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RESEARCH ARTICLE

Deciphering the interactions between plant species and their main fungal root pathogens in mixed grassland communities

Eline A. Ampt[1] | Davide Francioli[1,2] | Jasper van Ruijven[1] | Sofia I. F. Gomes[1] | Jose G. Maciá-Vicente[1] | Aad J. Termorshuizen[3] | Lisette M. Bakker[1] | Liesje Mommer[1]

[1]Plant Ecology and Nature Conservation Group, Wageningen University, Wageningen, The Netherlands

[2]Microbial Biogeochemistry, Research Area Landscape Functioning, Leibniz Center for Agricultural Landscape Research e.V. (ZALF), Müncheberg, Germany

[3]Aad Termorshuizen Consultancy, Doorwerth, The Netherlands

Correspondence
Eline A. Ampt
Email: eline.ampt@wur.nl

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Abstract

1. Plant diversity can reduce the risk of plant disease, but positive, and neutral effects have also been reported. These contrasting relationships suggest that plant community composition, rather than diversity per se, affects disease risk. Here, we investigated how the diversity and composition of plant communities drive root-associated pathogen accumulation belowground.
2. In a temperate grassland biodiversity experiment, containing 16 plant species (forbs and grasses), we determined the abundance of root-associated fungal pathogens in individual plant species growing in monocultures and in four-species mixtures through Illumina MiSeq amplicon sequencing.
3. In the plant monocultures, we identified three major fungal pathogens that differed in host range: *Paraphoma chrysanthemicola*, associated with roots of forb species of the Asteraceae family, *Slopeiomyces cylindrosporus*, associated with grass species, and *Rhizoctonia solani*, associated with multiple forb and grass species. In mixtures, there was no significant reduction in relative abundance of these pathogens in their host species as compared to monocultures. However, in mixtures, there was a significant increase in relative abundance of each pathogen in several non-host and host plant species. Across mixtures, plant community composition affected pathogen relative abundance in individual plant species. This effect was driven by the presence of a particular neighbouring plant species (depending on the pathogen), rather than functional group composition (i.e. grass/forb ratio) or averaged pathogen pressure (based on monocultures) of all neighbours. Specifically, the presence of neighbour host species *Achillea millefolium* significantly increased *P. chrysanthemicola*, but decreased *R. solani* relative abundance in several host and non-host plant species in mixtures.
4. *Synthesis*. Our results indicate that interactions between different plant species—both host and non-hosts—and fungal pathogens underlie the effects of plant

Eline A. Ampt and Davide Francioli contributed equally.

diversity on root pathogen abundance. Non-host species may act as pathogen reservoirs in diverse plant communities, as they harboured certain pathogens in mixtures, but not in monocultures. Additionally, particular host species can strongly affect pathogen abundance in other (host and non-host) plant species in plant mixtures, suggesting clear effects of species identity in the diversity–disease relationship. Below-ground disease risk thus depends on plant community composition rather than diversity per se, via specific interactions between plant species and their root-associated pathogens.

KEYWORDS
biodiversity ecosystem functioning, diversity–disease relationship, pathogen amplification, pathogen build-up, pathogen dilution, plant–soil (belowground) interactions, soil-borne pathogens

1 | INTRODUCTION

Increasing concerns about biodiversity loss have raised awareness that plant diversity positively affects ecosystem functioning (IPBES, 2019; Tilman, 2001). Numerous studies across grassland and forest ecosystems have established that plant diversity increases plant productivity (Barry et al., 2020; Cardinale et al., 2012). On average, plants in species-rich mixtures perform better than in monoculture. Initially, this positive effect of plant diversity was attributed to resource complementarity among plant species (Barry et al., 2019; Mueller et al., 2013), but several studies that manipulated soil biota have led to a paradigm shift in the field (van Ruijven et al., 2020). In experiments where soil was either sterilised (Hendriks et al., 2013; Schnitzer et al., 2011) or treated with fungicides (Maron et al., 2011), the positive effect of plant diversity on plant productivity disappeared, suggesting that fungal root pathogens play a role in the biodiversity–productivity relationship. It is hypothesised that the accumulation of fungal root pathogens at low plant diversity, which reduces plant productivity, is reduced at higher plant diversity. The reduction of pathogens with diversity, that is, 'pathogen dilution', has been observed across a broad range of host–pathogen systems (Keesing et al., 2006; Keesing & Ostfeld, 2021). For a pathogen specialised on a single host species, a reduced density of this host in more diverse communities often leads to pathogen dilution (Boudreau, 2013; Burdon & Chilvers, 1982; Collins et al., 2020). However, many (fungal root) pathogens have multiple host species (Sarmiento et al., 2017; Semchenko et al., 2022). Pathogen dilution may still occur with generalist pathogens if (a) there is variation in host quality among host species and (b) pathogen transmission from 'high-quality' hosts is reduced in more diverse communities (Hersh et al., 2012; Keesing & Ostfeld, 2021). In contrast, an increase in a generalist pathogen, that is, 'pathogen amplification', may occur if an increase in diversity includes the addition of high-quality hosts (Keesing et al., 2006). Consequently, pathogen accumulation in a diverse community will probably depend on both the host range of a pathogen and plant community composition, rather than on plant diversity per se.

Recent high-throughput sequencing studies in natural ecosystems, such as grasslands and forests, have shown that plant community composition can affect fungal (pathogen) communities in soils (Delgado-Baquerizo et al., 2018; Leff et al., 2018; Makiola et al., 2022; Peay et al., 2013; Schmid et al., 2021). For example, communities with a higher abundance of trees that prioritise root growth over defence against pathogens harboured a higher pathogen diversity in soils (Prada-Salcedo et al., 2021). Furthermore, Heinen et al. (2020) showed that relative abundance of a plant functional group (grasses), a common measure of plant community composition in diverse grasslands, was positively associated with the relative abundance of grass specialist fungal pathogens in bulk soil in mixtures of six plant species.

Yet, to understand the contribution of different plant species to the accumulation of individual fungal root pathogens in diverse communities, it is necessary to know which plant species are colonised by which fungal pathogen species. A study comparing fungal root pathogens in a biodiversity experiment found that more than 50% of the pathogenic fungal operational taxonomic units (OTUs) found in plant monocultures was not observed in mixtures of eight plant species (Mommer et al., 2018), consistent with pathogen dilution. However, the study of Mommer et al. (2018) focussed on the composition of pathogen communities in mixed root samples rather than on the abundance of individual fungal pathogen species in different plant species. The latter is needed to reveal the role of plant community composition in the diversity–disease relationship.

Here we determined the relative abundance of root-associated fungal pathogens in individual plant species growing in monocultures and mixtures of different combinations of four plant species in a 4-year-old grassland biodiversity experiment. We hypothesised that the composition of the neighbouring plant community, rather than plant diversity (i.e. plant species richness), determines the accumulation of root-associated pathogen species in plant species in mixtures as compared to monocultures. To test this, we investigated the accumulation of root-associated fungal pathogens in the roots of several individual plant species in plant monocultures and mixtures of different combinations of four plant species (Figure 1).

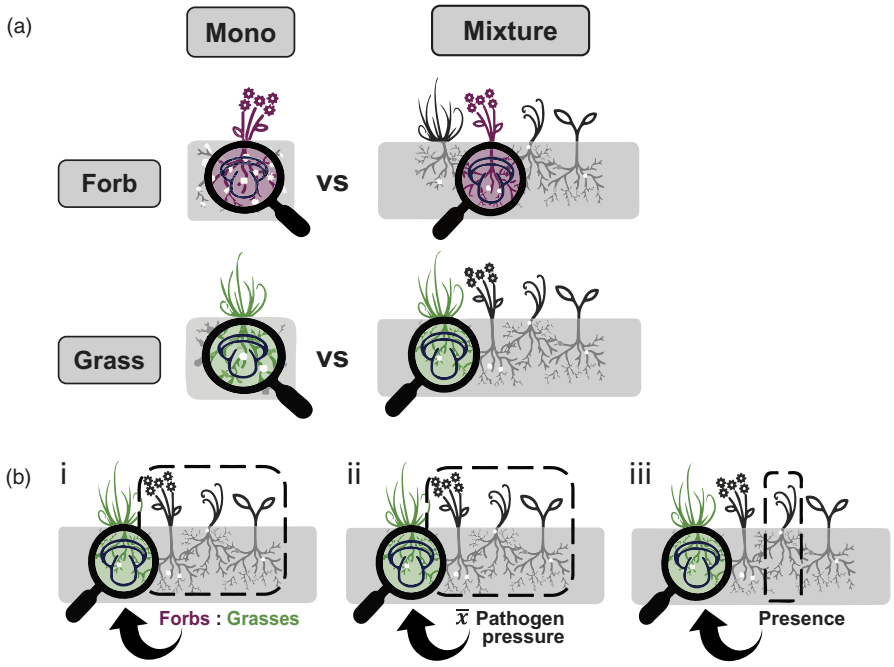

FIGURE 1 Overview of study approach investigating the accumulation of root-associated fungal pathogens in individual plant species. (a) First, effects of plant species identity and functional group (forb/grass) on pathogen accumulation were assessed in monocultures. Then, the effect of plant diversity (monocultures vs. 4-species mixtures) on pathogen accumulation in individual plant species was tested by comparing monocultures and 4-species mixtures. (b) Potential effects of plant community composition on pathogen accumulation in plant species were assessed by comparing mixtures of different combinations of four plant species. Three descriptors of plant community composition were tested: (i) functional group composition of neighbour species, (ii) mean pathogen pressure ($\bar{x}$) of neighbour species (based on monoculture pathogen abundance of these neighbour species) or (iii) the presence of particular neighbour species.

