


Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity

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Abstract

Plants are known to influence belowground microbial community structure along their roots, but the impacts of plant species richness and plant functional group (FG) identity on microbial communities in the bulk soil are still not well understood. Here, we used 454-pyrosequencing to analyse the soil microbial community composition in a long-term biodiversity experiment at Jena, Germany. We examined responses of bacteria, fungi, archaea, and protists to plant species richness (communities varying from 1 to 60 sown species) and plant FG identity (grasses, legumes, small herbs, tall herbs) in bulk soil. We hypothesized that plant species richness and FG identity would alter microbial community composition and have a positive impact on microbial species richness. Plant species richness had a marginal positive effect on the richness of fungi, but we observed no such effect on bacteria, archaea and protists. Plant species richness also did not have a large impact on microbial community composition. Rather, abiotic soil properties partially explained the community composition of bacteria, fungi, arbuscular mycorrhizal fungi (AMF), archaea and protists. Plant FG richness did not impact microbial community composition; however, plant FG identity was more effective. Bacterial richness was highest in legume plots and lowest in small herb plots, and AMF and archaeal community composition in legume plant communities was distinct from that in communities composed of other plant FGs. We conclude that soil microbial community composition in bulk soil is influenced more by changes in plant FG composition and abiotic soil properties, than by changes in plant species richness per se.

KEYWORDS

α -diversity, β -diversity, arbuscular mycorrhizal fungi, microbial diversity, plant community diversity, rhizobia

1 | INTRODUCTION

Soil microbes are known to be critical to terrestrial ecosystem functioning and are key determinants of plant community composition

and diversity (Bardgett & van der Putten, 2014; Van der Heijden, Bardgett, & Van Straalen, 2008). The majority of plant–soil microbe studies have focussed on the interface between plant roots and soil, the rhizosphere (Buée et al., 2009; Mellado-Vázquez et al., 2016;

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Philippot, Raaijmakers, Lemanceau, & van der Putten, 2013). However, less is known about the contribution of plant communities in driving the composition and diversity of microbes in bulk soil (Vos, Wolf, Jennings, & Kowalchuk, 2013). Here, we examine how plant diversity and soil abiotic properties influence bulk soil microbial community richness and composition.

Increasing plant species richness has generally been shown to lead to increased soil microbial biomass (Eisenhauer et al., 2010; Scherber et al., 2010; Zak, Holmes, White, Peacock, & Tilman, 2003) and activity (Eisenhauer et al., 2010). One explanation is that increased plant species richness diversifies the resource pool so that the biomass of a larger variety of soil microbes may be sustained (Milcu, Partsch, Langel, & Scheu, 2006; Sauheitl, Glaser, Dippold, Leiber, & Weigelt, 2010). Another possibility is that increasing plant species richness enhances the amount of primary production, which in turn may increase the biomass of soil fungi and bacteria (De Deyn, Quirk, & Bardgett, 2011). Plant community diversity effects on ecosystems might be due to plant species richness per se or to richness of their traits, or functions (Díaz & Cabido, 2001). It has been proposed that variation in plant traits can, at least partially, explain variation in soil microbial community composition (Bardgett, Mommer, & De Vries, 2014; Cornwell et al., 2008; Legay et al., 2014; Reich, 2014).

Grasses, legumes, tall herbs and small herbs represent typical plant functional groups that may fulfil different roles in ecosystems because of their distinct traits (Hector, 1999; Roscher, Schumacher, & Baade, 2004). Several plant traits that are typical for a particular plant FG have been shown to be related to microbial community composition and functioning (Baxendale, Orwin, Poly, Pommier, & Bardgett, 2014; Cantarel et al., 2015; Cortois, Schröder-Georgi, Weigelt, van der Putten, & De Deyn, 2016; Legay et al., 2014). For example, specific root length (SRL) is different between small and tall herbs and SRL is related to arbuscular mycorrhizal fungi root colonization (Cortois et al., 2016). The C:N ratio of legumes is typically lower than in other plant FGs (Abbas et al., 2013), and C:N ratio is a good predictor of litter decomposition (Chen et al., 2016). Few studies have examined the effects of plant species richness and plant FG identity in long-term experiments in the field, and most have been limited to a subset of the soil microbial community (König et al., 2010; Schlatter, Bakker, Bradeen, & Kinkel, 2015; Waldrop, Zak, Blackwood, Curtis, & Tilman, 2006).

When examining plant impacts on soil-borne microbial communities, it is relevant to not only examine the species richness of individual communities, *that is*, α -diversity, but also the identity and abundance of each species in the community, *that is*, β -diversity, which can be expressed in (dis)similarity between microbial communities (Anderson et al., 2011; Whittaker, 1972), or in community evenness (Simpson, 1949). To date, few studies have attempted to relate plant species richness to microbial species richness in bulk soil, and their outcomes have been variable. While some studies have reported no correlation between species richness of plants and species richness of bacteria (Schlatter et al., 2015; Sugiyama, Zayed, & Okubo, 2008) or fungi (Waldrop et al., 2006), Sugiyama et al., (2008) found a positive correlation between plant species richness and

fungal richness in bulk soil. Differences in outcomes across studies may be due to the use of different methodologies (fingerprinting, cloning and sequencing, or pyrosequencing), which can vary in depth and phylogenetic resolution (Bent et al., 2007), or differences in the soil types and plant communities examined. Potential impacts of plant diversity may also be masked by differences in other environmental variables, such as pH, soil structure and soil moisture, which may vary across and between experimental field sites.

Although higher plant diversity may impact the heterogeneity of associated soil habitats, levels of plant and microbial species richness differ several orders of magnitude. Moreover, it is not clear if higher plant species richness should dictate higher soil microbial species richness (Vos et al., 2013). Indeed, the major observable changes in microbial communities related to differences in plant community structure and diversity originate from shifts in the relative abundances of particular microbial species, as opposed to changes in absolute microbial species richness (Schlatter et al., 2015; Waldrop et al., 2006). Soil analyses of grassland biodiversity experiments also have shown that plant functional groups can differ in their effects on the abundances of fungi and bacteria. For example, the presence of legumes generally decreased the biomass of soil fungi (Bartelt-Ryser, Joshi, Schmid, Brandl, & Balsler, 2005; Lange et al., 2014), small herbs increased total soil microbial biomass, and tall herbs and grasses did not change microbial biomass (Strecker et al., 2015). Although such studies show that plant functional group identity can impact the abundance of soil microbes, they do not provide insight into influences on soil microbial community structure. Changes in plant community composition may have different effects on bacterial than on fungal community composition (Sugiyama et al., 2008). It has been suggested that fungal communities are linked more tightly to standing vegetation because of associations with the living plant and saprotroph activity (Millard & Singh, 2010), or because fungi are more responsive to soil nutrient status than bacteria (Lauber, Strickland, Bradford, & Fierer, 2008).

