppinger-Dingley et al., 2016). These studies, however, were unable to investigate the community composition of soil microbes and how they may respond to plant diversity or even if they might have been involved in causing the above-cited phenotypic differences between plants selected in monoculture versus mixture.

Here we investigated whether (a) the imprint in soil of past plant species diversity, and (b) the plant characteristics associated with plant cultivation history in either monocultures or mixtures have an impact on the diversity and composition of newly established bacterial rhizosphere microbiomes. To differentiate clearly between plants and soils we use plant history to indicate whether a plant has a history of growing in monoculture or mixture. We use soil legacy to indicate whether the soil originates from under a plant monoculture or a plant mixture. Using bacterial soil inocula and plant offspring obtained from a large field biodiversity experiment (the Jena Experiment, Roscher et al., 2004), we set up reciprocal transplant experiments (see, e.g., Joshi et al., 2001) in which plants with a monoculture or mixture history were grown as single individuals in pots inoculated with bacteria isolated from plant-monoculture or plant-mixture soils and assessed the community structure of the bacterial rhizosphere microbiomes. We did this with eight different plant species, each with its own monoculture and mixture inocula. We hypothesized (a) that microbiomes obtained from mixture soil are more diverse and differ in composition from microbiomes obtained from monoculture soil; (b) that each plant species would have a distinct microbiome; and (c) that plants with a history in monoculture compared with plants with a history in mixture have different microbiomes, and that this effect may depend on the source of the rhizosphere soils. This final hypothesis was based on the expectation that plants might have evolved preferences for different rhizosphere microbiomes during the course of selection in monocultures versus mixtures. We used 16S rRNA gene sequencing to determine the community structure and

diversity of the bacterial communities. To characterize the overall impact of soil legacy, plant species identity and plant history on the community structure of rhizosphere microbiomes, we analysed the variation in operational taxonomic unit (OTU) richness, effective richness and evenness, variation in dissimilarities between microbiomes, and the differences in individual OTU abundances across treatments.

2 | METHODS

2.1 | Plant species

We used eight common European grassland plant species previously classified into different functional groups (Roscher et al., 2004): one grass (*Festuca rubra* L.), three small herbs (*Plantago lanceolata* L., *Prunella vulgaris* L. and *Veronica chamaedrys* L.), two tall herbs (*Galium mollugo* L. and *Geranium pratense* L.) and two legumes (*Lathyrus pratensis* L. and *Onobrychis viciifolia* Skop.). The studied species had undergone 11 years of selection in either plant monocultures or species mixtures from 2002 to 2014, interrupted by a plant propagation and soil mixing step after 8 years (see below and Figure 1).

2.2 | Producing plants with monoculture versus mixture history

Plant communities of 48 plots (12 monocultures, 12 two-species mixtures, 12 four-species mixtures and 12 eight-species mixtures) of a field biodiversity experiment in Jena, Germany (the Jena Experiment, see Roscher et al., 2004), were collected as cuttings in spring 2010, after 8 years of growth in their respective plant communities. These cuttings were transplanted in identical plant composition to an experimental garden in Zurich, Switzerland, for the first controlled sexual reproduction among "coselected" plants (for details see Zuppinger-Dingley et al., 2014). In the context of the set-up of a different experiment (see van Moorsel, Hahl et al., 2018), the top 30 cm of soil of the 48 plots was pooled, mixed and returned to the excavated locations in the Jena Experiment. Hence, after the first 8 years in which plants could build up their own microbiomes, all microbial populations were mixed to create a microbial pool of maximal diversity. In spring 2011, the seedlings produced from the seeds of the first controlled sexual reproduction were transplanted back to this mixed soil in the same plots of the Jena Experiment from where the parents had originally been collected and in the same community composition as the parents had been established. In the following 3 years, plants could again select and assemble their own microbiomes from a mixed pool of maximal diversity. Thus, a potential main effect of soil legacy would have arisen within the 3 years from 2011 to 2014. In contrast, potential plant-history effects or soil legacy and plant species interaction effects could develop over 11 years in total (Figure 1, upper part).

In March 2014, plant communities including rhizosphere soil of the re-established plots in the Jena Experiment were transplanted to plots in the experimental garden in Zurich for a second controlled sexual reproduction. The 1 × 1 m plots were filled with 30 cm of soil (1:1 mixture of garden compost and agricultural soil, pH 7.4, Gartenhumus, RICOTER Erdaufbereitung AG, Aarberg, Switzerland) and fenced with netting to minimize cross-pollination with plants outside the plots. Seeds of plants with a monoculture history were collected from monoculture plots and seeds of plants with a mixture history were collected from four- or eight-species mixture plots in the experimental garden. After collection, the seeds of the eight plant species were stored at 4°C for at least 2 months. This plant material was then used in the pot experiment in the glasshouse described below.

2.3 | Soil inoculum preparation

In March 2014, samples of rhizosphere soil attached to the roots of the plants from the Jena Experiment that were transported to Zurich for the second sexual reproduction were collected and stored at 4°C. To isolate rhizosphere soil, plant roots were excavated, excess bulk soil was removed and only soil directly attached to the roots was kept. The remaining soil and the attached roots were mixed. Subsamples of approximately 5 g were then randomly taken from this mixed total amount of rhizosphere soil obtained from an individual root system. The monoculture soils came from each of the eight plant monoculture plots (resulting in eight different monoculture soils) and the mixture soils came from six different eight-species plots and one four-species plant mixture plot (one for each species except for *G. mollugo* and *O. viciifolia* whose rhizosphere soil samples came from the same mixture plot) in the Jena Experiment. Eight- and four-species mixtures were then combined into a single treatment, "mixture history/legacy". Microbial communities of the sampled rhizosphere soil were isolated and propagated and subsequently used in the pot experiment in the glasshouse. To isolate microbial communities, we produced a microbial wash by passing 500 ml of deionized water and 25 g of rhizosphere soil through a series of sieves with the smallest mesh size of 25 µm (Koide & Li, 1989; Wagg et al., 2014). We chose this mesh size to exclude spores from arbuscular mycorrhizal fungi (AMF) in order to study the direct interaction between plants and the microbial community excluding AMF (Wagg et al., 2014). To propagate the isolated microbes, we established trap cultures in two replicates for each of the eight plant species but using seeds without the above-mentioned plant histories (seeds from Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). The trap cultures consisted of 2-L pots filled with an autoclaved (120°C for 99 min) sand–soil mixture (4:1) and planted with several trap plant individuals (surface-sterilized seeds pregerminated on 1% water-agar) per species and pot (Figure 1). At the same time each trap culture received 250 ml of microbial wash. After 5 months of growth in the glasshouse we pooled the soils of the replicated trap cultures per plant species and soil legacy (monoculture or mixture soil). Trap plant roots were cut into 3- to 5-cm fragments and used together with the soil as inoculum in the pot experiment described below.

2.4 | Setup of the pot experiment in the glasshouse

The experiment included three soil-legacy treatments: (a) control (no live inoculum); (b) microbes isolated from soil under plant monocultures (monoculture soil); and (c) microbes isolated from soil under plant mixtures (mixture soil). We filled 1-L pots with 5.6 dl of gamma-radiated (27–53 kGy) 1:1 (w/w) sand/soil mixture (RICOTER Erdaufbereitung AG) and added layers of live or autoclaved (99 min