2 | MATERIALS AND METHODS

2.1 | Study site

A common garden experiment consisting of 198 plots was established in April 2014 at the experimental fields of Wageningen University, the Netherlands (51°99′N 5°66′E). To establish this experiment, the original field soil was removed to a depth of 80 cm, and replaced with pure river sand in the lower layer (50–80 cm depth) and with a mixture of pure river sand and soil from an old field (3:1) in the upper 50 cm layer (Bakker et al., 2016). Plots were created using wooden frames which were open at the bottom (l×b×h, 70×70×25 cm) that were pushed 22 cm into the soil. In this experiment, 16 grassland plant species were included, which were equally divided into two plant functional groups: the grasses *Agrostis stolonifera*, *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Briza media*, *Festuca pratensis*, *Festuca rubra*, *Phleum pratense*, *Trisetum flavescens*; and the forbs *Achillea millefolium*, *Centaurea jacea*, *Galium mollugo*, *Leontodon hispidus*, *Leucanthemum vulgare*, *Prunella vulgaris*, *Ranunculus repens* and *Sanguisorba officinalis*. The experiment had three plant diversity levels, corresponding to plant species richness of 1, 4 or 16 species. The 16 grassland species were grown in six replicated monocultures (96 plots), 4-species mixtures (90 plots, 45 different species compositions) and 16-species mixtures (12 plots; details in Bakker et al., 2016). The latter were not included in this study, because these did not differ in plant, and thus neighbour composition. In 2017, a drought treatment was applied to half of the plots (details in Bakker, 2018), which also affected the root-associated fungal pathogen community structure in monocultures immediately after the drought (Francioli et al., 2020). However, in root samples used in this study, collected 1 year after the drought treatment (see below), no difference in total or pathogen root-associated fungal community structure between drought and control monoculture plots could be detected (PERMANOVA: Total: $F_{1,82} = 0.79$, $p = 0.86$; Pathogens: $F_{1,82} = 0.76$, $p = 0.71$). Therefore, we did not include the drought treatment as a factor in our analysis.

2.2 | Root sampling

In July 2018, we sampled roots from all 16 individual plant species in monocultures and from 8 individual 'focal' plant species in 4-species mixtures (grasses *A. odoratum*, *A. elatius*, *B. media*, *F. rubra*; and forbs *A. millefolium*, *C. jacea*, *L. hispidus* and *S. officinalis*). Focal plant species were selected based on frequency in mixture plots at the moment of sampling: these eight species were present in at least 12 four-species mixture plots. In total, 246 root samples from 142 plots (91 samples from 91 monocultures and 155 samples from 51 four-species mixtures) were collected. Using a soil auger (4 cm diameter), three individual plants per species per plot were dug up until a depth of 10 cm from the edge of the plots to avoid disturbance of the inner biomass sampling area. Only fine

roots that were still attached to the shoot were collected. These were washed under running tap water and pooled per plant species per plot. The pooled root samples were immediately stored at −20°C until molecular analysis.

2.3 | Fungal amplicon sequencing

DNA was extracted from root samples using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). Fungal DNA amplification targeted the rDNA ITS1 region using the ITS1F and ITS2 primer pair (White et al., 1990), following the protocol described in Mommer et al. (2018). The amplicons were sequenced on an Illumina MiSeq instrument with 2×300 bp kits at Plant Research International, Wageningen UR, Wageningen, the Netherlands. The raw reads were denoised and then clustered into amplicon sequence variants (ASVs) using the DADA2 pipeline (Callahan et al., 2016). ASVs were chosen over OTUs because they were found most effective for recovering the richness and composition of the root fungal community (Pauvert et al., 2019). In this study, we retained ASVs that were detected in at least two samples. A total of 5,494,497 fungal ITS high-quality reads were recovered from all the samples, which clustered in 1204 fungal ASVs. Samples were rarefied to 3058 reads, the minimum number of reads present in the sample with the lowest sequencing depth (three samples were excluded from further analysis as they showed a sequencing coverage <1000 reads). All sequences have been submitted to the European Nucleotide Archive (study accession number PRJEB49542). The taxonomic assignment of the ASVs was performed using the dynamic version of the developer's full-length ITS reference sequences of the UNITE database (version 8, 18.11.2018; Nilsson et al., 2019). In our dataset, Ascomycota was the most abundant phylum, comprising approximately 54% of the reads across all samples, followed by Basidiomycota (40%; Figure S1).

We used a two-step process to create a subset of ASVs representing potential root pathogens. First, we made a rough selection of ASVs classified as 'highly probable', 'probable' and 'possible' fungal pathogens using FUNGuild (Nguyen et al., 2016). In the second step, we kept only ASVs that were identified at the species level and reported in the literature to be root pathogens, following the procedures described by Francioli et al. (2020) and Mommer et al. (2018). All subsequent analyses, except for pathogen community structure analyses, were performed using the sum of reads for ASVs with the same species-level identification, and our use of the term 'species' therefore refers to such grouping based on 'species hypotheses' as assigned by comparisons with the UNITE database (Kõljalg et al., 2013; Nilsson et al., 2014), rather than to formal species concepts. Using this criterion, we selected the three most prevalent pathogen species in our dataset for all analyses: *Rhizoctonia solani*, *Paraphoma chrysanthemicola* and *Slopeiomyces cylindrosporus* (Table S1). These were the only pathogen species present in a minimum of two monoculture plots of at least one plant species.

2.4 | Statistical analysis

All statistical analyses were performed using R v.4.0.2 (R Core Team, 2020) with the aid of relevant packages.

2.4.1 | Host range of root-associated fungal pathogen communities

To test for differences in root-associated fungal pathogen community structure across plant species, we compared the relative abundance of root-pathogenic fungi among the roots of the 16 plant species in monocultures. We used Bray–Curtis dissimilarities among root-associated fungal pathogen communities, calculated after a Hellinger transformation of ASV read abundances (Legendre & Gallagher, 2001), as a response variable in PERMANOVA (Anderson, 2001; adonis function, VEGAN package; Oksanen et al., 2020) models, including plant functional group (grass or forb) and plant species identity as explanatory variables. As PERMANOVA may be sensitive to unbalanced designs and heterogeneity of variance (i.e. distance to group centroids, calculated using the betadisper function of vegan, Table S2), we used the Wd*-test as available in the MICECO package of R (Russel, 2021), which is robust to heterogeneous dispersion and unbalanced designs (Hamidi et al., 2019), to validate the PERMANOVA results (Alekseyenko, 2016; Anderson & Walsh, 2013). We visualised differences in root-associated fungal pathogen communities across plant species and plant functional groups with a principal coordinates analysis-based ordination.

In this study, we use significant differences in the relative abundance of pathogens in monocultures to define their host range. Based on the assumption that plant species with a high relative abundance of a pathogen can sustain growth of this pathogen, we considered plant species (or plant functional groups) with a significantly higher relative abundance than other species to be 'hosts'. All other species were assumed to be 'non-hosts' for the respective pathogen. Throughout the text, 'non-hosts' refer to these presumed non-host species, based on the pathogen associations in monocultures and literature research, even if some of the presumed non-hosts had positive pathogen abundances when growing in mixtures. To identify the distribution of the different pathogen species in monocultures, we first tested whether there was a phylogenetic signal in pathogen prevalence (% of plots where the pathogen was present) and median relative abundance using Blomberg's *K* (Blomberg et al., 2003), determined with the multiPhylosignal function, in the PICANTE package (Kembel et al., 2010). This analysis was based on the plant phylogenetic tree obtained from the Daphne phylogenetic database (Durka & Michalski, 2012) as in Francioli et al. (2020). Second, we tested whether plant functional group and plant species identity affected relative abundances of each of the pathogens in monoculture, using separate factorial generalised linear models (GLMs) with negative binomial errors for each pathogen. As a first step, we used a model with plant functional group as fixed factor. To better understand the effect of plant species identity within/beyond plant functional group, we then used a follow-up model with plant species identity as fixed factor. If plant functional group significantly affected pathogen relative abundance, we applied this follow-up

model to each plant functional group separately. These GLMs were fitted using the glm.nb function (MASS package; Venables & Ripley, 2002). Post-hoc pairwise comparisons were conducted with Tukey HSD tests in the MULTCOMP package (ghlt function; Hothorn, Bretz, & Westfall, 2008) with Holm–Bonferroni adjustment for multiple comparisons.

2.4.2 | Effects of plant diversity on root-associated fungal pathogen communities

To assess the effects of plant richness on root-associated fungal pathogen community structure, we compared samples from the eight focal species growing in monoculture and in 4-species mixtures. We used the same methods as described above for community structure in monocultures, but added plant diversity and its two-way interactions with both plant functional group and plant species identity as additional independent variables to our model. For those independent variables that showed significant effects in the PERMANOVA analysis, we compared treatment groups with pairwise PERMANOVA comparisons with Holm–Bonferroni adjustment for multiple comparisons (pairwise_adonis function, RANACAPA package; Kandlikar, 2020).