The analysis of specific microbial groups may also help to understand the impacts of plant community composition on soil microbes. Examples of interesting specific microbial groups are arbuscular mycorrhizal fungi (AMF), rhizobia, archaea and protists. Although AMF are generally thought to be rather nonspecific with respect to host range (Jansa, Smith, & Smith, 2008; Klironomos, 2000), results across gradients in plant composition would suggest at least a degree of host specificity in the field (Hedlund, Santa Regina, & van der Putten, 2003; Hiiesalu et al., 2014; König et al., 2010). Furthermore, it has been shown that also plant species identity can influence both the abundance (De Deyn et al., 2011) and identity of AMF species (Scheublin, Ridgway, Young, & Van Der Heijden, 2004; Vandenkoornhuysen et al., 2002). Both findings indicate that plant community diversity and plant FG composition both can influence AMF community composition in soil.

Given the stricter host specificity of rhizobia compared to AMF, it is not surprising that the number of rhizobial species in soil has been shown to increase with increasing legume biomass (Van der Heijden et al., 2006), as legumes often harbour multiple nitrogen-

fixing symbiont species at the same time (De Meyer, Van Hoorde, Vekeman, Braeckman, & Willems, 2011). Nevertheless, the main drivers of rhizobial intraspecific and interspecific diversity have generally been shown to be abiotic soil properties such as soil nitrogen and phosphate levels (Palmer & Young, 2000), pH (Rodríguez-Echeverría, Moreno, & Bedmar, 2014; Van Cauwenberghe, Michiels, & Honnay, 2015) and land management (Palmer & Young, 2000). Archaea have been shown to be important across various terrestrial ecosystems (Offre, Spang, & Schleper, 2013; Prosser, 2012), but no studies have been reported to date that examine how their richness and community structure are impacted by plant functional group identity or diversity. In contrast, soil protist communities, which are highly species diverse and integral to soil functioning (Geisen, 2016), have been reported to be responsive to plant identity (Turner et al., 2013), and to plant FG identity and richness (Glaser et al., 2015; Ledeganck, Nijs, & Beyens, 2003). Clearly, it may be hard to find general patterns of plant species richness and composition on soil microbial community diversity, but it is worthwhile to investigate whether or not we can find them.

The aim of this study was to investigate how the diversity and composition of microbial communities responds to plant community diversity. These relationships were examined within the context of the large-scale plant biodiversity experiment in Jena, Germany, in which plant communities varying from 1 to 60 plant species were sown and maintained within a replicated experimental design. We used 454-pyrosequencing of small subunit ribosomal RNA markers to determine the community structure and diversity of bacteria, archaea, fungi and protists in bulk soil samples collected 8 years into this field experiment. Community patterns were examined in the light of plant species diversity, plant functional group composition and abiotic factors within the experimental field sites. We hypothesized that sown plant diversity and plant functional group diversity would have a positive effect on soil microbial richness of bacteria, rhizobia, archaea, fungi, AMF and protists. However, given the intrinsically high diversity within soil communities (Torsvik & Øvreås, 2002), we expected any such effects to be minor. In addition, we hypothesized that microbial community composition would be impacted by plant functional group, and that this effect would be stronger for fungi than bacteria.

2 | METHODS

2.1 | Study site, experimental design and management

The study site used for sampling is the biodiversity long-term biodiversity grassland experiment at Jena, Germany, established in 2002. A total of 60 European grassland species (grasses, legumes, small herbs and tall herbs) were sown in plots of 20 × 20 m at sowing diversity levels of 1, 2, 4, 8, 16 and 60 plant species, and functional group richness was varied from 1 to 4 (Roscher et al., 2004). The experimental field site has four blocks, with block 1 situated closest to the river "Saale" and block 4 furthest away from the river, thereby

covering a soil texture gradient with different proportions of sand and silt and a pH gradient from 7.59 to 8.15, with decreasing acidity with increasing distance from the river. Each block is a replication of the functional group gradient and plant species richness gradient, whereby plant identities within each plant community at each level of plant species and functional group richness vary among the blocks to avoid sampling effects (Roscher et al., 2004). Plots are mown twice a year and hand-weeded three times per year to remove non-sown plant species; target plant species that disappear from the plots are not re-sown. Data on plant cover were obtained by the Jena Consortium (Marquard et al., 2013). Data on total soil organic carbon (TOC) were obtained by Maike Habekost and Gerd Gleixner (<https://doi.org/10.1594/pangaea.848944>). Data for soil texture were collected by Anke Hildebrand and Jussi Baade (<https://doi.org/10.1007/s11104-014-2373-5> and <https://doi.org/10.1111/j.1365-2486.2008.01697.x>) (Fischer et al., 2015; Steinbeiss et al., 2008). An overview of the soil characteristics across the experimental site is provided (Table S1).

2.2 | Soil sampling

In September 2010, soil samples were collected across 82 experimental plots: 16 monocultures, 16 2-species mixtures, 16 4-species mixtures, 16 8-species mixtures, 14 16-species mixtures and 4 60-species mixtures. Per plot, five 2.5-cm-diameter soil cores were taken to a depth of 15 cm deep at random vegetation-bearing sampling points. These five soil samples were pooled and sieved using a 2-mm mesh to remove plant roots and other large organic material. A subsample from each pooled sample was taken to determine soil pH, using standard methods, and the remainder frozen and kept at -20°C until DNA extraction.

2.3 | DNA extraction and amplification

DNA was extracted from 0.25 g soil with the PowerSoil[®] DNA Isolation Kit (MoBio, Carlsbad, CA, USA). We amplified 16S rRNA gene fragments from bacteria and archaea using the primer combination 515f/806r (Bates et al., 2011). The amplification mixture contained 0.1 pmol/ μl of each primer, 1 μl DNA, 200 μM dNTPs, 0.056 U FastStart[™] High Fidelity PCR System (ROCHE) and 10× PCR buffer, in a total reaction volume of 25 μl . The PCR amplification of 16S had an initial annealing temperature of 95°C (5 min), then 30 cycles with denaturation at 95°C (30 s), annealing at 53°C (1 min), extension at 72°C (1 min) and final extension 72°C (10 min). We amplified 18S rRNA gene fragments from fungi and protists with primer FR 1 and the modified version of FF390 5'-CGWTAACGAACGAGACCT-3' designed to also include the Glomeromycota (Kuramae et al., 2013). The amplification mixture contained 0.1 pmol/ μl of each primer, 1 μl DNA, 200 μM dNTPs, 0.056 U FastStart[™] High Fidelity PCR System (ROCHE), 10× PCR buffer, 2.5 μM MgCl_2 and 0.5 mg/ml bovine serum albumin. The thermalcycling program consisted of an initial annealing temperature at 95°C (5 min), followed by 30 cycles with denaturation at 95°C (30 s), annealing at 58°C (30 s), extension at

72°C (1 min) and final extension at 72°C (10 min). The primers included unique tags for the 82 plots from which the samples were taken. Amplicons were purified prior to sequencing with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) protocol, according to the manufacturer's protocol. After quantification of PCR products, equal amounts of each sample were combined on a 96-well sequence plate, one full plate for each set of primers. The samples were subjected to Roche 454 automated sequencer and GS FLX system using titanium chemistry (Macrogen Seoul, Korea).