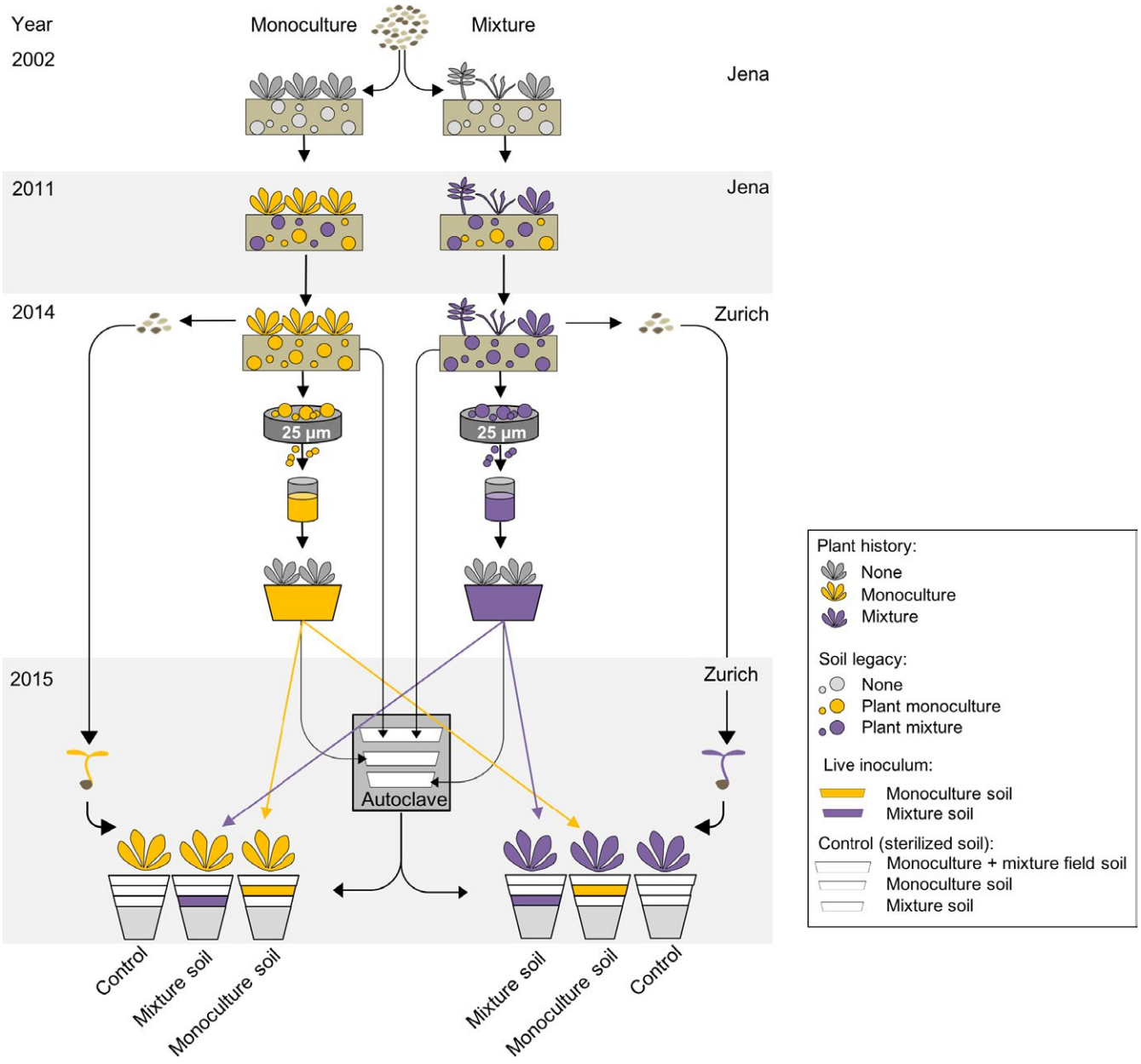

FIGURE 1 Experimental design. Plant monocultures and mixtures were sown in the Jena Experiment in 2002 and maintained until 2010. In 2010, the soil of the plots was pooled and placed back at the same locations. In spring 2011, plant seedlings were planted in the mixed soil in identical species composition as their parents. The soil communities were allowed to reassemble with their original plant communities for three more years from 2011 to 2014. In spring 2014, rhizosphere soils from eight plant species were collected and the plants were used for a second controlled seed production. Soil microorganisms smaller than 25 µm in diameter (i.e., excluding mycorrhizal spores) were then isolated. The isolated microorganisms were allowed to accumulate in trap-cultures for 5 months with neutral trap plants for each of the eight plant species. To create soil treatments for the subsequent pot experiment in the glasshouse, we filled pots with sterile soil (in grey) and added live inoculum of either microbes isolated from plants grown in monoculture (monoculture soil, indicated in yellow) or microbes isolated from plants grown in mixture (mixture soil, indicated in purple). To standardize the nutrient composition between pots, we added an 0.8-dl autoclaved counterpart of the remaining inocula to each pot (indicated in white, for details see Section 2, and Hahl, 2017). The control soil treatment received the same amount of each inoculum, but all inocula were autoclaved. Finally, we added 1 dl of the gamma-radiated sand–soil mixture to avoid cross-contamination of the live soil inocula between pots (not shown). A single plant with a history of growing either in monoculture or mixture (drawn in yellow or purple, respectively), germinated from the seeds of second controlled seed production, was then planted in each pot. Yellow and purple refer to monoculture and mixed culture soil legacies/plant histories [Colour figure can be viewed at wileyonlinelibrary.com]

at 120°C) inoculum from three sources: from trap cultures of monoculture soil treatments, from trap cultures of mixture soil treatments and from field soil (see Figure 1). Monoculture soil treatment comprised 0.8 dl of live inoculum from trap culture of monoculture soil, 0.8 dl of autoclaved inoculum from field soil and 0.8 dl of autoclaved inoculum from trap culture of mixture soil. Mixture soil treatment comprised 0.8 dl of live inoculum from trap culture of mixture soil, 0.8 dl of autoclaved inoculum from field soil and 0.8 dl of autoclaved inoculum from trap culture of monoculture soil. Control soil treatment comprised 0.8 dl of autoclaved inoculum from each of the three sources. Finally, 1 dl of the gamma-radiated sand–soil mixture was added as the uppermost layer to each pot to avoid cross-contamination of the live soil inocula between pots. Autoclaved field soil was used for comparison with a fourth soil treatment, which is not included in the analyses presented here (see Hahl, 2017).

Seeds were surface-sterilized and germinated for 2–4 weeks (depending on pretested germination times of each species) before the pot experiment on 1% water-agar. One pregerminated plant seedling with a monoculture or mixture history of one of the eight test species was planted in each pot. The experiment included in total three soil-legacy treatments (monoculture and mixture soil and control), eight plant species and two plant-history treatments (monoculture vs. mixture). The design of the experiment was a full 3 × 8 × 2 factorial. Each treatment combination was replicated seven times, resulting in 336 pots which we randomly arranged within seven experimental blocks in the glasshouse. After 19–23 weeks of plant growth, we collected rhizosphere soil samples from each pot containing a live plant and stored the samples at −80°C.

2.5 | Library preparation and sequencing

DNA was isolated from 500 mg of rhizosphere soil using the FastDNA SPIN Kit for Soil (MP Biomedicals, Illkirch-Graffenstaden, France) following the manufacturer's instructions. Samples from a subset of 150 plants were chosen for the molecular analyses (Supporting Information Table S1). We carried out targeted polymerase chain reaction (PCR) in duplicate to amplify the variable region V4 of the prokaryotic rRNA gene using primers 515f (GTGCCAGCMGCCGCGGTAA) combined with 5′ Illumina adapter, forward primer pad and forward primer linker and barcoded 806r (GGACTACHVGGGTWTCTAAT) combined with Illumina 3′ adapter, Golay barcode, reverse primer pad and reverse primer linker (Supporting Information Table S2, Bates et al., 2011). PCR conditions for amplification of the V4 region consisted of an initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and elongation at 72°C for 1 min followed by a final elongation at 72°C for 10 min. The PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). The amplicon concentrations were measured with Fragment Analyzer and the Standard Sensitivity NGS Fragment Analysis kit (Advanced Analytical Technologies, Inc., Heidelberg, Germany). Then, 60 ng of each sample was pooled and paired-end sequenced (2 × 300 bp) on the Illumina MiSeq 300 system (Beijing Genomics Institute, Bejing, China). Short reads were deposited at the Sequence Read Archive (SRA; accession number SRP105254).

2.6 | Identification and annotation of OTUs

Operational taxonomic units were generated with UPARSE (version 8.1.1861, Edgar, 2013) following the example and the tutorial given for paired-end Illumina data (drive5.com/uparse/). Reads were first quality-checked with FastQC (bioinformatics.babraham.ac.uk/projects/fastqc). Following removal of primer sequences (Supporting Information Table S2) and low-quality bases with Trimmomatic (version 0.33 with parameters ILLUMINACLIP:primerSeqs:2:30:10:8:1 SLIDINGWINDOW:5:15 MINLEN:100 [Bolger, Lohse, & Usadel, 2014]), paired-end reads were merged and filtered using usearch (with parameters -fastq_maxdiffs 25 -fastq_maxdiffpct 10 for merging and -fastq_trunclen 250 -fastq_maxee 0.25 for filtering, Edgar, 2013). Duplicated sequences were then removed with fqtrim (version 0.9.4, Pertea, 2009). The remaining sequences were clustered with usearch (with parameter -minsize 2, Edgar, 2013) to obtain 10,205 OTU sequences (Supporting Information File S1). OTU sequences were annotated with the taxonomy data from SILVA (Quast et al., 2013) using SINA with a minimal similarity of 90% and the 10 nearest neighbours (www.arb-silva.de/aligner, Yilmaz et al., 2014, Supporting Information Table S3). We chose this slightly lower threshold than the previously suggested minimal within-genus similarity of 95% (Stackebrandt & Goebel, 1994) because it has recently been shown that most of 158 genera assessed exhibit within-genera similarities well below 95% (Rossi-Tamisier, Benamar, Raoult, & Fournier, 2015). OTU abundances were finally obtained by counting the number of sequences (merged and filtered) matching the OTU sequences (usearch with parameters -usearch_global -strand plus -id 0.97, Edgar, 2013, Supporting Information Table S4). Three samples (Sample 77, Sample 265 and Sample 364) were removed from all further analysis because they had very low counts (six, 12 and one in total). OTUs annotated as chloroplast and mitochondria were removed to avoid any potential bias caused by plant DNA. To avoid sequencing artefacts, OTU sequences with fewer than 50 counts in total or with counts in fewer than five samples were removed from all further analyses (4,321 OTUs remained after this filter).