To assess whether the three most abundant pathogens in monoculture were amplified or diluted in the eight focal plant species in mixtures compared to monocultures, we tested whether plant diversity, plant functional group and plant species identity had an effect on the relative abundance of each of the pathogens, using separate factorial GLMs with negative binomial errors for each pathogen. We first constructed a model with plant diversity and plant functional group and their interaction as fixed factors. To better understand the effect of plant species identity and plant diversity within/beyond plant functional group, we then used a follow-up model with plant diversity and plant species identity and their interaction as fixed factors. If plant functional group significantly affected pathogen relative abundance, we applied this follow-up model to each focal plant functional group separately. These GLMs were fitted using the glm.nb function, except when there was complete separation in the data (i.e. when all samples of a factor level have 0 reads of a pathogen and there is thus no variation to estimate), in which case we used bias reduction methods (mean bias reduction for the regression parameters and median bias reduction for the dispersion parameter; negative binomial regression with logarithmic link function and the inverse transformation for the dispersion parameter) using the brnb function in the BRGLM2 package (Kosmidis, 2021) as described in Kenne Pagui et al. (2022). Post-hoc pairwise comparisons were conducted with Tukey HSD tests in the MULTCOMP package (ghlt function) with Holm–Bonferroni adjustment for multiple comparisons.

2.4.3 | Effects of plant community composition in mixtures

We refer to 'neighbour plant species' as the three plant species other than a focal species in a plot. We tested the following three descriptors of plant community composition as potential predictors of fungal root pathogen accumulation in the eight focal plant species in mixtures (Figure 1b):

1. neighbour functional group ratio: percentage of neighbour plant species in the plot of the same ('own') functional group as the focal plant species (continuous predictor).
2. mean pathogen pressure of neighbour species: based on monoculture pathogen abundance of these neighbour plant species (continuous predictor).
3. presence of particular neighbour species: presence of a neighbour plant species in a plot as a fixed factor (binary predictor) for each neighbour plant species in forward model selection (stepAIC function). Only neighbour plant species that were present in at least three plots with the focal plant species were included in the model selection. We then assessed whether the neighbour plant species that were included as predictors in the final model significantly affected pathogen abundance in the focal plant species through likelihood ratio tests with resampling (manyglm function).

For each descriptor, we separately performed our analyses based on neighbour plant species as they were planted (i.e. always three neighbour plant species, presented in supplemental Figures S4, S6, S8, S10, S12, S14) and on neighbour plant species that were still present in a plot at the moment of sampling (i.e. 4 years after the initial establishment). The impact of 'as planted' vs 'remaining' neighbour species analyses on the results was negligible. We therefore only present the results of the 'remaining' neighbour species analyses in the main text. For each combination of focal plant species and pathogen species, we constructed separate GLMs (negative binomial error distribution, manyglm function with Likelihood Ratio Test resampling under the null hypothesis, using the MVABUND package; Wang et al., 2020). Pathogen relative abundance in the focal plant species in mixtures was always the dependent variable, while the models differed in the independent variable (predictor), which examined the effect of neighbour plants in the 4-species mixtures. Model assumptions were assessed with plots of residuals (Dunn–Smyth residuals for GLMs with negative binomial errors, Dunn & Smyth, 1996; Warton et al., 2016), obtained through qresid function (STATMOD package; Dunn & Smyth, 1996) for glm.nb fits and through plot.manyglm function for manyglm fits (MVABUND package).

3 | RESULTS

3.1 | Effects of plant identity on root-associated fungal pathogen community in monocultures

We found a total of 914 fungal ASVs (classified into 259 fungal species), including 56 root pathogenic ASVs that belonged to nine fungal species (Table S1) across the roots of all 16 plant species in monoculture. The three most prevalent pathogen species were *Rhizoctonia solani* (present in 64% of monoculture plots, 27 ASVs), *Paraphoma*

chrysanthemicola (69%, 15 ASVs) and *Slopeiomyces cylindrosporus* (35%, 3 ASVs). Subsequent analyses focus on these three pathogens.

Plant functional group explained 9.1% of root-associated fungal pathogen community structure in the 16 plant species in monoculture, while plant species identity explained an additional 22.1% (PERMANOVA and Wd*-test; Table S2; Figure S2), indicating that root pathogen communities were not only different between plant functional groups, but also between plant species. The variation in root-associated fungal pathogen community structure was also different between plant species and between plant functional groups (PermDISP; Table S2). Furthermore, the effects of plant functional group and plant species on root-associated fungal pathogen community structure also emerged when only the eight focal plant species in monoculture were included (Table S2).

Each of the three most prevalent fungal pathogens was associated with different host ranges of plant species and/or functional groups in the monocultures (Figure 2; Table S3). The prevalence and relative abundance of the pathogen *P. chrysanthemicola* were significantly affected by plant phylogeny (prevalence: $K = 0.93$, $p < 0.01$; relative abundance: $K = 0.72$, $p < 0.01$; Figure 2). It was found in all monoculture plots of forb species belonging to the Asteraceae family (*L. vulgare*, *A. millefolium*, *C. jacea* and *L. hispidus*) and its relative abundance in these forb species was higher than in the two most distantly related forb species *S. officinalis* and *R. repens* (Forb species: $X^2_{7,37} = 34.98$, $p < 0.001$; Figure 2, Table S3). Furthermore, *P. chrysanthemicola* relative abundance was higher in forb than in grass species (plant functional group: $X^2_{1,87} = 33.08$, $p < 0.001$), and equally low in all grass species ($X^2_{7,37} = 7.77$, $p = 0.35$; Figure 2; Table S3).

The relative abundance of the pathogen *S. cylindrosporus* was significantly higher in grasses than in forbs ($X^2_{1,87} = 38.92$, $p < 0.001$): it was found in all grass species, while it was absent in all forb monocultures except one plot (Figure 2; Table S3). Its prevalence depended on plant phylogeny ($K = 0.71$, $p < 0.01$), but its relative abundance did not ($K = 0.11$, $p < 0.78$; Figure 2). The latter was reflected in significant differences in *S. cylindrosporus* relative abundances within the grasses: it was highest in *A. elatius* and lowest in *T. flavescens* and *B. media* ($X^2_{7,36} = 15.85$, $p < 0.05$; Figure 2; Table S3).

The pathogen *R. solani* was found in all plant species except one (Table S3), and its prevalence and relative abundance were independent of plant phylogeny ($K = 0.15$, $p = 0.68$; and $K = 0.20$, $p = 0.43$, respectively; Figure 2). Its relative abundance also did not differ between plant functional groups ($X^2_{1,87} = 3.25$, $p = 0.07$), but it differed greatly between plant species ($X^2_{15,73} = 49.51$, $p < 0.001$). *R. solani* relative abundance was highest in the two forbs *A. millefolium* and *R. repens*, and the two grasses *F. rubra* and *P. pratense* (Table S3).

3.2 | Effects of plant diversity on root-associated fungal pathogen community structure in eight plant species

All pathogen species that were present in the monocultures of the eight focal plant species were also present in these plant species grown in different 4-species mixtures (Table S1). Furthermore, we

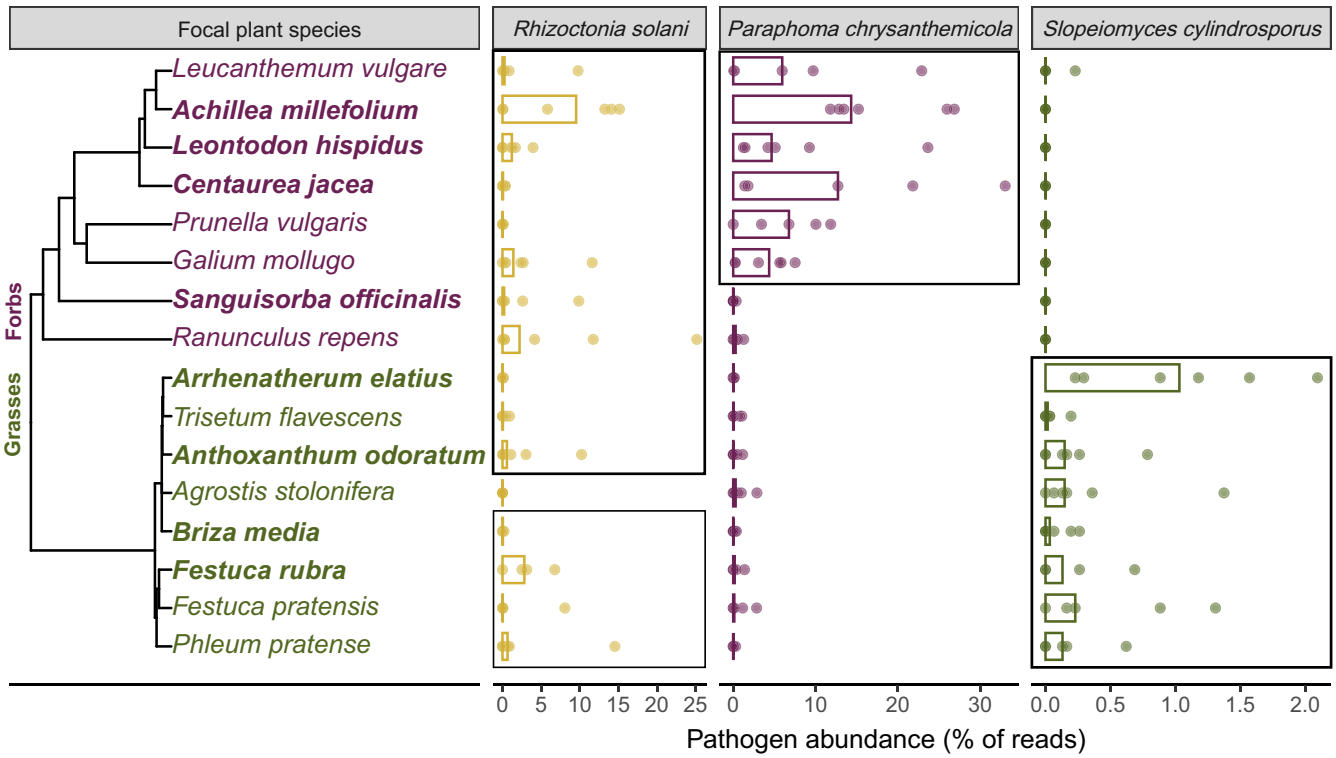

FIGURE 2 Associations of the three most prevalent fungal pathogen species with forb (purple) and grass (green) species in monocultures. Plant species in bold are the 8 'focal species' that were also sampled in mixtures. Species within a black border were designated as hosts for either *R. solani*, *P. chrysanthemicola* or *S. cylindrosporus* (see Table S3 for stats). Points indicate individual samples ($n = 4–6$) and horizontal bars indicate median relative abundance (% of reads) of a pathogen per plant species. Pathogen species are coloured as in Figures 3 and 4.

detected 498 fungal ASVs (67 fungal species), including 38 root pathogen ASVs (3 pathogen species) that were not found in monocultures of the eight plant species. However, the three fungal pathogen species found solely in mixtures represented only 0.6%–1.3% of the samples (Table S1).