2.4 | Sequence processing

Sequences were analysed using a Snakemake workflow (Köster & Rahmann, 2012) that follows the SOP for 454 data in MOTHER version 1.33.2 (Schloss et al., 2009). Flowgrams were denoised and quality filtered using the `shhh.flows` command (Quince, Lanzen, Davenport, & Turnbaugh, 2011), which includes de-multiplexing and trimming of the flowgrams. To further reduce sequence errors the `pre.cluster` command was used to merge sequences that were within two mismatches of each other. Chimeric sequences were removed using `chimeras.uchime` command (Edgar, Haas, Clemente, Quince, & Knight, 2011). Clustering of reads into OTUs was performed at a 97% identity threshold using the `dist.seqs` command and average neighbour clustering. A 97% threshold does not allow clear species or genus distinctions of the reads; however, it does provide robust diversity comparisons that avoid artificial inflation of diversity due to methodological issues such as PCR errors (Behnke et al., 2011; Wintzingerode, Göbel, & Stackebrandt, 1997). Sequences were aligned and classified with SINA (Pruesse, Peplies, & Glöckner, 2012) against the SILVA 119 database (Quast et al., 2013). The initial attempt to classify sequences with the RDP database (Wang, Garrity, Tiedje, & Cole, 2007) resulted in a large proportion of OTUs classified as bacteria but without further taxonomic depth. For each OTU, a consensus taxonomy was determined based on the lowest taxonomic ancestor, using the `classify.otu` command. Taxonomic classification and OTU clustering data are combined into the BIOM format (McDonald et al., 2012) for further downstream statistical analysis.

2.5 | Data analysis

OTUs that had two reads or fewer were removed from the data set. Rarefaction was performed using R (version 3.0.3) (R Development Core Team) with package "vegan" 2.2-1 (Oksanen et al., 2015), using the `Rarefy` command. Rarefaction curves were made to identify samples with insufficient sampling depth (Fig. S1). For bacterial and for fungal samples, the cut-off was set at 4,000 reads per sample, resulting in 69 and 67 sequence libraries for bacteria and fungi, respectively. For archaea, the cut-off was set at 750 reads per sample resulting in 77 sequence libraries, and for protists, the cut-off was set at 100 (66 sequence libraries). For N-fixers, a threshold of 15 reads per sample was set (48 sequence libraries), and for AMF the cut-off was at 100 reads per sample (59 sequence libraries). Prior to further analyses, the observed number of reads was rarefied

to the cut off made in the previous step. Bar-plots of the relative OTU abundances of bacteria plus archaea and fungi plus protists in relation to plant species richness were made in Excel (Microsoft Excel).

Hand weeding was necessary to maintain the plant species diversity treatments. We tested how the periodically removed plant species may have influenced the observed relationships between microbial, plant species and plant functional group diversities. We found that the effect of actual plant species numbers (both sown and those periodically removed by hand weeding) on microbial species richness was not significant for none of the six microbial groups examined. Because removal of weed species can be seen as a soil disturbance factor, weed cover percentage per plot was included in the models described below.

To test for a correlation between plant sown diversity and OTU richness or microbial community evenness (inverse Simpson's index), a linear mixed effect model was used with block included as a random factor and pH, TOC, soil texture and weed cover percentage as co-variables. Sown plant diversity was fit in the models and presented in figures as a logarithm with base 2. This was done to have a more equal distribution of step size between the levels of sown plant diversity. The model was made in R with the package "nlme" (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2013), and ANOVA was used to produce test statistics. Corresponding figures were made in R with the package "ggplot2" (H. Wickham, 2009). To further investigate the positive correlation between fungal OTU richness and plant sown diversity, the partitioning of OTU turnover and nestedness were calculated as described by Baselga (2010). Multiple site dissimilarity measure was used to obtain values for the partitioning of nestedness and turnover. Differences in plant sown diversity between plots were calculated with Euclidean distance. Subsequently pairwise dissimilarity measure was used to tests for a correlation between nestedness or turnover and plant sown diversity via a mantel test (1,000 permutations).

To test whether plant sown diversity and abiotic soil properties influence microbial community composition, distance-based redundancy analysis (db-RDA) was performed with forward selection of the explanatory plant and soil variables. Community distances were calculated with the Bray–Curtis measure, and explanatory variables were included into the model if P_{adj} was <0.05 . If the microbial community composition could not be explained by any of the variables in the model, nonmetric multidimensional scaling (NMDS) was used to show the sample distribution of the microbial communities in the different plots. All plots with mixtures of plant functional groups were excluded when testing for differences in microbial diversity between the plant functional groups across the plant species richness gradient.

To test for a correlation between plant functional group richness and OTU richness, a linear mixed effect model was used with block included as a random factor and pH, TOC, soil texture and weed cover percentage as co-variables. The model was made in R with the package "nlme" (Pinheiro et al., 2013), and ANOVA was used to produce test statistics. To test the responses of OTU

richness to plant functional group identity, Poisson generalized linear model (GLM) was used with sown plant diversity and plant functional group identity and their interaction as predicting factors, while using pH, SOM, soil texture and weed cover percentage as co-variables; block effect was not included because the functional groups were not evenly distributed over the blocks. The interaction between plant sown diversity and plant functional group was removed from the model if the reduced model was a better predictor for microbial OTU richness. Post hoc Tukey's test was used to identify which plant functional groups were different from one another. To test for plant functional group identity effects on microbial community composition, db-RDA with forward selection was used as described above. The NMDS, db-RDA analyses and corresponding plots were performed in CANOCO (version 5.0), and the linear models and graphs were made in R with package "ggplot2" (H. Wickham, 2009).

3 | RESULTS

3.1 | Diversity of soil bacteria and fungi

Amplification of 16S rRNA gene fragments yielded in total 4,025 bacterial, 23 archaeal and 826 unclassified OTUs, respectively, at a 97% similarity threshold. Amplification of eukaryotic 18S rRNA fragments yielded 431 fungal, 174 protist, 9 plant and 374 unclassified OTUs, respectively. All unclassified OTUs and OTUs of plant origin were excluded from further analyses. We could classify 84% of the

bacterial and 71% of the fungal OTUs to at least an order level of taxonomic resolution. For the bacteria, 39% of OTUs could be identified to the genus level, whereas only 11% of the fungi could be identified to at least the genus level.

The most dominant taxonomic group of bacteria was the *Chloroflexi*, based on the relative abundance of the sequence reads (Figure 1a). The most diverse bacterial groups were *Proteobacteria* and *Planctomycetes* with 1,045 and 655 OTUs, respectively (Figure 1a). A total of 19 putative rhizobial OTUs were recovered across the experimental fields (Table S2). The most dominant taxonomic group of eukaryotes was *Ascomycota*, which was also the most diverse fungal group with 177 OTUs (Figure 1b). In total, 19 AMF OTUs (phylum *Glomeromycota*) were recovered across all plant communities (Figure 1b). Of the main protist supergroups, Rhizaria were well represented in our data, even though the FR1 primer used has some fungal specificity. Although protists represent a relatively small proportion ($\approx 2\%$) of the total eukaryotic community, their diversity was considerable, with 174 detected OTUs.