2.7 | Data normalization and identification of differentially abundant OTUs

Variation in OTU relative abundance was analysed with a generalized linear model in R with the package DESeq2 (version 1.14.1, Love, Huber, & Anders, 2014) according to a factorial design with three explanatory factors, namely soil legacy (control, monoculture soil and mixture soil), plant species identity (*F. rubra*, *G. mollugo*, *G. pratense*, *L. pratensis*, *O. viciifolia*, *P. lanceolata*, *P. vulgaris* and *V. chamaedrys*) and plant history (monoculture and mixture). All individual factor combinations were coded as a unique level of a combined single factor (Supporting Information Table S1). Specific combinations of levels were then compared with linear contrasts

(Neter & Wassermann, 1974). The four main contrasts compared (a) the two different plant histories, (b) the control soil and the microbial soils, (c) the two different soil legacies of the microbial soils, and (d) each plant species to all other plant species. To test for interactions, each contrast was tested across the entire data set and within the individual soil-legacy treatments or different plant species. Contrasts (b), (c) and (d) were not tested separately within the two plant-history treatments because these only had weak effects on the composition of the microbiomes. Within each comparison, *p*-values were adjusted for multiple testing (Benjamini–Hochberg), and OTUs with an adjusted *p*-value (false discovery rate, FDR) below 0.01 and a minimal $\log_2$ fold-change (i.e., the difference between the $\log_2$-transformed, normalized OTU counts) of 1 were considered to be differentially abundant (Supporting Information Table S5). Normalized OTU counts were calculated accordingly with DESeq2 and $\log_2(x + 1)$-transformed to obtain the normalized OTU abundances. Sequencing data were not rarefied (McMurdie & Holmes, 2014).

2.8 | Visualization of between-sample distances

Distances between all samples using all 4,321 OTUs passing the filter were visualized (Figure 2) with a redundancy analysis (RDA). The RDA was conducted in R with the package vegan (version 2.4-4, function rda(); Oksanen et al., 2017). Input data were the normalized and $\log_2$-transformed OTU counts as response variables and the treatment factors with all interactions as explanatory terms.

Sample clustering using the 2,089 differentially abundant OTUs were visualized (Figure 5) with t-SNE (van der Maaten, 2014; van der Maaten & Hinton, 2008). We calculated 100 maps starting from different random seeds and selected the map with the lowest final error. Individual maps were calculated in R with the package Rtsne (version 0.13; van der Maaten & Hinton, 2008, van der Maaten, 2014). Parameters for the function Rtsne() were pca = FALSE, theta = 0. Input data were the normalized and $\log_2$-transformed read counts. Note that t-SNE differs from other ordination methods commonly used in ecology such as principal component or coordinate analysis (PCA) by focusing on high resolution of local distances in an ordination whereas PCA can better capture overall variation in a data set (van der Maaten & Hinton, 2008).

2.9 | Overall bacterial community structure

To assess the overall impact of the treatment factors on the dissimilarity between the microbiome bacterial community structure, we analysed the variation in dissimilarities between microbiomes with a multivariate ANOVA (Table 2) in R with the package vegan (version 2.4-4, function adonis(); Oksanen et al., 2017). We used the Manhattan distance as a dissimilarity measure because it has been shown to be consistently more preferable than the Euclidean distance for high-dimensional data (Aggarwal, Hinneburg, & Keim, 2001).

To assess the impact of the treatment factors on the genetic composition of the microbiomes, we performed a multivariate ANOVA with UniFrac distances (Lozupone, Lladser, Knights, Stombaugh, & Knight, 2011). To calculate UniFrac distances we used the R-package GUniFrac OTU (version 1.1, function GUniFrac()) with the parameter alpha controlling the weight on abundant lineages set to 0, 0.5 and 1. As input we used the normalized OTU counts and a midpoint rooted tree of OTU sequences. The genetic tree was obtained with QIIME with the scripts "align_seqs.py -m pynast" and "make_phylogeny.py -t fasttree" (version 1.9.1, Caporaso et al., 2010). The results for the three different alphas were similar and only results for $\alpha = 0.5$ are shown.

2.10 | Enrichment and depletion of bacterial phyla

To test for enrichment/depletion of bacterial phyla occurrences in a given set of OTUs (e.g., OTUs with significant difference in abundance between monoculture and mixture soils), we constructed for each phylum a contingency table with the within/outside phyla counts for the given set of OTUs and all OTUs passing the filter. We then tested for significance with Fisher's exact test. The *p*-values were adjusted for multiple testing (Benjamini–Hochberg), and phyla with an adjusted *p*-value (FDR) below 0.05 were considered to be significantly enriched/depleted (Supporting Information Table S7).

3 | RESULTS

3.1 | Characterization of OTUs

Amplification of 16S rRNA gene fragments yielded an initial set of 10,205 OTUs (Supporting Information File S1, Tables S3 and S4). After removing OTUs with similarity to chloroplast sequences (86 OTUs) and mitochondrial sequences (57 OTUs) and low-abundance

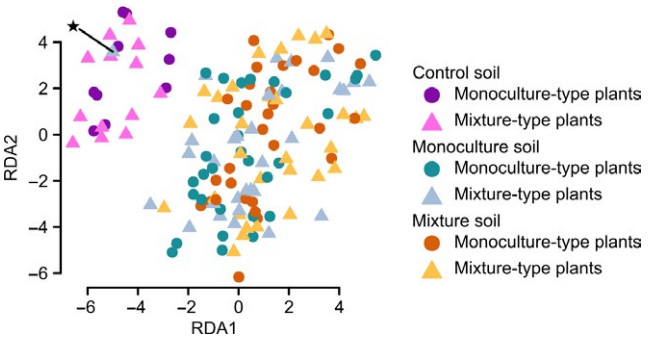

FIGURE 2 Redundancy analysis (RDA) using the normalized operational taxonomic unit (OTU) abundances of all samples sequenced. The two first RDA axes explained 17.4% of the overall variance and separated the control soil from the microbial soils. The constrained components accounted for 60% of the total variance. The star marks "Sample492" (*Lathyrus pratensis*, mixture history, monoculture soil), which clustered among the samples from the control soil. This sample was excluded as an outlier from the analysis of differential OTU abundance [Colour figure can be viewed at wileyonlinelibrary.com]

OTUs with <50 counts in total or with counts in fewer than five samples (5,741 OTUs), 4,321 OTUs remained. Of these, 3,955 and 41 were classified as bacteria and archaea, respectively (325 remained unclassified or unknown). Within the bacterial domain, the ten most abundant phyla accounted for 82.6% of all OTUs (Supporting Information Table S6); they were *Proteobacteria* (10.6% *Alpha*-, 2.5% *Beta*-, 9.0% *Gamma*-, 12.3% *Delta*- and 0.3% other/unknown *Proteobacteria*, respectively), *Planctomycetes* (9.0%), *Bacteroidetes* (7.42%), *Chloroflexi* (6.5%), *Firmicutes* (6.3%), *Actinobacteria* (4.7%), *Parcubacteria* (4.4%), *Acidobacteria* (3.6%), *Verrucomicrobia* (3.2%) and *Gemmatimonadetes* (3.0%).

To evaluate the overall differences between the microbiomes of the different soil legacies, plant species and plant histories, we conducted an RDA (Oksanen et al., 2017) using the normalized OTU abundances as response variables and the treatment factors with all interactions as explanatory terms (Figure 2). The two first RDA axes explained 17.4% of the overall variance and separated the control soil from the monoculture and mixture soils. An exception was "Sample492", which grouped among the samples from the control soil, even though it came from a microbial soil (see Figure 2). This sample was therefore removed as an outlier from all subsequent analyses. Overall, the results clearly indicated that soils with live inocula had rhizosphere communities that were distinct from the rhizosphere communities that developed in control soil.

3.2 | OTU richness, effective richness and evenness

To characterize the overall impact of the treatment factors on the bacterial community structure, we analysed the variation in OTU richness, effective richness (exponent of the Shannon index, Magurran, 2004) and evenness (Pielou, 1975) among the different treatments (Figure 3, Table 1). The results for the three indices were similar, with plant species, soil legacy and their interaction being significant. Within the soil legacy factor, the contrast comparing the control soil to the microbial soils was significant, but differences between the two microbial soil legacies were not. However, the interactions of these two contrasts with plant species were both significant, except for that of the control soil versus microbial soil contrast with plant species in the analysis for effective species richness. All three indices were higher in the microbial soils than in the control soil. In contrast, the difference between the two different microbial soil legacies was specific to the plant species. For example, OTU richness was significantly higher in mixture soil compared to monoculture soil in *Festuca rubra* (+382 species on average), tended to be higher in *Onobrychis viciifolia* and *Plantago lanceolata* (+239 and +166 species on average, respectively), but tended to be lower in *Geranium pratense* (−232 species on average, Figure 3). Similar results were obtained with the multivariate ANOVA testing for the impact of the treatment factors on the bacterial community composition (Table 2) and the genetic community composition (Table 3). Here, the main-effect contrast comparing the two microbial soil legacies with each other was also significant. For genetic community composition, the interactions of the contrast comparing the control soil to the microbial soil and plant history and their three-way interaction with plant species were also significant, providing evidence that plants with monoculture versus mixture history did pick up genetically different microbiomes from monoculture versus mixture soil at least in some species.