The root-associated fungal pathogen community structure was different in roots from monocultures compared to mixtures, independent of plant functional group and plant species identity (PERMANOVA, Table 1; Figure S3). Plant functional group (4.7%) and plant species identity (8.9%) together explained 13.6% of the variance in pathogen community structure across monocultures and 4-species mixtures (PERMANOVA, Table 1; Figure S3). These main effects of plant diversity (PD), plant functional group (PFG) and plant species (PS) identity were confirmed by the Wd*-test (PD: Wd* = 1.74, $p = 0.03$; PFG: Wd* = 9.23, $p = 0.001$, PS: Wd* = 4.32, $p = 0.001$), which is not sensitive to the differences in dispersion (i.e. variation) of community structure that were found between monocultures and mixtures for the species *A. elatius* and *C. jacea* (PermDISP: PS × PD: $F_{7,173} = 2.82$, $p < 0.01$). Specifically, for these two plant species, roots in mixtures did harbour a significantly larger variation in root-associated fungal pathogen community structure than those from monocultures the grass *A. elatius* (35.3% larger variation in mixtures; post-hoc: $t = 4.40$, $p < 0.001$) and the forb *C. jacea* (22.8% larger variation in mixtures; post-hoc: $t = 2.98$, $p < 0.01$). Overall, this variation was not significantly different between grasses and forbs (PermDISP: PFG: $F_{1,185} = 0.05$, $p = 0.83$).

3.3 | Effects of plant diversity on the three main root pathogens in eight plant species

For all three pathogen species there were small, but non-significant, decreases in relative abundance in several host plant species in mixtures compared to monocultures. However, there was a significant increase in relative abundance of *P. chysanthemicola* and *S. cylindrosporus* in non-host species and of *R. solani* in several host species in mixtures (Figure 3). Specifically, the relative abundance of the pathogen *R. solani* was significantly increased in mixtures compared to monocultures in two forb host (*C. jacea* and *L. hispidus*) and two grass host species (*A. elatius* and *B. media*; Plant functional group × plant diversity: $X^2_{1,195} = 0.18$, $p = 0.67$; Plant species × plant diversity: $X^2_{1,195} = 18.42$, $p < 0.05$; Figure 3a,b). The relative abundance of the pathogen *P. chrysanthemicola* was significantly increased in all four grass non-host species (Grasses: Plant diversity: $X^2_{1,194} = 12.10$, $p < 0.001$; Figure 3c,d). In the forbs, the relative abundance of the pathogen was significantly increased in the non-host *S. officinalis*, but was not affected by diversity in the three Asteraceae host species (Forbs: Plant species × plant diversity: $X^2_{3,95} = 13.87$, $p < 0.01$; Figure 3c,d). For the pathogen *S. cylindrosporus*, there was significant amplification in all four forb non-host species (Forbs: plant diversity: $X^2_{1,101} = 6.82$, $p < 0.01$; Figure 3e), but not in any grass host species (Grasses: plant diversity: $X^2_{1,194} = 2.15$, $p = 0.14$; Figure 3e).

3.4 | Effects of plant community composition on the three main root pathogens in mixtures

Within the 4-species mixtures, the percentage of neighbour plant species with the same functional group as the focal plant species did not significantly relate to the relative abundance of any of the three main root pathogens in a focal plant species (Figure S5). In addition, mean pathogen pressure of neighbour plant species (based on monoculture pathogen abundance of these neighbour plant species) was also not a good predictor for pathogen relative abundance of the focal plant species in mixtures (Figure S7), except for the pathogen *P. chrysanthemicola* in two focal plant species. *P. chrysanthemicola* relative abundance in *C. jacea* and *A. elatius* roots was higher when these focal plant species were with neighbour plant species with a high average relative abundance of this pathogen in monoculture (Figure S7b).

The presence of particular neighbour plant species in mixtures was significantly associated with the relative abundance of the three main pathogens in focal plant species. For *R. solani*, the presence of a particular neighbour plant species was associated with a reduced relative abundance of the pathogen in six focal-neighbour plant combinations (Figures S9 and S15) compared to mixtures without the neighbour plant species. Specifically, the presence of the forb *A. millefolium* as a neighbour decreased the relative abundance of *R. solani* in two focal plant species (the forbs *C. jacea* and *S. officinalis*, both hosts), but not in the two other focal plant species (the grasses *A. elatius* or *B. media*, both hosts) where there was sufficient power to test for *A. millefolium* neighbour effects (Figure 4). In contrast, the presence of *A. millefolium*

TABLE 1 Effects of plant functional group (PFG; forb vs. grass), plant species (PS) and plant diversity (PD; 1 vs. 4-species mixture) on root-associated pathogen fungal (RAF) community structure analysed with PERMANOVA. Bold indicates significance ($p < 0.05$). Terms were tested sequentially in PERMANOVA

	Pathogen RAF community structure			
Parameter	**df**	***F***	**R^2**	***p***
Plant Functional Group (PFG)	**1**	**9.90**	**0.047**	**0.001**
Plant Species (PS)	**6**	**3.14**	**0.089**	**0.001**
Plant Diversity (PD)	**1**	**1.73**	**0.008**	**0.046**
PFG × PD	1	1.48	0.007	0.091
PS × PD	6	1.00	0.028	0.470
Residuals	173		0.820	
Total	188		1	

as a neighbour increased the relative abundance of *P. chrysanthemicola* in three (*C. jacea*, host; *S. officinalis* and *A. elatius*, both non-hosts) of these four focal plant species (Figure 4). Due to the dominant effect of *A. millefolium* on *P. chrysanthemicola*, we further tested the potential effects of other neighbour plant species on this pathogen in plots where *A. millefolium* was not present as a neighbour to ensure that the effects of other neighbour plant species were not masked by the effect of *A. millefolium*. This resulted in seven focal-neighbour species combinations where the presence of the neighbour was associated with a reduced relative abundance of *P. chrysanthemicola* in the focal plant species (two host and three non-host species, Figures S11 and S15). In addition, there were three focal-neighbour plant species combinations where the presence of the neighbour was associated with an increased relative abundance of this pathogen in the focal plant species (one host and two non-host species; Figures S11 and S15). Finally, for *S. cylindrosporus*, the presence of a particular neighbour plant species was associated with a reduced relative abundance of the pathogen in three focal plant species (two host and one non-host species) and an increased relative abundance of the pathogen in one focal plant species (a non-host species; Figures S13 and S15).

4 | DISCUSSION

Here, we identified three main root-associated fungal pathogens and determined their relative abundance in individual plant species growing in monocultures and mixtures differing in species composition. In monocultures, we found different host ranges for the three fungal root pathogens. In mixtures, we did not find significant reductions in the relative abundance of pathogens in hosts species, but we did find significant increases in several host and non-host species, consistent with pathogen amplification. Furthermore, across mixtures, the presence of particular neighbour plant species was associated with a significant change in the accumulation of specific root-associated pathogens. Below, we discuss the implications of these results for our understanding of the plant–pathogen interactions in diverse plant communities that lead to root pathogen dilution or amplification.

4.1 | Pathogen host range

The three main fungal root pathogen species in our study appear to differ in host range, as defined by their relative abundances in monocultures. These host ranges are in line with those reported in the literature for these pathogens. *P. chrysanthemicola* was primarily associated with forb species of the Asteraceae family in our monocultures. It has originally been described as a pathogen of *Chrysanthemum morifolium*, which belongs to the Asteraceae (Peerally & Colhoun, 1969). More recently, it has been found in roots of several Asteraceae species (Wehner et al., 2014) and found to be pathogenic to *L. vulgare* (Hendriks et al., 2015; Mommer et al., 2018). *S. cylindrosporus* was only present in grass monocultures and is indeed considered a pathogen of Poaceae species (Klaubauf et al., 2014) and has recently also been found in grassland soils on which several grass species had been grown (Hannula et al., 2021; Heinen et al., 2020). The third pathogen, *R. solani*, is often described as a generalist pathogen, able to infect plant species from many monocot and dicot families (Anderson, 1982) and it was indeed present in all but one grass and forb species in our monocultures. However, the host range of *R. solani* is known to differ at a higher taxonomic resolution, that is, between strains that belong to different anastomosis groups (AGs; González García et al., 2006). Most AGs have different ranges of forb species as hosts, while some other AGs are known as grass pathogens (Ogoshi, 1987). These groups could not be differentiated based on ITS sequences as used in our study. Therefore, we cannot rule out that in our analysis different *R. solani* AGs were clustered into a single pathogen species. This may also explain why there was no significant differentiation of *R. solani* abundance between groups of plant species in monocultures. Identifying these AGs and determining their host range is an interesting avenue for future studies.