3.2 | Sown plant diversity effects on microbial richness and community composition

The mean number of bacterial OTUs per plot did not increase significantly with increasing sown plant diversities ($F_{1,58} = 1.793$, $p = .186$) (Table S3, Figure 2a). Fungi, however, showed a trend of increasing OTU richness with increasing sown plant diversity ($F_{1,56} = 3.960$, $p = .052$) (Table S3, Figure 2d). There was also no correlation

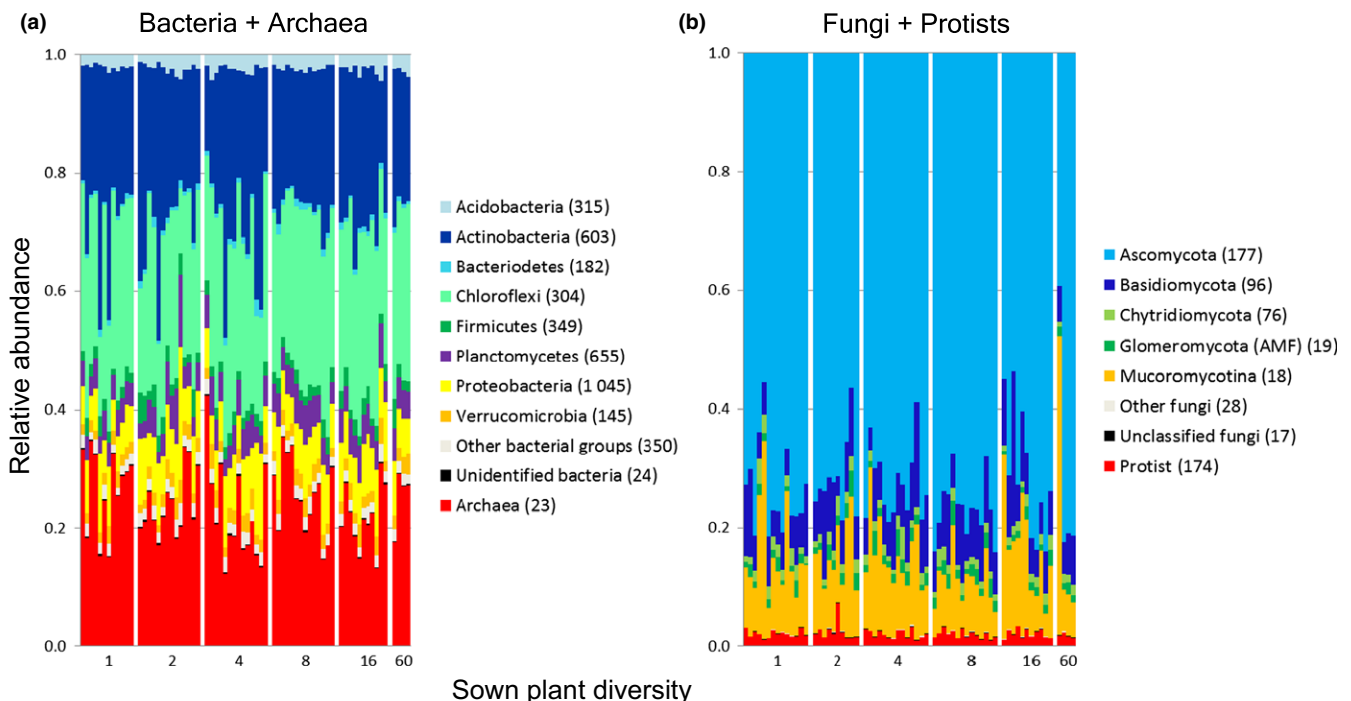


FIGURE 1 Relative composition of the prokaryotic (a) and eukaryotic (b) communities in relation to sown diversity. Each stacked bar represents the observed microbial taxonomic groups in a plot as a fraction of all observed taxa. Plots were grouped according to level of sown diversity (1, 2, 4, 8, 16, and 60 plant species per plot) that was artificially maintained by hand weeding. In the legend, taxonomic groups are followed by a number between brackets, which indicates the total number of different OTUs that could be classified to that group