Nevertheless, the overall bacterial community structure was primarily determined by soil legacy, plant species identity and the interaction between the two. In contrast, the main effect of plant history and its interactions were rarely significant (Tables 1 and 2), indicating that plants with a history in monoculture versus mixture had not evolved strong preferences for different rhizosphere microbiomes and that home versus away effects (for associations between monoculture or mixture plants reciprocally combined with monoculture or mixture soil) were not important.

3.3 | Differential OTU abundance

To identify the OTUs that contributed to the differences in the bacterial communities, we tested each OTU for differential abundance between the different soil legacies, plant species identities and plant histories. We therefore combined these three treatment factors and their interactions into a single factor with 3 × 2 × 8 levels and compared specific combinations of levels with linear contrasts (see Section 2 for details). Of the 4,321 OTUs tested, 2,089 showed one or several significant comparisons (Table 4). Comparisons between the different soil-legacy treatments were often significant (e.g., 975 OTUs were different between the control soil and the microbial soils). Likewise, contrasts comparing each plant species to all other plant species were frequently significant (e.g., 493 OTUs were different between *F. rubra* and all other plant species). In agreement with the significant interaction between soil-legacy treatments and plant species identities in the analysis of bacterial richness, the number and identity of the OTUs identified as significantly differentially abundant between soil-legacy treatments or among plant species varied if they were tested within a given plant species or soil-legacy treatment, respectively (Figure 4, Supporting Information Figures S1 and S2). The contrasts comparing the two plant histories across the entire data set, within microbial soil legacies (monoculture or mixture soil) and within plant species were almost never significant (fewer than 13 OTUs in every case). Together, these results confirmed that the composition of the rhizosphere microbiomes was mainly determined by the microbial community developed over time in the field biodiversity experiment and its interaction with the particular plant species that provided the "root interface" in the field and later on in the pot experiment in the glasshouse. Overall, when looking at the bacterial community composition in terms of the normalized abundances of the 2,089 bacterial OTUs which were significant in any of the comparisons, the rhizosphere microbiomes cluster according to plant species and often also according to soil legacy, whereas plant histories did not form any apparent clusters (Figure 5).

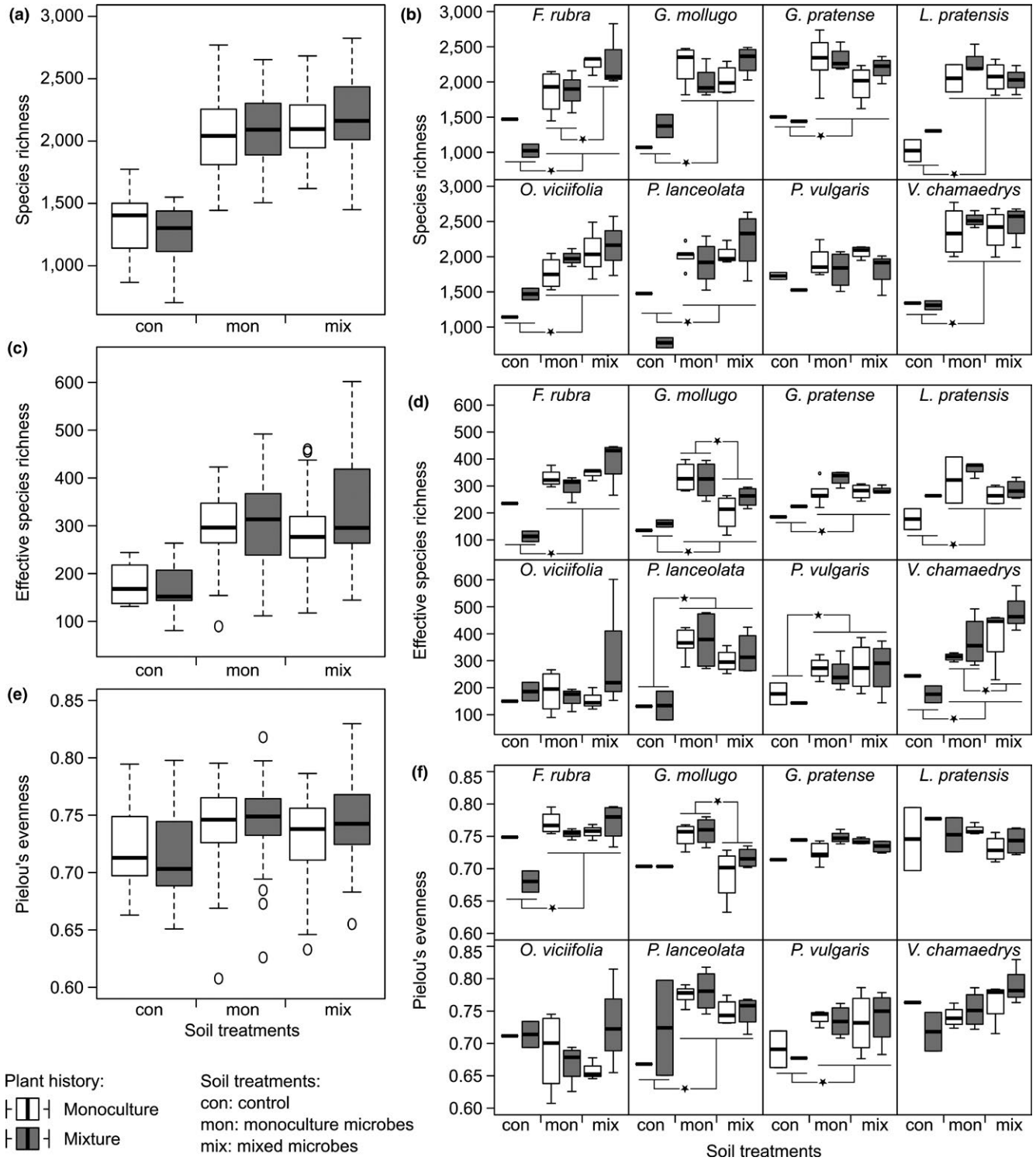

FIGURE 3 Microbial richness (number of operational taxonomic units) in the rhizosphere of plants with a history in monoculture (white bars) and plants with a history in mixture (grey bars) across all plant species (a) and for each plant species separately (b). Effective species richness (exp(Shannon Index)) in the rhizosphere of plants with a history in monoculture (white bars) and plants with a history in mixture (grey bars) across all plant species (c) and for each plant species separately (d). Pielou's evenness in the rhizosphere of plants with a history in monoculture (white bars) and plants with a history in mixture (grey bars) across all plant species (e) and for each plant species separately (f). Significant ($p < 0.05$) soil contrasts are indicated in the panels with the individual species (b, d and f). The ANOVA results for the full model using all data (a, c and e) are given in Table 1. Boxplots: the bottom and top of the boxes correspond to the lower and upper quartiles and the centre line marks the median. Whiskers extend to the lowest/highest values unless these values are lower/higher than the first/third quartile minus/plus 1.5 times the interquartile range, which equals the third minus the first quartile

3.4 | Soil legacy and plant species shape bacterial rhizosphere communities

Differences between the microbiomes from monoculture versus mixture soils were highly specific to each particular plant species (see Figure 4). In total, 864 OTUs were significantly differentially abundant between the two microbial soils if tested separately for each plant species. The majority of them (563 OTUs) were unique to a given plant species. Only 138 significantly differentially abundant OTUs were identified if tested across all plant species (of which 23 were not among the 864 with significant differences in the plant species-specific comparisons). In contrast, 74.0% of all OTUs identified as differentially abundant between the control soil and the two microbial soil-legacy treatments were also significantly different if tested across all plant species (975 of 1,317 OTUs). On average per plant species, 91 OTUs were more abundant in microbiomes from mixture than from monoculture soil and 63 OTUs were more abundant in microbiomes from monoculture than from mixture soil. Except for *G. pratense*, microbiomes from mixture soil always had a higher number of OTUs with increased abundance than microbiomes from monoculture soil. This was also true if tested across all plant species, where 106 and 32 OTUs exhibited increased abundance in microbiomes from mixture and monoculture soil, respectively (see Table 4). Together, the microbiomes from soil with a legacy of plant species mixtures generally contained more individual OTUs with higher abundance than the microbiomes from soil with a legacy of monocultures.

3.5 | Taxonomy of OTUs differentially abundant in monoculture versus mixture soils

We assessed the taxonomy of the OTUs that were significantly differentially abundant between microbiomes from monoculture and mixture soils (Figure 4b, Supporting Information Table S7). OTUs with increased abundance in microbiomes from monoculture soils were enriched for *Bacteroidetes* (75 observed, 46 expected) and depleted for *Firmicutes* (one observed, 13 expected) and unknown phyla (14 observed, 36 expected). OTUs with increased abundance in microbiomes from mixture soils were enriched for *Bacteroidetes* (87 observed, 61 expected) and depleted for *Firmicutes* (five observed, 17 expected) and unknown phyla (12 observed, 47 expected); in addition, they were also depleted for *Actinobacteria* (11 observed, 26 expected). Hence, *Bacteroidetes* was the phylum most responsive to the plant diversity level in the field. In contrast, *Firmicutes* was the least responsive phylum.