4.2 | Effects of plant diversity on fungal root pathogens in plant species

Previous work found a reduction of the number of different root pathogenic OTUs with increased plant diversity (1, 4 and 8 plant species; Mommer et al., 2018). Here, we focused on the relative abundance of three root pathogens at the level of individual species and did not find a significant reduction of pathogen accumulation with increasing plant diversity. This suggests that, on average, the presence of three heterospecific neighbouring plant species could not prevent root pathogen accumulation in individual host plant species. This finding may be due to the fact that the difference in host density between monocultures and mixtures was not large enough to significantly reduce root pathogen accumulation. This may have two reasons. First, the relative abundance of each focal plant species was often relatively high compared to the other three plant species in mixtures (based on above-ground biomass; Figure S16). This was partly due to the fact that we selected those species that were present in most, if not all plots in which they were planted. Second, the host range of all three pathogens included at least six of the 16 plant species in the biodiversity experiment. Therefore, most mixtures likely contained one or more other host species in addition to the focal host species, which may impede dilution of the pathogens in more diverse communities. Overall, the absence of dilution in our study is in line with a recent study in forests, which found that community-level disease prevalence of the foliar oomycete pathogen *Phytophthora ramorum* decreased with tree diversity (species richness), while disease risk in individual host species did not (Rosenthal et al., 2021). Similarly, in a forest biodiversity experiment, average fungal pathogen infection (% of symptomatic leaf area) decreased with tree species richness, but individual host species varied in this response, including multiple species that showed no effect of tree diversity on fungal infestation (Rutten et al., 2021).

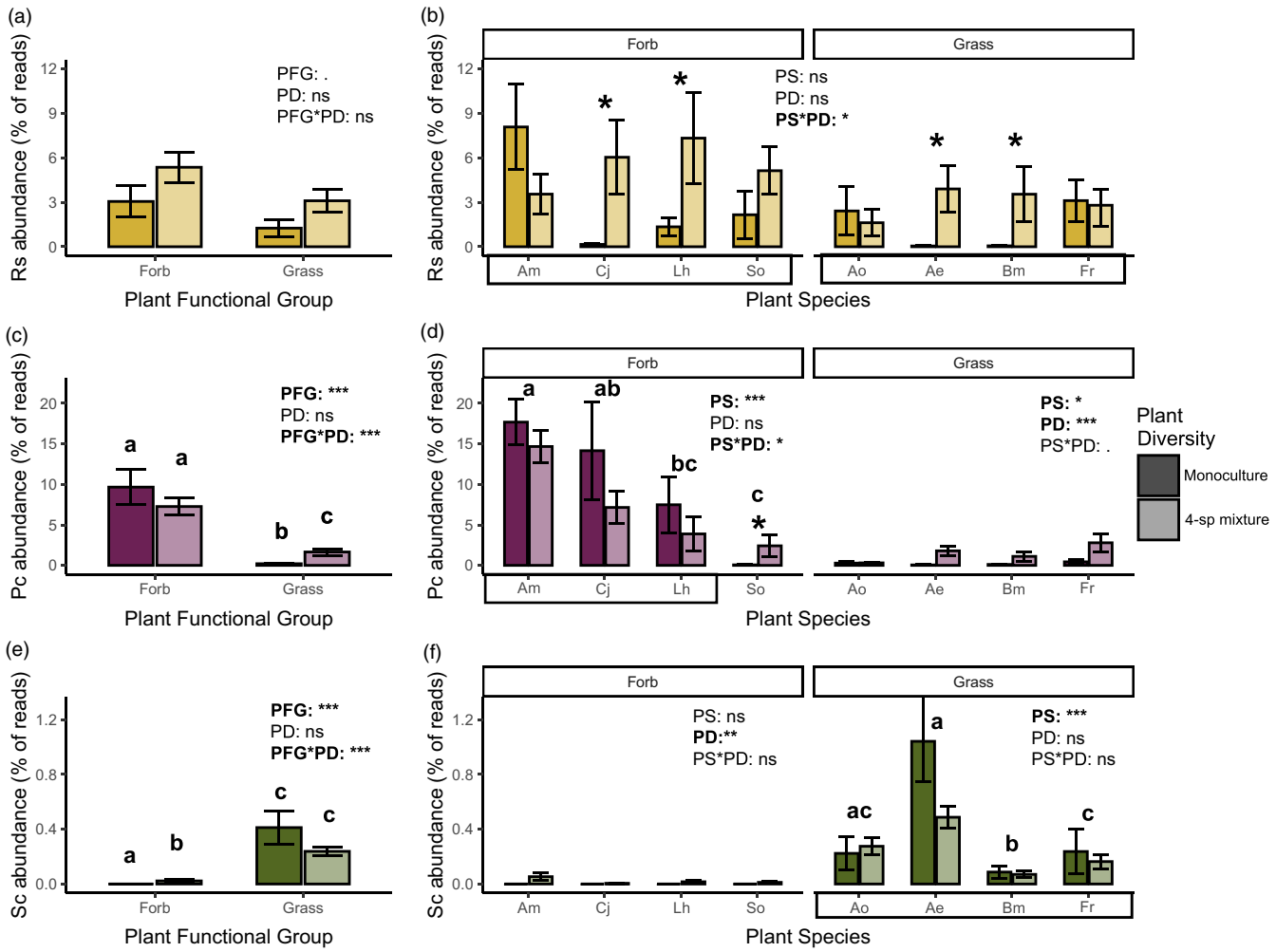

FIGURE 3 Root-associated fungal pathogen relative abundance in plant species depends on plant functional group (PFG), plant species identity (PS) and plant diversity (PD). (a, b) *Rhizoctonia solani* (Rs) relative abundance, (c, d) *Paraphoma chrysanthemicola* (Pc) relative abundance, (e, f) *Slopeiomyces cylindrosporus* (Sc) relative abundance. Relative abundance expressed as percentage of reads (mean ± SE). Species within a black border were designated as hosts for either *R. solani* (b), *P. chrysanthemicola* (d) or *S. cylindrosporus* (f) based on monocultures (see Figure 2; Table S3). Asterisks represent significance levels of factor terms in negative binomial GLM (. $p < 0.1$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Asterisks and letters above bars indicate significant post-hoc differences (asterisk indicates a significant effect of PD within plant species; different letters indicate significant differences between plant species). Species abbreviations are *Achillea millefolium* (Am), *Centaurea jacea* (Cj), *Leontodon hispidus* (Lh), and *Sanguisorba officinalis* (So), *Anthoxanthum odoratum* (Ao), *Arrhenatherum elatius* (Ae), *Briza media* (Bm), *Festuca rubra* (Fr).

Together, these results show that the importance of assessing pathogen dilution at the individual host level to understand how species interactions underly diversity–disease relationships also applies to below-ground pathogens.

Intriguingly, in many plant species that we assumed non-hosts because of the low relative abundance of the pathogens in their roots, we did find amplification of root pathogens in mixtures compared to monocultures. This suggests that these plant species might actually be less preferred hosts, whose roots are more easily colonised by the pathogen in mixed plant communities than in monocultures. Several mechanisms may contribute to this effect, such as (1) pathogen spillover, that is, transmission of the pathogen from other (higher quality) host neighbours (Grossman et al., 2019; Power & Mitchell, 2004), and/or for example (2) shifts in microbial community dynamics within the plant due to the presence of heterospecific neighbour plants. Importantly, the associations of the pathogens with less preferred hosts in mixtures may not induce disease symptoms in these plant species. Many plant-pathogenic fungi can also have a non-pathogenic lifestyle through endophytic root colonisation (i.e. without causing disease symptoms; Hernandez-Escribano et al., 2018; Kia et al., 2017; Lofgren et al., 2018; Selosse et al., 2018). Indeed, the three pathogens in our study have frequently been found as root endophytes of grassland plant species in previous studies (e.g. Glynou et al., 2018; Mesny et al., 2021; Pereira et al., 2019; Sánchez Márquez et al., 2010). However, less preferred host species may serve as pathogen reservoirs (Dhingra & Coelho Netto, 2001; Malcolm et al., 2013) in mixed plant communities. Revealing whether these plant species indeed function as pathogen reservoirs and their role in disease epidemiology in diverse plant communities will be a necessary next step.

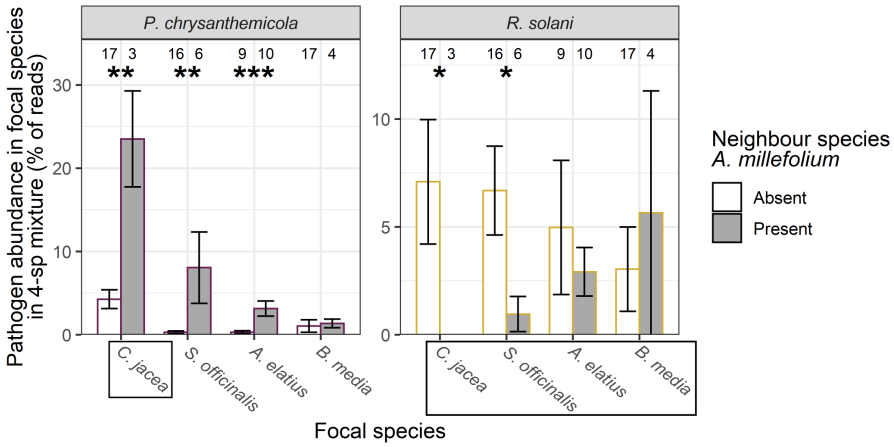

FIGURE 4 Effect of the presence of *A. millefolium* as neighbour species (present at the time of sampling) on relative abundance (% of reads) of *P. chrysanthemicola* and *R. solani* in focal species roots in 4-species mixtures. Species within a black border were designated as hosts for either *P. chrysanthemicola* or *R. solani* based on monocultures (see Figure 2; Table S3). Note that only the four focal species with ≥3 plots with *A. millefolium* as a neighbour were included in the analysis. Asterisks indicate significant difference in pathogen relative abundance in focal species roots between 4-species mixtures with and without *A. millefolium* as neighbour species (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). *A. millefolium* as neighbour species was included as fixed term in final model after forward model selection (based on AIC) per focal species per pathogen [generalised linear model (GLM) with negative binomial errors, Likelihood Ratio Test].