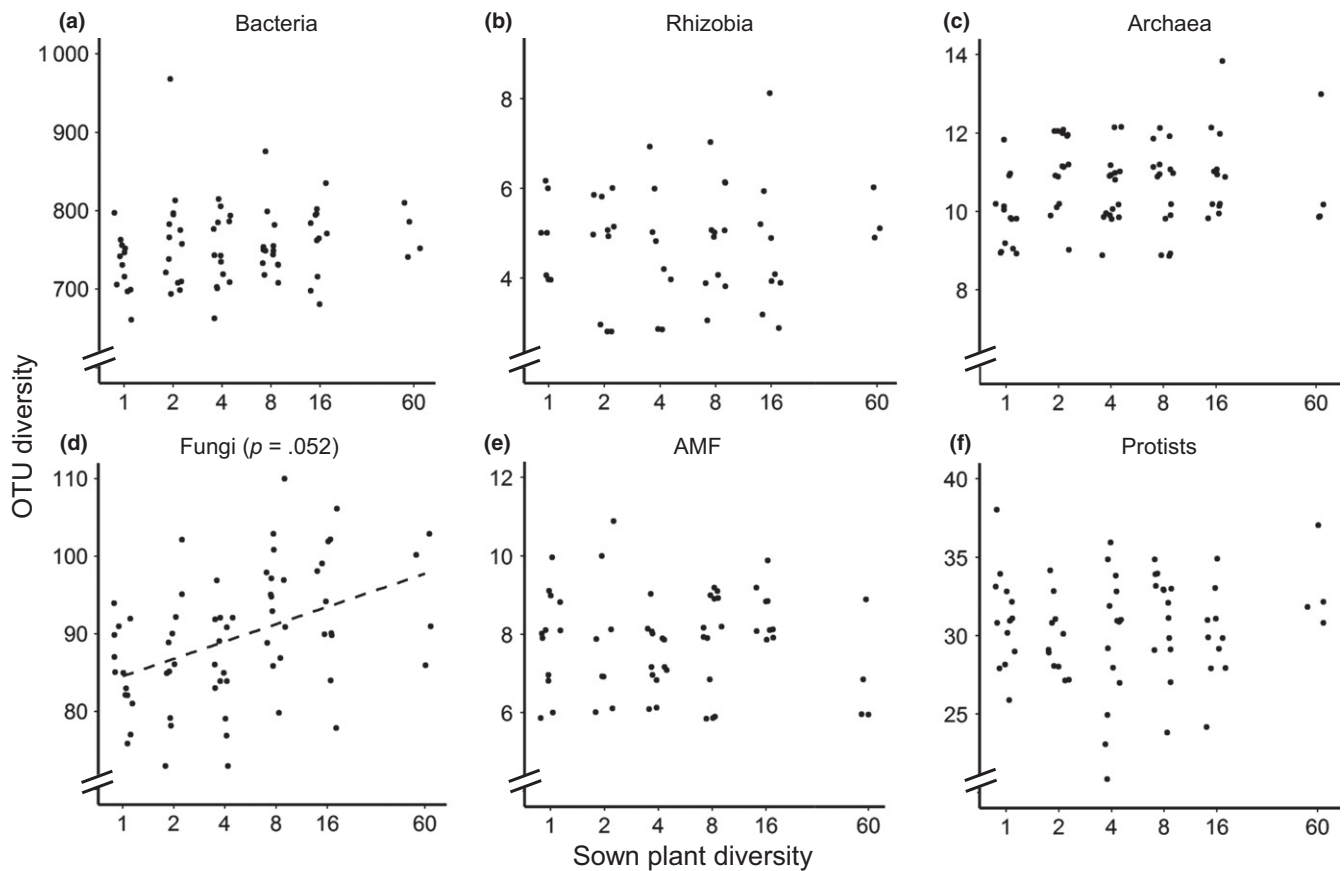


FIGURE 2 Relationship between (log) plant sown diversity and OTU diversity of bacteria (a), rhizobia (b), archaea (c), fungi (d), AMF (e) and protists (f). To avoid overlap, the dots in the graph are spread around their actual value. The trend line in Figure 2d was based on fixed effects only

observed between sown plant richness and the OTU richness of rhizobia ($F_{1,37} = 0.517$, $p = .477$), AMF ($F_{1,48} = 0.081$, $p = .777$), archaea ($F_{1,66} = 0.524$, $p = .472$) or protists ($F_{1,55} = 0.275$, $p = .602$) (Table S3, Figure 2b,c,e,f).

Neither of the abiotic soil properties measured, nor the disturbance of the soil expressed in weed cover, showed a significant correlation with microbial richness parameters (Table S3). To investigate the trend of increased fungal OTU richness and sown plant diversity further, the partitioning of nestedness and turnover was calculated. Turnover ($r = .036$, $p = .324$) and nestedness ($r = .035$, $p = .272$) were not significantly correlated with sown plant diversity. Calculations based on multiple site dissimilarities showed that the majority of variation is partitioned to turnover ($\beta_{JTU} = 0.958$) and not to nestedness ($\beta_{JNE} = 0.006$).

Community evenness is a measure for how well abundance is distributed over all species in the communities. Higher levels of evenness represent a community with similar abundances per species, whereas low levels of evenness represent communities with more extreme variation in abundances per species. Plant sown diversity was not correlated with bacterial ($F_{1,58} = 0.194$, $p = .661$) or fungal ($F_{1,56} = 1.704$, $p = .197$) community evenness (Table S4, Fig. S2a, d). Plant sown diversity was positively related with evenness for rhizobial communities ($F_{1,37} = 5.280$, $p = .027$) (Fig. S2b,

Table S4), but there was no relation between plant sown diversity and community evenness of AMF, archaea or protists (Fig. S2; Table S4). Weed cover percentage, soil texture and TOC influenced microbial community evenness (Table S4). Bacterial evenness was unaffected by the measured soil abiotic factors, whereas fungal evenness decreased with increasing percentage weed cover. Rhizobial and AMF community evenness were also unaffected by soil abiotic factors. Community evenness of archaea was positively correlated with the percentage TOC ($F_{1,66} = 14.801$, $p < .001$). Soil structure affected evenness of the protist communities, with higher evenness related to a higher sand fraction ($F_{1,55} = 4.197$, $p = .045$) and lower community evenness with an increased fraction of silt ($F_{1,55} = 4.232$, $p = .044$) and clay ($F_{1,55} = 4.209$, $p = .045$).

Sown plant diversity had no effect on community composition on any of the microbial groups investigated, but abiotic soil properties did. The community composition of bacteria and fungi was influenced by soil sand fraction, which explained 4.7% of the variation in community composition of bacteria (db-RDA pseudo- $F = 3.3$, $P_{\text{adj}} = 0.012$), as well as 4.7% for fungi (db-RDA pseudo- $F = 3.2$, $P_{\text{adj}} = 0.014$) (Figure 3a,d). The composition of the rhizobial communities was not related to any of the abiotic soil properties measured (Figure 3b). Sand proportion could explain 6.75 of the variation in AMF communities (db-RDA pseudo- $F = 4.1$,