4 | DISCUSSION

Here we tested three hypotheses: (a) that microbiomes obtained from soil from plant mixtures are more diverse and differ in composition from microbiomes obtained from soil from plant monocultures; (b) that plant species differ in their microbiomes; and (c) that plants with a history of growing in monoculture or in species

TABLE 1 Analysis of variance of microbial richness (number of operational taxonomic units), effective species richness (exp(Shannon Index)) and Pielou's evenness

		Species richness			Eff. species richness			Pielou's evenness		
Source of variation	***df***	***F***	***p***	**%-SS**	***F***	***p***	**%-SS**	***F***	***p***	**%-SS**
Plant history (Ph)	1	0.01	0.925	0	2.21	0.141	0.55	1.46	0.230	0.48
Soil legacy (Sl)	2	100.75	**<0.001**	31.07	33.99	**<0.001**	16.87	6.88	0.002	4.58
Control versus microbial soil (C)	1	199.55	**<0.001**	30.77	67.97	**<0.001**	16.87	12.98	**<0.001**	4.31
Monoculture versus Mixture soil (M)	1	1.95	0.166	0.30	0.00	0.975	0.00	0.79	0.378	0.26
Plant species (Sp)	7	5.19	**<0.001**	5.61	8.11	**<0.001**	14.09	8.90	**<0.001**	20.72
Ph × Sl	2	1.4	0.251	0.43	2.15	0.123	1.07	1.43	0.244	0.95
Ph × C	1	2.25	0.137	0.35	1.99	0.162	0.49	0.91	0.343	0.30
Ph × M	1	0.55	0.459	0.09	2.32	0.131	0.58	1.95	0.166	0.65
Ph × Sp	7	1.25	0.283	1.35	0.73	0.649	1.26	0.48	0.846	1.12
Sl × Sp	14	2.37	**0.007**	5.12	2.69	**0.002**	9.36	2.59	**0.003**	12.05
C × Sp	7	2.48	**0.022**	2.68	1.74	0.109	3.02	2.52	**0.020**	5.86
M × Sp	7	2.26	**0.035**	2.44	3.65	**0.002**	6.34	2.66	**0.015**	6.19
Ph × Sl × Sp	14	1.08	0.381	2.34	0.74	0.726	2.58	1.07	0.396	4.97
Ph × C × Sp	7	1.09	0.373	1.18	0.52	0.817	0.90	0.97	0.455	2.27
Ph × M × Sp	7	1.07	0.386	1.16	0.97	0.460	1.68	1.16	0.331	2.71
Residuals	98			15.11			24.32			32.59

Notes. Contrasts among soil-legacy treatments and their interactions are indented and printed in italics. Significant *p*-values are highlighted in bold. %-SS: proportion of total sum of squares; *df*: degrees of freedom; *p*: error probability.

TABLE 2 Multivariate analysis of variance of dissimilarities between microbial communities

Source of variation	*df*	SS	MS	*F*	*p*
Plant history (Ph)	1	5,179,156.5	5,179,156.5	0.80	0.769
Soil legacy (Sl)	2	217,988,671.7	108,994,335.9	16.78	**0.001**
Control versus microbial soil (C)	1	187,773,678.1	187,773,678.1	28.90	**0.001**
Monoculture versus Mixture soil (M)	1	30,214,993.6	30,214,993.6	4.65	**0.001**
Plant species (Sp)	7	396,818,364.0	56,688,337.7	8.73	**0.001**
Ph × Sl	2	12,960,987.9	6,480,493.9	1.00	0.373
Ph × C	1	7,885,628.8	7,885,628.8	1.21	0.168
Ph × M	1	5,075,369.1	5,075,369.1	0.78	0.810
Ph × Sp	7	46,319,383.0	6,617,054.7	1.02	0.411
Sl × Sp	14	282,148,085.7	20,153,434.7	3.10	**0.001**
C × Sp	7	91,109,620.4	13,015,660.1	2.00	**0.001**
M × Sp	7	191,038,465.3	27,291,209.3	4.20	**0.001**
Ph × Sl × Sp	14	86,097,936.9	6,149,852.6	0.95	0.513
Ph × C × Sp	7	44,940,444.8	6,420,063.5	0.99	0.522
Ph × M × Sp	7	41,157,492.0	5,879,641.7	0.90	0.778
Residuals	98	636,709,175.9	6,497,032.4		

Note. Contrasts among soil-legacy treatments and their interactions are indented and in italic type. Significant *p*-values are highlighted in bold. The lowest *p*-value was set to 0.001 because the results were obtained with 999 permutations. SS/MS refer to sum of squares/mean squares of the dissimilarity measure (Manhattan distance between samples calculated with the $\log_2$-transformed normalized operational taxonomic unit sequence counts).

mixtures associate with different rhizosphere microbiomes if planted in pots inoculated with soil taken from monoculture or mixtures of the same species.

Overall, we found clear differences between control and microbial soil treatments in bacterial richness, diversity, evenness and composition, thus confirming the anticipated establishment of our microbial treatments. Differences between the two microbial soil types were less pronounced. For bacterial richness, effective richness and evenness, the difference depended on the species (Table 1). In contrast, the overall community structure assessed with a multivariate ANOVA was clearly different between the two microbial soil types (Table 2). Similarly, we identified 864 OTUs which were significantly differentially abundant between monoculture and mixture soil legacies (i.e., 20% of all tested OTUs, Table 4). In all analyses we observed a strong interaction between the soil legacy effects and the plant species. However, we found almost no significant differences between microbiomes of plants with a monoculture versus mixture history, even when testing for interactions of plant history with the other factors (see Tables 1–3). In summary, this supports our first (a) and second (b) hypotheses but provides little evidence for our third (c) hypothesis. Hence, our findings demonstrate that the composition of rhizosphere microbiomes is shaped by the soil environment from which they originate and where legacy effects of plant diversity play an important role, and by the identity of the host plant species with which they associate during plant growth (both during the 11 years in the field biodiversity experiment and the 5 months in the glasshouse experiment). However, plant history, i.e. whether plants have been selected in monoculture or mixture, is not reflected in differences between rhizosphere microbiomes. As a corollary, this suggests that the differences observed between monoculture- and mixed culture-history plants in Zuppinger-Dingley et al. (2014) were unlikely to be due to the plants being associated with different microbiomes.

It is important to note that the mixture and monoculture soil inocula included microbial filtrates from eight different plant monoculture plots, six different eight-species plant-mixture plots (one for each species except for *Galium mollugo* and *Onobrychis viciifolia* whose rhizosphere soil samples came from the same mixture plot) and one four-species plant-mixture plot in the Jena Experiment. Hence, both plant history and soil legacy were of relatively high diversity in the mixture. It is possible that results would have been different if we had included plants and microbial inocula from, for example, two-species mixtures instead of mostly eight-species mixtures. In this respect, note that to a certain extent, the average monoculture soil represents a mixture influenced by eight plant species. Likewise, the average mixture soil represents a mixture influenced by seven different plant communities (36 plant species in total). The overall comparison between monoculture and mixture soil across all plant species thus resembles a comparison of soil influenced by eight plant species compared to 36 plant species (without considering the potential effects of plant diversity on each individual soil sample from the Jena Experiment). The effect of soil legacy on the bacterial diversity of plant rhizospheres may therefore be better understood when comparisons are made within each plant species.

TABLE 3 Multivariate analysis of variance of UniFrac distances

Source of variation	*df*	SS	MS	*F*	*p*
Plant history (Ph)	1	0.05	0.05	0.87	0.636
Soil legacy (Sl)	2	1.51	0.75	12.13	**0.001**
Control versus microbial soil (C)	1	1.31	1.31	21.09	**0.001**
Monoculture versus Mixture soil (M)	1	0.20	0.20	3.17	**0.001**
Plant species (Sp)	7	3.59	0.51	8.23	**0.001**
Ph × Sl	2	0.16	0.08	1.27	0.286
Ph × C	1	0.11	0.11	1.69	**0.028**
Ph × M	1	0.05	0.05	0.84	0.708
Ph × Sp	7	0.43	0.06	0.99	0.498
Sl × Sp	14	2.20	0.16	2.52	**0.004**
C × Sp	7	0.95	0.14	2.19	**0.001**
M × Sp	7	1.25	0.18	2.85	**0.001**
Ph × Sl × Sp	14	0.99	0.07	1.14	0.335
Ph × C × Sp	7	0.61	0.09	1.39	**0.001**
Ph × M × Sp	7	0.39	0.06	0.89	0.888
Residuals	98	6.10	0.06		

Note. Contrasts among soil-legacy treatments and their interactions are indented and in italics. Significant *p*-values are highlighted in bold. The lowest *p*-value was set to 0.001 because the results were obtained with 999 permutations. SS/MS refer to sum of squares/mean squares of the dissimilarity measure.