4.3 | Effects of plant community composition on fungal root pathogens in plant species

In mixtures, plant community composition affected root-associated fungal pathogen accumulation in plant species. Two community descriptors, neighbour functional group composition and the average predicted pathogen abundance based on monocultures, were poor predictors of pathogen accumulation in mixtures. However, the presence of specific neighbour plant species was a good predictor of root pathogen accumulation in mixtures. Effects of the presence of certain plant species in communities on pathogen accumulation have previously been shown, but only in foliar fungi in forests (Field et al., 2020; Hantsch et al., 2014; Rosenthal et al., 2021; Setiawan et al., 2014). Our findings thus add to a growing body of evidence that suggests that specific plant species can have key roles in pathogen accumulation in mixed communities. Differences in plant species' (1) effects on pathogen growth (e.g. host quality) and (2) density may induce these specific effects. This suggests that it is important to focus on the characteristics of specific neighbour plant species rather than using a metric of community composition to understand the diversity–disease relationship.

We found that a particular neighbour plant species, *A. millefolium*, was associated with both an increase in the pathogen *P. chrysanthemicola* and a decrease in *R. solani* in several plant species. The decrease in *R. solani* accumulation was surprising, as the roots of this neighbour plant species harboured a high relative abundance of both pathogens in monoculture and mixtures. Such reducing effects of a host neighbour species may provide an additional explanation about why community descriptors were poor predictors of pathogen accumulation in plant species in mixtures. The increase in *P. chrysanthemicola* in plant species with *A. millefolium* as a neighbour is likely due to accumulation of the pathogen in the *A. millefolium* roots, which may act as nutrient source for further exploration and potential exploitation of nearby heterospecific roots. The simultaneous decrease in *R. solani* in these plant species with *A. millefolium* as neighbour is harder to explain. One possible explanation is competition between the two pathogens. Although co-infections by multiple pathogens are common in plants, interactions between co-infecting pathogens may be antagonistic when they compete for the same limited resources. This has recently been shown for foliar fungal pathogens (Dutt et al., 2021), but it is unknown whether *R. solani* and *P. chrysanthemicola* would occupy the same niche when co-infecting a host. Moreover, another explanation could be induced systemic resistance, that is, a plant defence response triggered by one pathogen, that blocks subsequent infections by other pathogens (Dutt et al., 2022). Alternatively, competitive or antagonistic interactions with other soil microbes (Raaijmakers et al., 2009) that are stimulated by *A. millefolium* neighbour plants may suppress *R. solani* accumulation. Furthermore, any neighbouring plant species whose presence was associated with a reduced pathogen abundance in focal plant species might directly inhibit pathogen growth through antifungal compounds in root exudates (Baetz & Martinoia, 2014; Yang et al., 2014). Additionally, neighbouring plant species may indirectly affect pathogen accumulation in plant species via resource competition, thereby increasing or decreasing the plant species' abundance ('host regulation'; Keesing et al., 2006).

5 | CONCLUSIONS

In this study, we assessed root pathogen relative abundance in individual plant species in plant monocultures and mixtures to shed more light on the below-ground diversity–disease relationship. We found that plant diversity did not significantly reduce root pathogen

accumulation in host species, but often amplified fungal root pathogen accumulation in non-host and host plant species. Our approach thus reveals unexpected potential root pathogen reservoirs in diverse plant communities. Furthermore, we show that fungal root pathogen accumulation in diverse communities depends on interactions between individual pathogen and neighbour plant species. The effects of neighbour plant species in mixtures could not be predicted from root-associated pathogen accumulation in monocultures alone. This highlights the need for further mechanistic understanding of the below-ground interactions that underly diversity–disease relationships. As we cannot directly infer disease incidence from pathogen accumulation in roots, an important next step will be to test whether root-associated relative abundance of a pathogen is indeed related to (a) the susceptibility of these plant species and (b) their ability to sustain pathogen growth and promote pathogen transmission. Our current identification of these potential key pathogens and a first insight into their interactions with individual plant species in diverse communities paves the way to understanding below-ground diversity–disease relationships.

AUTHOR CONTRIBUTIONS

Eline A. Ampt, Davide Francioli, Jasper van Ruijven and Liesje Mommer designed the project. Lisette M. Bakker, Jasper van Ruijven and Liesje Mommer set up and maintained the biodiversity experiment. Eline A. Ampt, Davide Francioli, Lisette M. Bakker, Jasper van Ruijven and Liesje Mommer collected the root samples. Davide Francioli and Eline A. Ampt performed the molecular analysis. Davide Francioli performed the bioinformatic analysis. Eline A. Ampt and Davide Francioli analysed the data with input from Jasper van Ruijven, Sofia I. F. Gomes and Jose G. Maciá-Vicente. Eline A. Ampt, Jasper van Ruijven and Liesje Mommer wrote the draft of the manuscript. All authors contributed substantially to the writing of the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Liesje Mommer is an Associate Editor for *Journal of Ecology*, but took no part in the peer review or decision-making process for this paper.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.h70rxwdnd (Ampt et al., 2022).

ORCID

Eline A. Ampt https://orcid.org/0000-0002-1783-6184
Davide Francioli https://orcid.org/0000-0001-6572-3574
Jasper van Ruijven https://orcid.org/0000-0003-0003-2363
Jose G. Maciá-Vicente https://orcid.org/0000-0002-7174-7270
Aad J. Termorshuizen https://orcid.org/0000-0003-3058-0088
Liesje Mommer https://orcid.org/0000-0002-3775-0716

REFERENCES

Alekseyenko, A. V. (2016). Multivariate Welch *t*-test on distances. *Bioinformatics*, *32*(23), 3552–3558. https://doi.org/10.1093/bioinformatics/btw524

Ampt, E. A., Francioli, D., van Ruijven, J., Gomes, S. I. F., Maciá-Vicente, J., Termorshuizen, A. J., Bakker, L. M., & Mommer, L. (2022). Data from: Deciphering the interactions between plant species and their main fungal root pathogens in mixed grassland communities. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.h70rxwdnd

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1046/j.1442-9993.2001.01070.x

Anderson, M. J., & Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, *83*(4), 557–574. https://doi.org/10.1890/12-2010.1

Anderson, N. A. (1982). The genetics and pathology of *Rhizoctonia Solani*. *Annual Review of Phytopathology*, *20*(1), 329–347. https://doi.org/10.1146/annurev.py.20.090182.001553

Baetz, U., & Martinoia, E. (2014). Root exudates: The hidden part of plant defense. *Trends in Plant Science*, *19*(2), 90–98. https://doi.org/10.1016/j.tplants.2013.11.006

Bakker, L. M. (2018). *The positive effect of biodiversity: Using root traits to understand effects of plant diversity and drought on grassland productivity* (PhD thesis). Wageningen University. https://doi.org/10.18174/443242

Bakker, L. M., Mommer, L., & van Ruijven, J. (2016). Can root trait diversity explain complementarity effects in a grassland biodiversity experiment? *Journal of Plant Ecology*, *515*, rtw111. https://doi.org/10.1093/jpe/rtw111

Barry, K. E., Mommer, L., van Ruijven, J., Wirth, C., Wright, A. J., Bai, Y., Connolly, J., De Deyn, G. B., de Kroon, H., Isbell, F., Milcu, A., Roscher, C., Scherer-Lorenzen, M., Schmid, B., & Weigelt, A. (2019). The future of complementarity: Disentangling causes from consequences. *Trends in Ecology & Evolution*, *34*(2), 167–180. https://doi.org/10.1016/j.tree.2018.10.013

Barry, K. E., van Ruijven, J., Mommer, L., Bai, Y., Beierkuhnlein, C., Buchmann, N., de Kroon, H., Ebeling, A., Eisenhauer, N., Guimarães-Steinicke, C., Hildebrandt, A., Isbell, F., Milcu, A., Neßhöver, C., Reich, P. B., Roscher, C., Sauheitl, L., Scherer-Lorenzen, M., Schmid, B., … Weigelt, A. (2020). Limited evidence for spatial resource partitioning across temperate grassland biodiversity experiments. *Ecology*, *101*(1), 1–13. https://doi.org/10.1002/ecy.2905

Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, *57*(4), 717–745. https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

Boudreau, M. A. (2013). Diseases in intercropping systems. *Annual Review of Phytopathology*, *51*(1), 499–519. https://doi.org/10.1146/annurev-phyto-082712-102246

Burdon, J. J., & Chilvers, G. A. (1982). Host density as a factor in plant disease ecology. *Annual Review of Phytopathology*, *20*(1), 143–166. https://doi.org/10.1146/annurev.py.20.090182.001043

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., MacE, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., & Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, *486*(7401), 59–67. https://doi.org/10.1038/nature11148

Collins, C. D., Bever, J. D., & Hersh, M. H. (2020). Community context for mechanisms of disease dilution: Insights from linking epidemiology and plant–soil feedback theory. *Annals of the New York Academy of Sciences*, *1469*(1), 65–85. https://doi.org/10.1111/nyas.14325

Delgado-Baquerizo, M., Fry, E. L., Eldridge, D. J., de Vries, F. T., Manning, P., Hamonts, K., Kattge, J., Boenisch, G., Singh, B. K., & Bardgett, R. D. (2018). Plant attributes explain the distribution of soil microbial communities in two contrasting regions of the globe. *New Phytologist*, *219*(2), 574–587. https://doi.org/10.1111/nph.15161

Dhingra, O. D., & Coelho Netto, R. A. (2001). Reservoir and non-reservoir hosts of bean-wilt pathogen, *Fusarium oxysporum* f. sp. *phaseoli*. *Journal of Phytopathology*, *149*(7–8), 463–467. https://doi.org/10.1046/j.1439-0434.2001.00664.x

Dunn, P. K., & Smyth, G. K. (1996). Randomized quantile residuals. *Journal of Computational and Graphical Statistics*, *5*(3), 236–244.