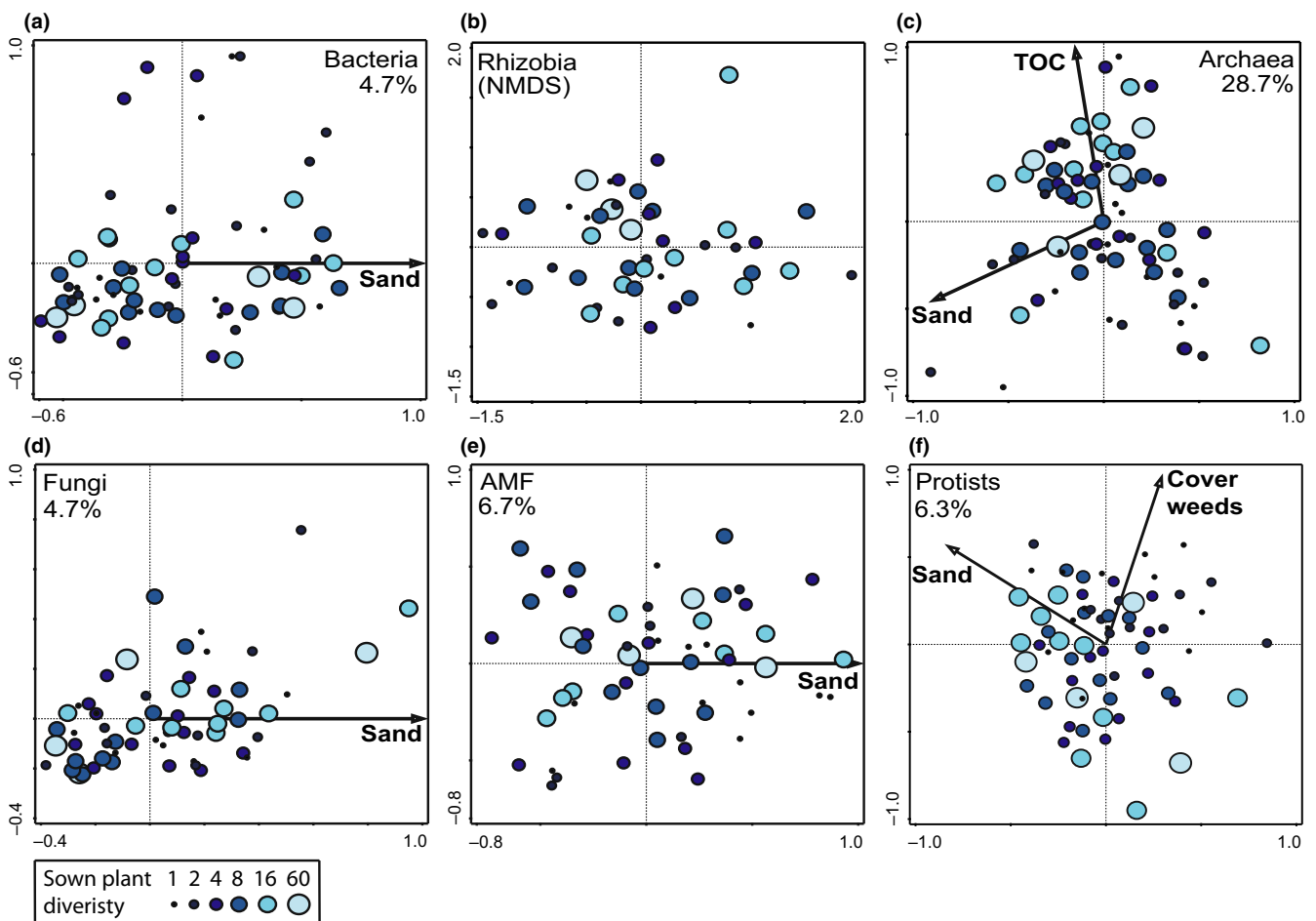


FIGURE 3 Distance-based redundancy analysis (db-RDA) plot showing the relationship of abiotic soil factors and (log) plant sown diversity (SD) to community composition of bacteria (a), archaea (c), fungi (d), AMF (e), and protists (f). The ordination is based on Bray–Curtis distance. With forward selection, factors were chosen that significantly ($P_{\text{adj}} < 0.05$) contributed to the model. In each window, the percentage of explained variation is shown. Rhizobial community composition could not be explained by any of the factors measured, therefore a nonmetric multidimensional scaling (NMDS) of the community is shown instead (b)

$P_{\text{adj}} = 0.012$; Figure 3e). Archaea communities responded to percentage sand (db-RDA pseudo- $F = 20.5$, $P_{\text{adj}} = 0.012$) and TOC (db-RDA pseudo- $F = 7.6$, $P_{\text{adj}} = 0.012$). Sand and TOC percentage together explained 28.7% of the variation in archaeal community composition (Figure 3c). Protist communities were influenced by weed cover percentage (db-RDA pseudo- $F = 2.0$, $P_{\text{adj}} = 0.042$) and sand (db-RDA pseudo- $F = 2.2$, $P_{\text{adj}} = 0.014$). TOC and sand percentage together explained 6.3% of the variation in protist community composition (Figure 3f).

3.3 | Plant functional group (FG) effects on microbial richness and community composition

Plant FG richness did not significantly affect OTU richness of any of the microbial groups (Table S5). Abiotic soil properties also did not influence microbial richness, which is in line with results presented above (Table S3), except for weed cover percentage. Fungal OTU richness decreased with increasing weed cover percentage ($F_{1,56} = 5.259$, $p = .026$) (Table S5).

To test the effect of plant FG identity on microbial richness and community composition, we confined analyses to plots with plant species belonging to only a single plant FG. Identity of plant FG significantly affects the OTU richness of bacteria, with significant differences between all plant FGs except between grasses and tall herbs (Table S6, Fig. S3a). Plant FG identity had no effect on OTU richness of fungi or any of the other microbial groups examined (Table S6, Fig. S3).

Plant functional group identity did not explain a significant proportion of the variation in bacterial, fungal or rhizobial community composition (Figure 4). In contrast, AMF communities in legume plots were however distinct from those in grass and herb plots (Figure 4e) and explained 16.1% of the variation in AMF communities (db-RDA pseudo- $F = 4.2$, $P_{\text{adj}} = 0.02$). Archaeal communities in legume plots were also distinct from those in grass and herb plots (Figure 4c), and FG explained 14.0% of the variation in archaeal communities (db-RDA pseudo- $F = 6.7$, $P_{\text{adj}} = 0.02$). Plant FG identity had no significant explanatory power in relation to protist community composition (Figure 4f). Abiotic soil properties did not explain a