4.1 | Factors affecting the diversity of rhizosphere microbiomes

Indeed, differences in diversity indices, composition and individual OTU abundances between microbiomes from monoculture and mixture soil depended on the plant species (interactions M × Sp and PH × M × Sp in Tables 1 and 2, contrast 3 in Table 4). Given that plant species identity was confounded with species composition of plant mixtures in the Jena Experiment, it is possible that in part these differences were due to key plant species being present or absent in these plant mixtures in the Jena Experiment. However, a more diverse plant community always has a greater probablility of containing some particular species. In this sense, the presence of a key species may also be considered as a biodiversity effect, sometimes referred to as the "selection probability effect" (Niklaus, Baruffol, He, Ma, & Schmid, 2017). The observed species-specific effects are in line with a variety of responses previously reported in the literature. Studies examining the correlation of plant species diversity and soil bacterial richness found positive correlations (Garbeva et al., 2006; Stephan et al., 2000), negative correlations (Schlatter et al., 2015) or no correlation (Dassen et al., 2017; Grüter et al., 2006). Nevertheless, positive correlations seem to predominate both in our study and in the sum of previous studies. Because the habitats and resources in the rhizosphere tend to vary between different plant species (Berg & Smalla, 2009; Eisenhauer et al., 2017; Hooper et al., 2000), increasing plant species diversity could provide a greater variety of resources and habitats for microbes and thereby result in higher richness of rhizosphere microbiomes from mixture than from monoculture soil.

4.2 | Factors affecting the differences in abundance of individual OTUs

When we compared differential relative abundances of individual OTUs between the rhizosphere microbiomes from mixture and from monoculture soils, the number of OTUs with increased relative abundance in treatments with mixture soil was higher in all plant species except *Geranium pratense* (Figure 4 and Table 4). In *G. pratense*, overall bacterial richness tended to be higher in microbiomes from monoculture than from mixture soil (Figure 3b). One explanation could be the selection history of *G. pratense*. Whereas all the other species grew in mixed-species field plots with herbs and grasses, legumes or both, *G. pratense* was growing in a monofunctional group mixture (eight species of tall herbs) in the Jena Experiment. This may have resulted in a more monoculture-like selective environment for the associated microbes. Plant functional groups have been shown to influence bacterial abundance (Stephan et al., 2000; Bartelt-Ryser et al., 2005; Latz et al., 2012; Latz et al., 2016; Lange et al., 2014) and richness (Dassen et al., 2017; Stephan et al., 2000) in soil. Dassen et al. (2017) suggested that plant functional groups are more important determinants of bacterial richness than plant species. Together, our results suggest that individual OTU abundances and overall bacterial richness in the rhizosphere generally increase with increasing plant species diversity to the extent that this feeds back to newly establishing plants, but that they are also positively influenced by plant functional diversity.

5 | CONCLUSIONS

Our results suggest that plant species diversity generally leaves a legacy which increases the diversity of soil bacteria that assemble in the rhizospheres of subsequently grown plant species. The extent of this effect is further modulated by the identity of the host plant species. These findings support our hypothesis that when plants and soil microbial communities develop together for prolonged periods in the field in plant monocultures and mixtures, the diversity and composition of rhizosphere bacterial communities associated with subsequently grown plants can diverge.

ACKNOWLEDGEMENTS

We thank M. Furler, H. Martens, D. Topalovic, D. Trujillo Villegas and T. Zwimpfer for technical assistance, Y. Xu, E. De Luca, M. Brezzi and A. Grossniklaus for help with the experiment and Agroscope for providing facilities. This study was supported by the Swiss National Science Foundation (grant numbers 147092 and 166457

TABLE 4 The number of operational taxonomic units (OTUs) exhibiting significant differential abundance in any of the contrasts tested in this study (FDR ≤ 0.01 and abs(logFC) ≥ 1; see Section 2)

	All	*Festuca rubra*	*Galium mollugo*	*Geranium pratense*	*Lathyrus pratensis*	*Onobrychis viciifolia*	*Plantago lanceolata*	*Prunella vulgaris*	*Veronica chamaedrys*	Total (*n*)
1. PH: mixture- versus monoculture history	1 (0/1)	5 (4/1)	6 (5/1)	0	3 (1/2)	1 (1/0)	6 (6/0)	11 (4/7)	1 (0/1)	32
(a) Within control soil	12 (6/6)	—	—	—	—	—	—	—	—	—
(b) Within monoculture soil	0	0	1 (1/0)	0	2 (1/1)	0	0	5 (0/5)	0	8
(c) Within mixture soil	0	0	0	0	0	0	1 (1/0)	2 (1/1)	0	3
2. SO: control versus microbial soils	975 (157/818)	321 (76/245)	267 (92/175)	136 (29/107)	171 (46/125)	339 (37/302)	291 (69/222)	296 (77/219)	368 (54/314)	1,317
3. SO: mixture versus monoculture soil	138 (106/32)	137 (109/28)	135 (85/50)	209 (67/142)	150 (109/41)	103 (77/26)	126 (69/57)	241 (144/97)	135 (69/66)	864
4. Plant species: one versus all others	—	493 (189/304)	128 (84/44)	230 (164/66)	218 (161/57)	261 (147/114)	286 (132/154)	301 (116/185)	505 (427/78)	1,287
(a) Within control soil	—	95 (60/35)	36 (33/3)	54 (50/4)	54 (52/2)	35 (24/11)	68 (42/26)	62 (48/14)	77 (69/8)	383
(b) Within monoculture soil	—	459 (180/279)	215 (145/70)	419 (289/130)	156 (100/56)	327 (194/133)	300 (177/123)	349 (174/175)	593 (497/96)	1,464
(c) Within mixture soil	—	343 (176/167)	220 (129/91)	282 (140/142)	390 (283/107)	280 (206/74)	333 (162/171)	406 (199/207)	451 (390/61)	1,422

Note. Numbers in parentheses refer to the number of OTUs having higher abundance in the first and the second group of the contrast, respectively. Contrasts included either all species or each species individually (columns). The contrast comparing the different plant histories (PH) was tested across the entire data set and within the specific soil-legacy treatments. Considering that plant history had a weak effect on the composition of the microbiomes, the other contrasts comparing the different soil-legacy treatments (SO) were only tested across the entire data set and within the individual plant species but not within the specific plant-history treatments. Likewise, the contrast comparing one specific plant species with all others (SP) was only tested across the entire data set and within the specific soil-legacy treatments. Because there were only three samples from the control soil per species (i.e., one with monoculture and two with mixture plants or vice versa), the contrast 1.a) was only tested across all species.