Durka, W., & Michalski, S. G. (2012). Daphne: A dated phylogeny of a large European flora for phylogenetically informed ecological analyses. *Ecology*, *93*(10), 2297. https://doi.org/10.1890/12-0743.1

Dutt, A., Andrivon, D., & Le May, C. (2022). Multi-infections, competitive interactions, and pathogen coexistence. *Plant Pathology*, *71*(1), 5–22. https://doi.org/10.1111/ppa.13469

Dutt, A., Anthony, R., Andrivon, D., Jumel, S., Le Roy, G., Baranger, A., Leclerc, M., & Le May, C. (2021). Competition and facilitation among fungal plant parasites affect their life-history traits. *Oikos*, *130*(4), 652–667. https://doi.org/10.1111/oik.07747

Field, E., Castagneyrol, B., Gibbs, M., Jactel, H., Barsoum, N., Schönrogge, K., & Hector, A. (2020). Associational resistance to both insect and pathogen damage in mixed forests is modulated by tree neighbour identity and drought. *Journal of Ecology*, *108*(4), 1511–1522. https://doi.org/10.1111/1365-2745.13397

Francioli, D., van Ruijven, J., Bakker, L., & Mommer, L. (2020). Drivers of total and pathogenic soil-borne fungal communities in grassland plant species. *Fungal Ecology*, *48*, 100987. https://doi.org/10.1016/j.funeco.2020.100987

Glynou, K., Nam, B., Thines, M., & Maciá-Vicente, J. G. (2018). Facultative root-colonizing fungi dominate endophytic assemblages in roots of nonmycorrhizal Microthlaspi species. *New Phytologist*, *217*(3), 1190–1202. https://doi.org/10.1111/nph.14873

González García, V., Portal Onco, M. A., & Rubio Susan, V. (2006). Biology and systematics of the form genus *Rhizoctonia*. *Spanish Journal of Agricultural Research*, *4*(1), 55–79.

Grossman, J. J., Cavender-Bares, J., Reich, P. B., Montgomery, R. A., & Hobbie, S. E. (2019). Neighborhood diversity simultaneously increased and decreased susceptibility to contrasting herbivores in an early stage forest diversity experiment. *Journal of Ecology*, *107*(3), 1492–1505. https://doi.org/10.1111/1365-2745.13097

Hamidi, B., Wallace, K., Vasu, C., & Alekseyenko, A. V. (2019). Wd*-test: Robust distance-based multivariate analysis of variance. *Microbiome*, *7*(1), 51. https://doi.org/10.1186/s40168-019-0659-9

Hannula, S. E., Heinen, R., Huberty, M., Steinauer, K., De Long, J. R., Jongen, R., & Bezemer, T. M. (2021). Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nature Communications*, *12*(1), 1–13. https://doi.org/10.1038/s41467-021-25971-z

Hantsch, L., Bien, S., Radatz, S., Braun, U., Auge, H., & Bruelheide, H. (2014). Tree diversity and the role of non-host neighbour tree species in reducing fungal pathogen infestation. *Journal of Ecology*, *102*(6), 1673–1687. https://doi.org/10.1111/1365-2745.12317

Heinen, R., Hannula, S. E., De Long, J. R., Huberty, M., Jongen, R., Kielak, A., Steinauer, K., Zhu, F., & Bezemer, T. M. (2020). Plant community composition steers grassland vegetation via soil legacy effects. *Ecology Letters*, *23*(6), 973–982. https://doi.org/10.1111/ele.13497

Hendriks, M., Mommer, L., de Caluwe, H., Smit-Tiekstra, A. E., van der Putten, W. H., & de Kroon, H. (2013). Independent variations of plant and soil mixtures reveal soil feedback effects on plant community overyielding. *Journal of Ecology*, *101*(2), 287–297. https://doi.org/10.1111/1365-2745.12032

Hendriks, M., Raaijmakers, J. M., Reijers, V., van de Mortel, J., de Kroon, H., & Mommer, L. (2015). The role of root-associated fungi as drivers of the plant diversity-productivity relationship. In *Effects of plant–soil feedback on root distribution, plant competition and community productivity* (PhD thesis). Radboud University Nijmegen.

Hernandez-Escribano, L., Iturritxa, E., Elvira-Recuenco, M., Berbegal, M., Campos, J. A., Renobales, G., García, I., & Raposo, R. (2018). Herbaceous plants in the understory of a pitch canker-affected *Pinus radiata* plantation are endophytically infected with *Fusarium circinatum*. *Fungal Ecology*, *32*, 65–71. https://doi.org/10.1016/j.funeco.2017.12.001

Hersh, M. H., Vilgalys, R., & Clark, J. S. (2012). Evaluating the impacts of multiple generalist fungal pathogens on temperate tree seedling survival. *Ecology*, *93*(3), 511–520. https://doi.org/10.1890/11-0598.1

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

IPBES (2019). In S. Díaz, J. Settele, E. S. Brondízio, H. T. Ngo, M. Guèze, J. Agard, A. Arneth, P. Balvanera, K. A. Brauman, S. H. M. Butchart, K. M. A. Chan, L. A. Garibaldi, K. Ichii, J. Liu, S. M. Subramanian, G. F. Midgley, P. Miloslavich, Z. Molnár, D. Obura, et al. (Eds.), *Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the intergovernmental science-policy platform on biodiversity and ecosystem services*. IPBES Secretariat.

Kandlikar, G. (2020). *Ranacapa: Utility functions and 'shiny' app for simple environmental DNA visualizations and analyses*. R package version 0.1.0. https://github.com/gauravsk/ranacapa

Keesing, F., Holt, R. D., & Ostfeld, R. S. (2006). Effects of species diversity on disease risk. *Ecology Letters*, *9*(4), 485–498. https://doi.org/10.1111/j.1461-0248.2006.00885.x

Keesing, F., & Ostfeld, R. S. (2021). Dilution effects in disease ecology. *Ecology Letters*, *24*(11), 2490–2505. https://doi.org/10.1111/ele.13875

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*(11), 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Kenne Pagui, E. C., Salvan, A., & Sartori, N. (2022). Improved estimation in negative binomial regression. *Statistics in Medicine*, *41*(13), 2403–2416. https://doi.org/10.1002/sim.9361

Kia, S. H., Glynou, K., Nau, T., Thines, M., Piepenbring, M., & Maciá-Vicente, J. G. (2017). Influence of phylogenetic conservatism and trait convergence on the interactions between fungal root endophytes and plants. *The ISME Journal*, *11*(3), 777–790. https://doi.org/10.1038/ismej.2016.140

Klaubauf, S., Tharreau, D., Fournier, E., Groenewald, J. Z., Crous, P. W., de Vries, R. P., & Lebrun, M.-H. (2014). Resolving the polyphyletic nature of *Pyricularia* (*Pyriculariaceae*). *Studies in Mycology*, *79*(1), 85–120. https://doi.org/10.1016/j.simyco.2014.09.004

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., … Larsson, K.-H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, *22*(21), 5271–5277. https://doi.org/10.1111/mec.12481

Kosmidis, I. (2021). *brglm2: Bias reduction in generalized linear models*. R package version 0.8.2. https://cran.r-project.org/package=brglm2

Leff, J. W., Bardgett, R. D., Wilkinson, A., Jackson, B. G., Pritchard, W. J., De Long, J. R., Oakley, S., Mason, K. E., Ostle, N. J., Johnson, D., Baggs, E. M., & Fierer, N. (2018). Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *The ISME Journal*, *12*(7), 1794–1805. https://doi.org/10.1038/s41396-018-0089-x

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, *129*(2), 271–280. https://doi.org/10.1007/s004420100716

Lofgren, L. A., LeBlanc, N. R., Certano, A. K., Nachtigall, J., LaBine, K. M., Riddle, J., Broz, K., Dong, Y., Bethan, B., Kafer, C. W., & Kistler, H. C. (2018). *Fusarium graminearum*: Pathogen or endophyte of north American grasses? *New Phytologist*, *217*(3), 1203–1212. https://doi.org/10.1111/nph.14894

Makiola, A., Holdaway, R. J., Wood, J. R., Orwin, K. H., Glare, T. R., & Dickie, I. A. (2022). Environmental and plant community drivers of plant pathogen composition and richness. *New Phytologist*, *233*(1), 496–504. https://doi.org/10.1111/nph.17797