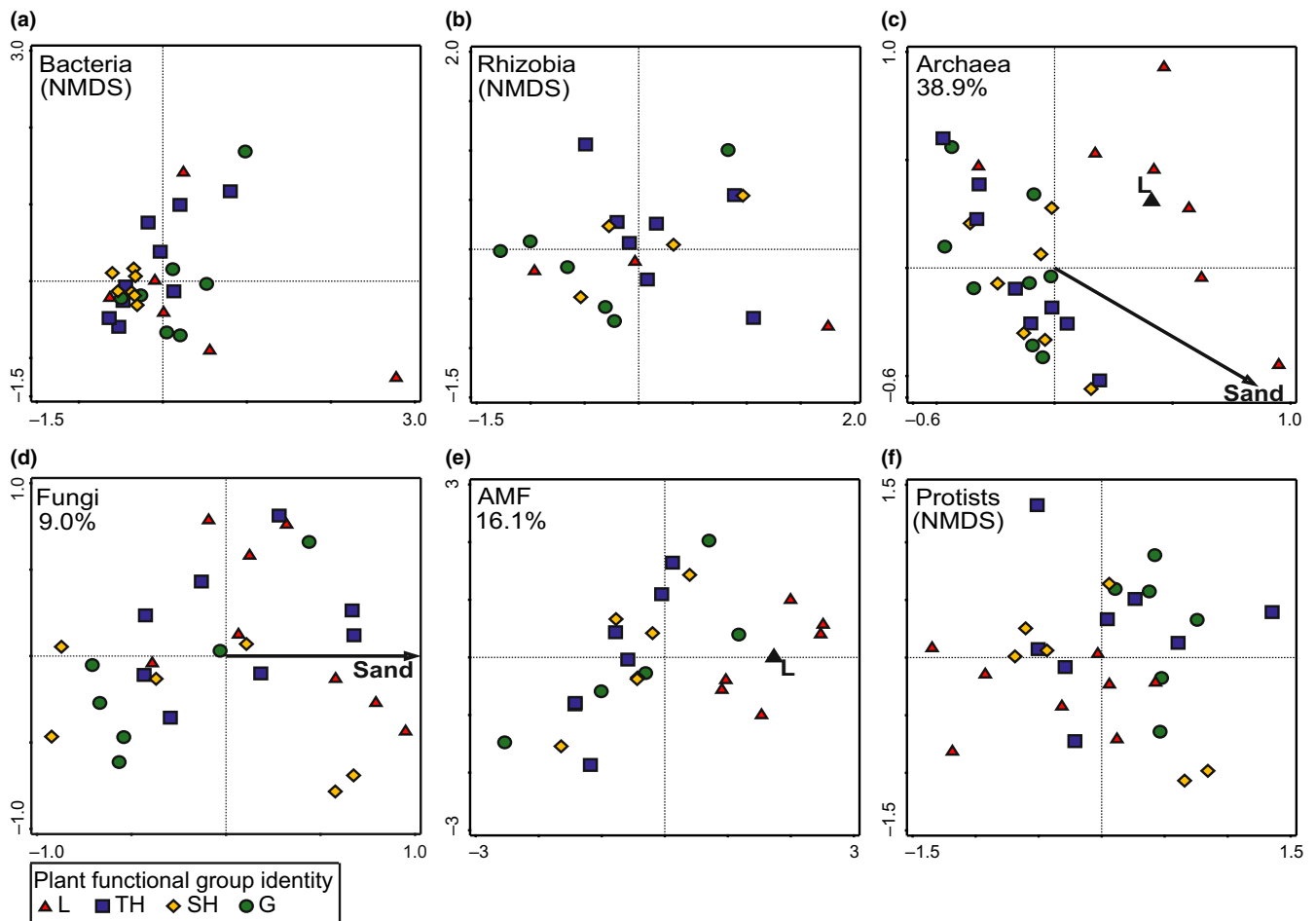


FIGURE 4 Distance-based redundancy analysis (db-RDA) plot showing the relationship of abiotic soil factors and plant functional group identities to community composition of archaea (c), fungi (d), and AMF (e). The plant functional groups tested were grasses (G; green), legumes (L; red), small herbs (SH; yellow) and tall herbs (TH; blue). The ordination is based on Bray–Curtis distance. With forward selection, factors were chosen that significantly ($P_{\text{adj}} < 0.05$) contributed to the model. In each window, the percentage of explained variation is shown. Community composition of bacteria (a), rhizobia (b) and protists (f) could not be explained by any of the factors measured; therefore, a nonmetric multidimensional scaling (NMDS) of the community is shown instead

significant proportion of the variation in bacterial, rhizobial, AMF or protist community composition. For the fungi and archaea, abiotic properties appeared relevant: percentage sand explained 9.0% of fungal community composition (db-RDA pseudo- $F = 2.6$, $P_{\text{adj}} = 0.02$) (Figure 4d), and 24.9% in archaeal community composition (db-RDA pseudo- $F = 9.9$, $P_{\text{adj}} = 0.02$).

4 | DISCUSSION

Our study is one of the first to simultaneously examine impacts of plant species richness and plant functional group identity on richness and community composition of bacteria, fungi, archaea and protists in a long-term controlled grassland biodiversity field experiment. Although there have been several studies analysing the effects of plant diversity on subsets of the microbial community in bulk soil (Grüter, Schmid, & Brandl, 2006; LeBlanc, Kinkel, & Kistler, 2015; Schlatter et al., 2015), few have been able to examine plant diversity

impacts on a range of microbial groups across a well-established experimental gradient of plant species diversity. Another important feature is that we targeted our sampling and analyses to bulk soil samples, as opposed to the rhizosphere, where plant–microbe interactions would be expected to be stronger. Previous studies have shown that plant diversity effects may be limited to the rhizosphere (Kowalchuk, Buma, de Boer, Klinkhamer, & van Veen, 2002), although plant diversity effects have also been found in bulk soil (Schlatter et al., 2015; Sugiyama et al., 2008). Here, we observed some evidence of plant community diversity on bulk soil microbial diversities, but such effects were rather limited and not observed across all microbial groups examined.

4.1 | Microbial species richness

Given the high diversity of soil-borne microbial communities, we did not expect large impacts of plant species richness per se on total soil microbial diversity. Rather, plant communities with disparate plant

traits and functional groups were expected to differentially affect the relative abundance of specific microbial groups in the soil. Regarding microbial species diversity, our results showed that fungal richness responded positively to plant sown diversity. Similar results for fungi and not for bacteria were found by Sugiyama et al. (2008) in a semi-natural grassland system. The positive plant–fungi diversity interaction may be due to selective effects associated with plant–fungal interactions (including AMF) and selection of saprotrophs by specific litter traits (Millard & Singh, 2010). It may also be the case that bacteria experience more stringent top-down control than fungi making plant diversity a less important driver of bacterial diversity (Wardle, 2002).

Our results are in contrast with a study on bacterial diversity in soils of the Cedar Creek biodiversity experiment, as Schlatter et al. (2015) reported a negative relationship between bacterial and plant species richness as determined by 454-pyrosequencing. The authors attributed this finding to increased resource competition in monocultures, which would favour antagonistic communities and subsequently drive higher bacterial diversity, as proposed by Kinkel, Bakker, and Schlatter (2011). One important aspect that may explain the different results between our study and that of Schlatter et al. (2015) is the sampling design. Where Schlatter et al. (2015) collected soil cores at the base of four target species, we sampled the soil at random locations within each plot. Alternatively, the soil type, soil history and plant species used may also differentially impact the development of bacterial diversity across these systems.

Contradictory to our first hypothesis, we did not observe a positive relationship between plant FG richness and microbial species richness. There are studies that tried to relate plant FG diversity to microbial basal respiration (Strecker et al., 2015) and to microbial biomass (Bartelt-Ryser et al., 2005; Lange et al., 2014), but we are not aware of any comparative studies for microbial species richness. With increasing plant FG richness, the number of plant traits also increases. Apparently, plant trait diversity does not necessary impact microbial diversity in the bulk soil.

With respect to the impact of different plant functional groups, we found that bacterial richness responded significantly to plant functional group identity. This contrasts results from a study in a steppe ecosystem, which showed that grasses and perennial forbs did not differentially affect bacterial species diversity (Zhang, Liu, Xue, & Wang, 2015). One explanation may be the absence of legumes in their study as legumes were generally the plant functional group to which the microbial groups responded strongest. However, we also found bacterial community richness to differ between short herbs and grasses and between short herbs and tall herbs. The underlying explanation for this is yet unknown.

Our study did not reveal a significant relationship between plant diversity and the diversity of AMF, whereas a previous study by König et al. (2010) reported such a relationship within the same Jena biodiversity experiment as we examined in the current study. Such a positive relationship was also observed by Hiiesalu et al. (2014). In contrast, Lekberg, Gibbons, Rosendahl, and Ramsey (2013) actually reported a negative relationship between plant species richness and

AMF species richness. These opposing results may be due to a number of factors that differ with our study including the different vegetation types studied (Öpik et al., 2008), different samples sources (within roots or soil) (Saks et al., 2014) or the different methods applied to determine AMF community richness (König et al., 2010). With respect to König et al. (2010), it should also be noted that the samples examined in our study were taken 3 years later and changes in AMF communities may have occurred in the intervening time. Furthermore, the season of sampling (spring vs. late summer) (Bennett et al., 2013; Dumbrell et al., 2011) may also have contributed to the differential results observed.