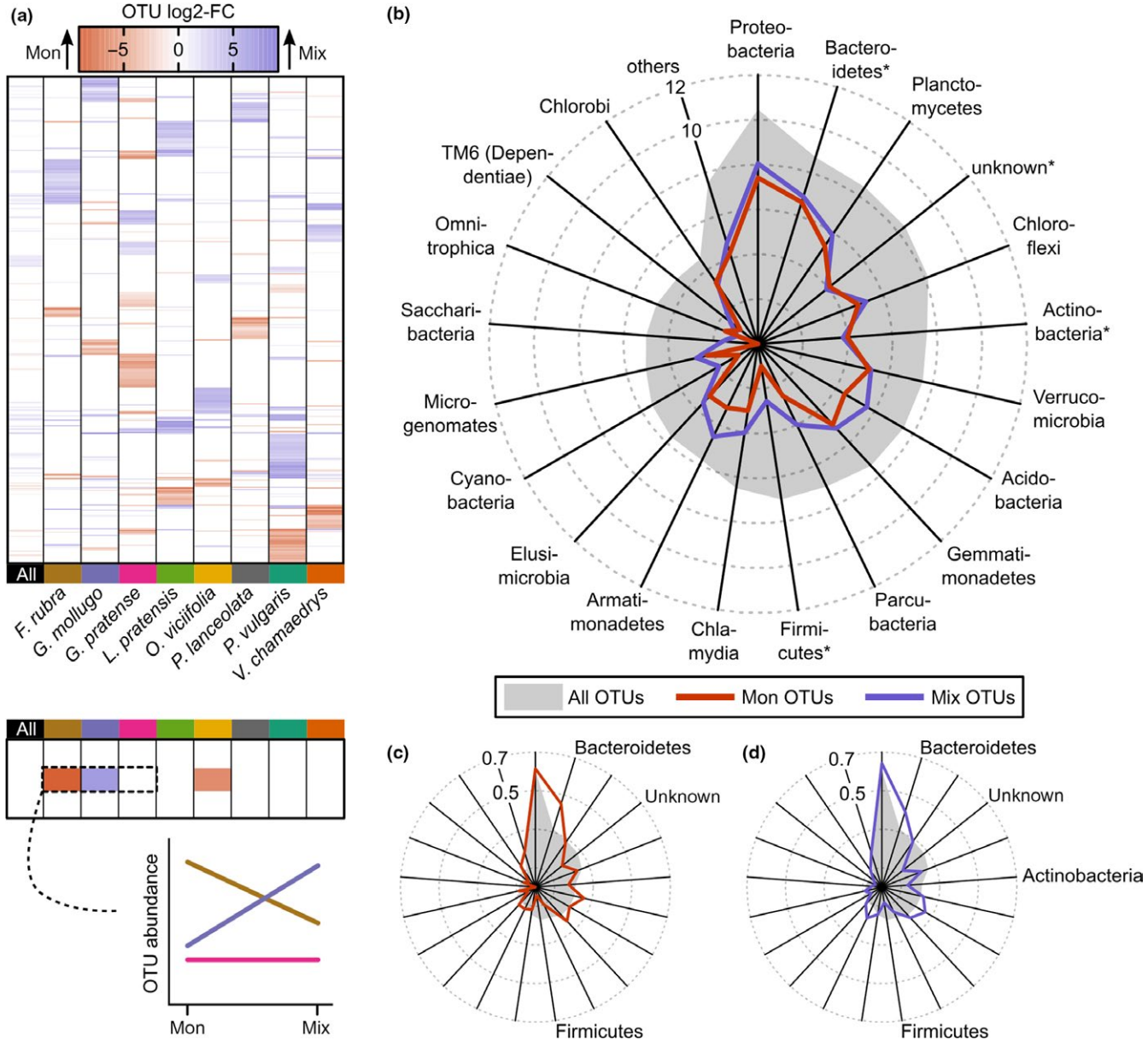

FIGURE 4 Operational taxonomic units (OTUs) with significant differences in abundance (log2-FC = $\log_2$ fold changes) between the two soil-legacy treatments monoculture (mon) and mixture soil (mix) across all eight plant species or for each plant species separately (contrast 3 in Table 2). (a) Top: heat map with differences in abundance of the significant OTUs. Each row corresponds to one OTU, and each column to the contrast tested across all plant species ("all") or separately for each plant species. Red or blue corresponds to an increased abundance of an OTU in microbiomes from monoculture or mixture soils, respectively. White indicates an insignificant difference (false discovery rate [FDR] > 0.01). Bottom: drawing illustrating how interactions between the plant species and the soil-legacy contrast can be inferred from the heat map. (b) OTU frequency ($\log_2$-transformed) of the phyla with at least 20 OTUs in the entire data set. The remaining phyla were summarized as "others". The grey polygon represents the background distribution (all 4,321 OTUs passing the filters described in the Section 2). Red and blue lines correspond to the frequencies of OTUs identified as significantly more abundant in microbiomes from monoculture (Mon OTUs) and mixture soils (Mix OTUs), respectively, within any of the plant species. Phyla with significant enrichment/depletion in either of these sets are marked with asterisks (two-sided Fisher's exact test, adjusted for multiple testing, FDR < 0.05). (c,d) Similar to (b), but with OTU frequencies normalized to the total number of OTUs and arc-sin-transformed. Only phyla with significant enrichment/depletion are labelled [Colour figure can be viewed at wileyonlinelibrary.com]

to B. Schmid) and the University Research Priority Program Global Change and Biodiversity of the University of Zurich. The Jena Experiment is supported by the German Science Foundation (FOR 1451, SCHM 1628/5-2). G. B. De Deyn was financially supported by NWO-ALW VIDI (grant number 864.11.003).

AUTHOR CONTRIBUTION

T.H., C.W., G.B.D.D. and B.S. designed the study. T.H. and S.J.V.M. carried out the experiment. T.H. performed the DNA extraction and sequencing preparation. M.W.S. processed the sequencing

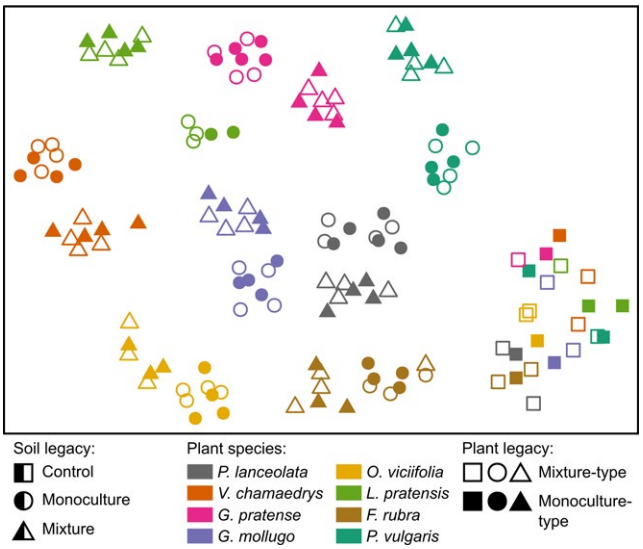

FIGURE 5 t-SNE map of all samples sequenced and analysed (excluding the outlier "Sample492"). Control soils (squares) cluster separately. Within plant species (different colours), microbiomes from monoculture soils (circles) and mixture soils (triangles) also cluster separately, although plants with a history in either monoculture or mixture within species and soil treatments are associated with similar microbiomes. The map was generated using normalized abundances of operational taxonomic units (OTUs) identified as significantly differentially abundant within any of the contrasts tested in this study (2,089 OTUs, Table 4). Note that t-SNE projection axes are arbitrary and dimensions are therefore not shown [Colour figure can be viewed at wileyonlinelibrary.com]

data and performed data analysis. The paper was written by M.W.S., T.H., S.J.V.M. and B.S. with all authors contributing to the final version.

DATA ACCESSIBILITY

Short-reads were deposited at the Short Read Archive (accession number SRP105254). Supplementary tables and files are accessible online and on Zenodo (https://doi.org/10.5281/zenodo.1309178).

ORCID

Marc W. Schmid https://orcid.org/0000-0001-9554-5318
Sofia J. van Moorsel https://orcid.org/0000-0003-1947-8971
Cameron Wagg https://orcid.org/0000-0002-9738-6901
Gerlinde B. De Deyn https://orcid.org/0000-0003-4823-6912
Bernhard Schmid https://orcid.org/0000-0002-8430-3214

REFERENCES

Aggarwal, C. C., Hinneburg, A., & Keim, D. A. (2001). On the surprising behavior of distance metrics in high dimensional space. In J. Van den Bussche, & V. Vianu (Eds.), *Database Theory—ICDT 2001. ICDT 2001. Lecture notes in computer science* (Vol. 1973, pp. 420–434). Berlin. Germany: Springer.

Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D., & Schmid, B. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters*, *9*, 1146–1156. https://doi.org/10.1111/j.1461-0248.2006.00963.x

Bardgett, R. D., & Wardle, D. A. (2003). Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*, *84*(9), 2258–2268. https://doi.org/10.1890/02-0274

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., & Balser, T. (2005). Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspectives in Plant Ecology, Evolution and Systematics*, *7*, 27–49. https://doi.org/10.1016/j.ppees.2004.11.002

Bates, S. T., Berg-Lyons, D., Caporaso, J. G., Walters, W. A., Knight, R., & Fierer, N. (2011). Examining the global distribution of dominant archaeal populations in soil. *The ISME Journal*, *5*(5), 908–917. https://doi.org/10.1038/ismej.2010.171

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere: Plant species, soil type and rhizosphere communities. *FEMS Microbiology Ecology*, *68*(1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Bever, J. D., Mangan, S. A., & Alexander, H. M. (2015). Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 305–325. https://doi.org/10.1146/annurev-ecolsys-112414-054306

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Broughton, L. C., & Gross, K. L. (2000). Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. *Oecologia*, *125*(3), 420–427. https://doi.org/10.1007/s004420000456

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, *64*(1), 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*, 335–336. https://doi.org/10.1038/nmeth.f.303

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., … Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, *486*(7401), 59–67.

Dakora, F. D., & Phillips, D. A. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil*, *245*(1), 35–47.