Malcolm, G. M., Kuldau, G. A., Gugino, B. K., & Jiménez-Gasco, M. d. M. (2013). Hidden host plant associations of soilborne fungal pathogens: An ecological perspective. *Phytopathology*, *103*(6), 538–544. https://doi.org/10.1094/PHYTO-08-12-0192-LE

Maron, J. L., Marler, M., Klironomos, J. N., & Cleveland, C. C. (2011). Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters*, *14*(1), 36–41. https://doi.org/10.1111/j.1461-0248.2010.01547.x

Mesny, F., Miyauchi, S., Thiergart, T., Pickel, B., Atanasova, L., Karlsson, M., Hüttel, B., Barry, K. W., Haridas, S., Chen, C., Bauer, D., Andreopoulos, W., Pangilinan, J., LaButti, K., Riley, R., Lipzen, A., Clum, A., Drula, E., Henrissat, B., … Hacquard, S. (2021). Genetic eterminants of endophytism in the Arabidopsis root mycobiome. *Nature Communications*, *12*(1), 1–15. https://doi.org/10.1038/s41467-021-27479-y

Mommer, L., Cotton, T. E. A., Raaijmakers, J. M., Termorshuizen, A. J., van Ruijven, J., Hendriks, M., van Rijssel, S. Q., van de Mortel, J. E., van der Paauw, J. W., Schijlen, E. G. W. M., Smit-Tiekstra, A. E., Berendse, F., de Kroon, H., & Dumbrell, A. J. (2018). Lost in diversity: The interactions between soil-borne fungi, biodiversity and plant productivity. *New Phytologist*, *218*(2), 542–553. https://doi.org/10.1111/nph.15036

Mueller, K. E., Tilman, D., Fornara, D. A., & Hobbie, S. E. (2013). Root depth distribution and the diversity–productivity relationship in a long-term grassland experiment. *Ecology*, *94*(4), 787–793. https://doi.org/10.1890/12-1399.1

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nilsson, R. H., Hyde, K. D., Pawłowska, J., Ryberg, M., Tedersoo, L., Aas, A. B., Alias, S. A., Alves, A., Anderson, C. L., Antonelli, A., Arnold, A. E., Bahnmann, B., Bahram, M., Bengtsson-Palme, J., Berlin, A., Branco, S., Chomnunti, P., Dissanayake, A., Drenkhan, R., … Abarenkov, K. (2014). Improving ITS sequence data for identification of plant pathogenic fungi. *Fungal Diversity*, *67*(1), 11–19. https://doi.org/10.1007/s13225-014-0291-8

Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*(D1), D259–D264. https://doi.org/10.1093/nar/gky1022

Ogoshi, A. (1987). Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Annual Review of Phytopathology*, *25*, 125–143.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). *Vegan: Community ecology package*. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Pauvert, C., Buée, M., Laval, V., Edel-Hermann, V., Fauchery, L., Gautier, A., Lesur, I., Vallance, J., & Vacher, C. (2019). Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology*, *41*, 23–33. https://doi.org/10.1016/j.funeco.2019.03.005

Peay, K. G., Baraloto, C., & Fine, P. V. A. (2013). Strong coupling of plant and fungal community structure across western Amazonian rainforests. *ISME Journal*, *7*(9), 1852–1861. https://doi.org/10.1038/ismej.2013.66

Peerally, M. A., & Colhoun, J. (1969). The epidemiology of root rot of chrysanthemums caused by *Phoma* sp. *Transactions of the British Mycological Society*, *52*(1), 115–123. https://doi.org/10.1016/s0007-1536(69)80165-8

Pereira, E., Vázquez De Aldana, B. R., Emeterio, L. S., & Zabalgogeazcoa, I. (2019). A survey of culturable fungal endophytes fromfestuca rubra subsp. pruinosa, a grass from marine cliffs, reveals a core microbiome. *Frontiers in Microbiology*, *10*(Jan), 1–14. https://doi.org/10.3389/fmicb.2018.03321

Power, A. G., & Mitchell, C. E. (2004). Pathogen spillover in disease epidemics. *The American Naturalist*, *164*(S5), S79–S89. https://doi.org/10.1086/424610

Prada-Salcedo, L. D., Goldmann, K., Heintz-Buschart, A., Reitz, T., Wambsganss, J., Bauhus, J., & Buscot, F. (2021). Fungal guilds and soil functionality respond to tree community traits rather than to tree diversity in European forests. *Molecular Ecology*, *30*(2), 572–591. https://doi.org/10.1111/mec.15749

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, *321*(1–2), 341–361. https://doi.org/10.1007/s11104-008-9568-6

Rosenthal, L. M., Simler-Williamson, A. B., & Rizzo, D. M. (2021). Community-level prevalence of a forest pathogen, not individual-level disease risk, declines with tree diversity. *Ecology Letters*, *24*(11), 2477–2489. https://doi.org/10.1111/ele.13871

Russel, J. (2021). *MicEco: Various functions for microbial community data*. R package version 0.9.16. https://zenodo.org/record/4733747#.YcWJqWjMLb0

Rutten, G., Hönig, L., Schwaß, R., Braun, U., Saadani, M., Schuldt, A., Michalski, S. G., & Bruelheide, H. (2021). More diverse tree communities promote foliar fungal pathogen diversity, but decrease infestation rates per tree species, in a subtropical biodiversity experiment. *Journal of Ecology*, *109*(5), 2068–2080. https://doi.org/10.1111/1365-2745.13620

Sánchez Márquez, S., Bills, G. F., Domínguez Acuña, L., & Zabalgogeazcoa, I. (2010). Endophytic mycobiota of leaves and roots of the grass Holcus lanatus. *Fungal Diversity*, *41*(1), 115–123. https://doi.org/10.1007/s13225-009-0015-7

Sarmiento, C., Zalamea, P.-C., Dalling, J. W., Davis, A. S., Stump, S. M., U'Ren, J. M., & Arnold, A. E. (2017). Soilborne fungi have host affinity and host-specific effects on seed germination and survival in a lowland tropical forest. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(43), 201706324. https://doi.org/10.1073/pnas.1706324114

Schmid, M. W., van Moorsel, S. J., Hahl, T., De Luca, E., de Deyn, G. B., Wagg, C., Niklaus, P. A., & Schmid, B. (2021). Effects of plant community history, soil legacy and plant diversity on soil microbial communities. *Journal of Ecology*, *109*(8), 3007–3023. https://doi.org/10.1111/1365-2745.13714

Schnitzer, S. A., Klironomos, J. N., Hillerislambers, J., Kinkel, L. L., Reich, P. B., Xiao, K., Rillig, M. C., Sikes, B. A., Callaway, R. M., Scott, A., Van Nes, E. H., Scheffer, M., Klironomos, J. N., Hillerislambers, J., Kinkel, L. L., Reich, P. B., Xiao, K., Rillig, M. C., Sikes, B. A., … Scheffer, M. (2011). Soil microbes drive the classic plant-productivity diversity pattern. *Ecology*, *92*(2), 296–303.

Selosse, M.-A., Schneider-Maunoury, L., & Martos, F. (2018). Time to re-think fungal ecology? Fungal ecological niches are often prejudged. *New Phytologist*, *217*(3), 968–972. https://doi.org/10.1111/nph.14983

Semchenko, M., Barry, K. E., de Vries, F. T., Mommer, L., Moora, M., & Maciá-Vicente, J. G. (2022). Deciphering the role of specialist and generalist plant–microbial interactions as drivers of plant–soil feedback. *New Phytologist*, *234*(6), 1929–1944. https://doi.org/10.1111/nph.18118

Setiawan, N. N., Vanhellemont, M., Baeten, L., Dillen, M., & Verheyen, K. (2014). The effects of local neighbourhood diversity on pest and disease damage of trees in a young experimental forest. *Forest Ecology and Management*, *334*, 1–9. https://doi.org/10.1016/j.foreco.2014.08.032

Tilman, D. (2001). Diversity and productivity in a Long-term grassland experiment. *Science*, *294*(5543), 843–845. https://doi.org/10.1126/science.1060391

van Ruijven, J., Ampt, E., Francioli, D., & Mommer, L. (2020). Do soil-borne fungal pathogens mediate plant diversity–productivity relationships? Evidence and future opportunities. *Journal of Ecology*, *108*(5), 1810–1821. https://doi.org/10.1111/1365-2745.13388

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed.). Springer.

Wang, Y., Naumann, U., Eddelbuettel, D., Wilshire, J., & Warton, D. (2020). *Mvabund: Statistical methods for analysing multivariate abundance data*. R package version 4.1.3. https://CRAN.R-project.org/package=mvabund

Warton, D. I., Lyons, M., Stoklosa, J., & Ives, A. R. (2016). Three points to consider when choosing a LM or GLM test for count data. *Methods in Ecology and Evolution*, *7*(8), 882–890. https://doi.org/10.1111/2041-210X.12552

Wehner, J., Powell, J. R., Muller, L. A. H., Caruso, T., Veresoglou, S. D., Hempel, S., & Rillig, M. C. (2014). Determinants of root-associated fungal communities within Asteraceae in a semi-arid grassland. *Journal of Ecology*, *102*(2), 425–436. https://doi.org/10.1111/1365-2745.12197

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, *18*(1), 315–322.

Yang, M., Zhang, Y., Qi, L., Mei, X., Liao, J., Ding, X., Deng, W., Fan, L., He, X., Vivanco, J. M., Li, C., Zhu, Y., & Zhu, S. (2014). Plant-plant-microbe mechanisms involved in soil-borne disease suppression on a maize and pepper intercropping system. *PLoS ONE*, *9*(12), e115052. https://doi.org/10.1371/journal.pone.0115052

SUPPORTING INFORMATION

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