4.2 | Microbial community composition

Our results showed that microbial community composition was significantly influenced by plant community composition, with stronger effects of plant functional group identity than sown plant diversity. However, this effect was not present in all microbial groups but notable in communities of rhizobia, AMF and archaea. We found that sown plant diversity was positively correlated with rhizobial community evenness, but not with evenness of the other microbial groups examined. Because rhizobia are associated with legumes, the larger number of legume species at higher sown plant diversity levels could have caused the rhizobial community to become more similar. In contrast to our results, a previous study that examined the same field experiment showed that the evenness of both bacterial and fungal communities increased with sown plant diversity (Lange et al., 2015). This contrasting result may be because of the fact that the study of Lange et al. (2015) was based on *terminal restriction fragment length polymorphism* (TRFLP) data, which provides a coarse level of taxonomic analysis of the community, and is only able to examine the most dominant community members.

Because of the tight association between rhizobia and legumes, we expected differently composed rhizobial communities in legume plots compared to the other plant FG plots. However, this was not the case. This may be due to the fact that rhizobia of specific species accumulate inside root hair cells (Gage, 2004), and not in bulk soil. AMF and archaeal community compositions in legume plots were different from those of other plant FG plots. These findings for AMF are in line with a study on plant roots by Scheublin et al. (2004) who showed that AMF communities were different between legume and nonlegume roots of plants from a nutrient-poor dune grassland system. Here, we show that, also in richer soil, and in the bulk compartment legumes accumulate distinct AMF communities. Previous work in the Jena experiment (König et al., 2010) showed that also in mixed plant communities presence of legumes, and to a lesser extend presence of small herbs, significantly affected the AMF community composition.

It is not well understood why AMF communities of legumes should differ from nonlegumes (Veresoglou, Chen, & Rillig, 2012). A possible mechanism may be related to differences in nitrogen and phosphorus stoichiometry of legumes as compared to other plants, due to the activities of nitrogen-fixing bacteria, leading to a shift

from N- to P- limitation (Aerts & Chapin, 1999; Roscher et al., 2011). This may select for more efficient P-acquiring AMF taxa in legumes. Community composition of archaea in legume plots differed from other plant FGs, which could be partially explained by soil carbon or nitrogen concentrations. Presence of legumes may decrease soil carbon concentrations (Lange et al., 2015), and our data show that archaeal community composition is partially explained by TOC. Another possibility is that the increased nitrogen levels arising from legume growth (Spehn et al., 2002) led to changes in the abundance or diversity of ammonia-oxidizing archaea. However, the taxonomic depth to which we could assign OTUs did not allow assessing whether affected populations were indeed ammonia oxidizers.

The absence of any effect of sown plant diversity or plant FG identity on protist community composition is remarkable considering that effects of plant identity (Turner et al., 2013) and plant FG (Glaser et al., 2015; Ledeganck et al., 2003) have been reported before. Protist community composition might not be directly linked to plant community composition but indirect via plant induced changes in abundance and composition of bacterial, fungal and nematode communities, which are all both prey and predators of protists (Geisen, 2016). In addition, our protist results represent mainly the Rhizarial community, whereas plant induced changes in the other protist supergroups could not be analysed.

4.3 | Sown plant diversity versus plant functional group

Interestingly, the influence of plant FG identity on microbial community composition was larger than the influence of sown plant diversity. Diversification of the plant community allowed new fungal species to establish. However, these additional microbial species are probably still relatively low in abundance because their effects are not visible in ordination analyses. The fungal groups that fulfil more general ecological roles in bulk soil, such as decomposition of soil organic matter, are probably present in relatively high abundance across both the low and high plant species richness plots. However, the low abundance species cannot be considered as unimportant because rare microbes have been shown to serve important functions in soil (Delgado-Baquerizo et al., 2016; Hol, Bezemer, & Biere, 2013; Philippot, Spor, et al., 2013).

Plant FG identity had effects on microbial community composition with a notable role for legumes. Plant FGs differ in litter quality, which is correlated with litter decomposition rate (Chen et al., 2016), and can alter the abundance of bacteria and fungi in soil differently (Bezemer et al., 2006; Chen, Chen, & Marschner, 2008; Güsewell & Gessner, 2009; Orwin et al., 2010). Beside effects of plant FG on soil nutrient status, we also expected to find plant FG phylogenetic effects on soil microbial community diversity too, although we did not test this explicitly. As grasses are phylogenetically more related, we expected their associated microbial communities to be less variable across different grass plots compared to short or tall herb

species. This expectation was not borne out from our results. Thereby our results illustrate that also species from one plant family can create diverging effects on soil microbial communities so that quantification of specific traits is warranted to understand the underlying mechanisms.

4.4 | Impacts of soil properties

Soil texture explained part of the variation in community composition for all the microbial groups analysed, except rhizobia. Soil texture can influence microbial richness (Sessitsch et al., 2001), abundance (de Vries et al., 2012) and community composition (Lauer, Ramirez, Aanderud, Lennon, & Fierer, 2013) via soil physical (e.g. pore size) and chemical properties. These factors can affect water holding capacity, drainage, the distribution of food resources and predator access to prey (Ritz & Young, 2004). Why rhizobia remained unaffected by the measured soil properties in our experiment cannot be well explained. Rhizobial community composition is known to respond to abiotic soil factors like pH (Van Cauwenberghe et al., 2015). However, in the Jena experiment, the pH gradient is relatively narrow and relatively high (pH 7.6–8.2). In the majority of the earlier soil microbial community studies, pH has been found to be a major factor in directing microbial community composition (Dumbrell, Nelson, Helgason, Dytham, & Fitter, 2010; Prober et al., 2015; Rousk et al., 2010; Tedersoo et al., 2015; Zhulina et al., 2015). These studies have been conducted across a larger soil pH range and on average at a lower pH than our study. Thereby, our study supports the idea that small variations in pH (when slightly above neutral pH) do not have such a strong effect on microbial communities.

5 | CONCLUSIONS

We found in our long-term grassland biodiversity experiment that increasing plant species richness led to higher fungal species richness in bulk soil, but had no impact on the richness of AMF, bacteria, protists or archaea. Plant species richness also did not significantly alter the community composition of bacteria, fungi and archaea. However, we found that plant FG identity do significantly impact species richness of bacteria, as well as the community composition of AMF and archaea with a notable role for legumes. We conclude that soil microbial community composition in bulk soil can be influenced more by changes in plant FG composition and abiotic soil properties, than by changes in plant species richness per se.

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DATA ACCESSIBILITY

Microbial community data and pH data have been archived in the Pangaea database (<https://doi.org/10.1594/pangaea.874990>).

AUTHOR CONTRIBUTION

S.D., W.H.v.d.P. and G.B.D.D. designed the study. R.C. and G.B.D.D. carried out the soil sampling. H.M. performed DNA extraction and sequencing preparations. M.d.H. processed the sequencing data and S.D. performed data analysis. The study was written by S.D., W.H.v.d.P. and G.B.D.D., and all authors commented on the manuscript.

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