Dassen, S., Cortois, R., Martens, H., de Hollander, M., Kowalchuk, G. A., van der Putten, W. H., & De Deyn, G. B. (2017). Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. *Molecular Ecology*, *26*(15), 4085–4098. https://doi.org/10.1111/mec.14175

De Deyn, G. B., Quirk, H., & Bardgett, R. D. (2011). Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. *Biology Letters*, *7*(1), 75–78. https://doi.org/10.1098/rsbl.2010.0575

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, *10*(10), 996–998. https://doi.org/10.1038/nmeth.2604

Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M. P., & Mommer, L. (2017). Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Scientific Reports*, *7*, 44641. https://doi.org/10.1038/srep44641

Eisenhauer, N., Reich, P. B., & Scheu, S. (2012). Increasing plant diversity effects on productivity with time due to delayed soil biota effects

on plants. *Basic and Applied Ecology*, *13*(7), 571–578. https://doi.org/10.1016/j.baae.2012.09.002

Eisenhauer, N., Barnes, A. D., Cesarz, S., Craven, D., Ferlian, O., Gottschall, F., … Türke, M. (2016). Biodiversity-ecosystem function experiments reveal the mechanisms underlying the consequences of biodiversity change in real world ecosystems. *Journal of Vegetation Science*, *27*(5), 1061–1070. https://doi.org/10.1111/jvs.12435

Eisenhauer, N., Milcu, A., Sabais, A. C. W., Bessler, H., Brenner, J., Engels, C., … Scheu, S. (2011). Plant diversity surpasses plant functional groups and plant productivity as driver of soil biota in the long term. *PLoS ONE*, *6*(1), e16055. https://doi.org/10.1371/journal.pone.0016055

Fakheran, S., Paul-Victor, C., Heichinger, C., Schmid, B., Grossniklaus, U., & Turnbull, L. A. (2010). Adaptation and extinction in experimentally fragmented landscapes. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(44), 19120–19125. https://doi.org/10.1073/pnas.1010846107

Garbeva, P., Postma, J., van Veen, J. A., & van Elsas, J. D. (2006). Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. *Environmental Microbiology*, *8*(2), 233–246. https://doi.org/10.1111/j.1462-2920.2005.00888.x

Gosling, P., Hodge, A., Goodlass, G., & Bending, G. D. (2006). Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems & Environment*, *113*(1–4), 17–35. https://doi.org/10.1016/j.agee.2005.09.009

Grüter, D., Schmid, B., & Brandl, H. (2006). Influence of plant diversity and elevated atmospheric carbon dioxide levels on belowground bacterial diversity. *BMC Microbiology*, *6*, 68.

Hahl, T. (2017). *Testing for co-adaptation of plants and soil organisms in a biodiversity experiment*. Doctoral Dissertation, University of Zurich, Switzerland. Retrieved from http://www.zora.uzh.ch/id/eprint/145156/

Hooper, D. U., Bignell, D. E., Brown, V. K., Brussard, L., Mark Dangerfield, J., Wall, D. H., … Wolters, V. (2000). Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. *BioScience*, *50*(12), 1049. https://doi.org/10.1641/0006-3568(2000)050[1049:IBAABB]2.0.CO;2

Joshi, J., Schmid, B., Caldeira, M. C., Dimitrakopoulos, P. G., Good, J., Harris, R., … Lawton, J. H. (2001). Local adaptation enhances performance of common plant species. *Ecology Letters*, *4*, 536–544. https://doi.org/10.1046/j.1461-0248.2001.00262.x

Koide, R. T., & Li, M. (1989). Appropriate controls for vesicular-arbuscular mycorrhiza research. *New Phytologist*, *111*, 35–44. https://doi.org/10.1111/j.1469-8137.1989.tb04215.x

Lange, M., Habekost, M., Eisenhauer, N., Roscher, C., Bessler, H., Engels, C., … Gleixner, G. (2014). Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. *PLOS ONE*, *9*, e96182.

Latz, E., Eisenhauer, N., Rall, B. C., Allan, E., Roscher, C., Scheu, S., & Jousset, A. (2012). Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. *Journal of Ecology*, *100*, 597–604.

Latz, E., Eisenhauer, N., Rall, B. C., Scheu, S., & Jousset, A. (2016). Unravelling Linkages between plant community composition and the pathogen-suppressive potential of soils. *Scientific Reports*, *6*, 23584. https://doi.org/10.1038/srep23584

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12). https://doi.org/10.1186/s13059-014-0550-8

Lozupone, C., Lladser, M. E., Knights, D., Stombaugh, J., & Knight, R. (2011). UniFrac: An effective distance metric for microbial community comparison. *ISME Journal*, *5*(2), 169–172. https://doi.org/10.1038/ismej.2010.133

Magurran, A. (2004). *Measuring biological diversity*. Oxford, UK: Blackwell Publishers.

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: Why rarefying microbiome data is inadmissible. *PLoS Computational Biology*, *10*(4), e1003531. https://doi.org/10.1371/journal.pcbi.1003531

Neter, J., & Wassermann, W. (1974). *Applied linear statistical models*. Homewood, IL: Richard D. Irwin Inc.

Niklaus, P. A., Baruffol, M., He, J.-S., Ma, K., & Schmid, B. (2017). Can niche plasticity promote biodiversity–productivity relationships through increased complementarity? *Ecology*, *98*, 1104–1116.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P.,McGlinn, D.,… Wagner, H. (2017). *Vegan: Community ecology package*. Retrieved from https://CRAN.R-project.org/package=vegan

Pertea, G. (2009). *Fqtrim 0.9.4*. Retrieved from https://doi.org/10.5281/zenodo.20552

Pielou, E. (1975). *Ecological diversity*. New York, NY: Wiley and Sons.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., … Glockner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596.

Reich, P. B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S. E., Flynn, D. F. B., & Eisenhauer, N. (2012). Impacts of biodiversity loss escalate through time as redundancy fades. *Science*, *336*(6081), 589–592.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W. W., … Schulze, E.-D. (2004). The role of biodiversity for element cycling and trophic interactions: An experimental approach in a grassland community. *Basic and Applied Ecology*, *5*(2), 107–121. https://doi.org/10.1078/1439-1791-00216

Roscher, C., Schumacher, J., Lipowsky, A., Gubsch, M., Weigelt, A., Pompe, S., … Schulze, E.-D. (2013). A functional trait-based approach to understand community assembly and diversity–productivity relationships over 7 years in experimental grasslands. *Perspectives in Plant Ecology, Evolution and Systematics*, *15*(3), 139–149. https://doi.org/10.1016/j.ppees.2013.02.004

Rossi-Tamisier, M., Benamar, S., Raoult, D., & Fournier, P.-E. (2015). Cautionary tale of using 16S rRNA gene sequence similarity values in identification of human-associated bacterial species. *International Journal of Systematic and Evolutionary Microbiology*, *65*, 1929–1934. https://doi.org/10.1099/ijs.0.000161

Schlatter, D. C., Bakker, M. G., Bradeen, J. M., & Kinkel, L. L. (2015). Plant community richness and microbial interactions structure bacterial communities in soil. *Ecology*, *96*(1), 134–142. https://doi.org/10.1890/13-1648.1

Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology*, *44*, 846–849. https://doi.org/10.1099/00207713-44-4-846

Stephan, A., Meyer, A. H., & Schmid, B. (2000). Plant diversity affects culturable soil bacteria in experimental grassland communities. *Journal of Ecology*, *88*(6), 988–998. https://doi.org/10.1046/j.1365-2745.2000.00510.x

Tilman, D., Reich, P. B., & Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, *441*(7093), 629–632.

van der Maaten, L. (2014). Accelerating t-SNE using tree-based algorithms. *Journal of Machine Learning Research*, *15*, 1–21.

van der Maaten, L., & Hinton, G. (2008). Visualizing data using t-SNE. *Journal of Machine Learning Research*, *9*, 2579–2605.

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., … Wardle, D. A. (2013). Plant–soil feedbacks: The past, the present and future challenges. *Journal of Ecology*, *101*(2), 265–276. https://doi.org/10.1111/1365-2745.12054

van Moorsel, S. J., Hahl, T., Wagg, C., De Deyn, G. B., Flynn, D. F. B., Zuppinger-Dingley, D., & Schmid, B. (2018). Community evolution

increases plant productivity at low diversity. *Ecology Letters*, *21*, 128–137.

van Moorsel, S. J., Schmid, M. W., Hahl, T., Zuppinger-Dingley, D., & Schmid, B. (2018). Selection in response to community diversity alters plant performance and functional traits. *Perspectives in Plant Ecology, Evolution and Systematics*, *33*, 51–61.

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(14), 5266–5270. https://doi.org/10.1073/pnas.1320054111

Whipps, J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, *52*, 487–511. https://doi.org/10.1093/jxb/52.suppl_1.487

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., … Glöckner, F. O. (2014). The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research*, *42*(D1), D643–D648. https://doi.org/10.1093/nar/gkt1209

Zuppinger-Dingley, D., Flynn, D. F. B., Brandl, H., & Schmid, B. (2015). Selection in monoculture vs. mixture alters plant metabolic fingerprints. *Journal of Plant Ecology*, *8*(5), 549–557. https://doi.org/10.1093/jpe/rtu043

Zuppinger-Dingley, D., Flynn, D. F. B., De Deyn, G. B., Petermann, J. S., & Schmid, B. (2016). Plant selection and soil legacy enhance long-term biodiversity effects. *Ecology*, *97*(4), 918–928.

Zuppinger-Dingley, D., Schmid, B., Petermann, J. S., Yadav, V., De Deyn, G. B., & Flynn, D. F. B. (2014). Selection for niche differentiation in plant communities increases biodiversity effects. *Nature*, *515*(7525), 108–111.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Schmid MW, Hahl T, van Moorsel SJ, Wagg C, De Deyn GB, Schmid B. Feedbacks of plant identity and diversity on the diversity and community composition of rhizosphere microbiomes from a long-term biodiversity experiment. *Mol Ecol*. 2019;28:863–878. https://doi.org/10.1111/mec.14987