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Research

Responses of rhizosphere fungi to the root economics space in grassland monocultures of different age

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Summary

- Recent studies on root traits have shown that there are two axes explaining trait variation belowground: the collaboration axis with mycorrhizal partners and the conservation ('fast – slow') axis. However, it is yet unknown whether these trait axes affect the assembly of soil-borne fungi. We expect saprotrophic fungi to link to the conservation axis of root traits, whereas pathogenic and arbuscular mycorrhizal fungi link to the collaboration axis, but in opposite directions, as arbuscular mycorrhizal fungi might provide pathogen protection.
- To test these hypotheses, we sequenced rhizosphere fungal communities and measured root traits in monocultures of 25 grassland plant species, differing in age. Within the fungal guilds, we evaluated fungal species richness, relative abundance and community composition.
- Contrary to our hypotheses, fungal diversity and relative abundance were not strongly related to the root trait axes. However, saprotrophic fungal community composition was affected by the conservation gradient and pathogenic community composition by the collaboration gradient. The rhizosphere AMF community composition did not change along the collaboration gradient, even though the root trait axis was in line with the root mycorrhizal colonization rate.
- Overall, our results indicate that in the long term, the root trait axes are linked with fungal community composition.

Introduction

The 'fast – slow axis' of the leaf economics spectrum is a major structuring principle in ecology (Wardle, 2004; Reich, 2014). Plant species on the 'fast' side of the gradient are characterized by high relative growth rates, high specific leaf area, high relative nitrogen content and photosynthesis rate, whereas resource-conservative 'slow' plant species show opposite patterns (Wright *et al.*, 2004). Trait differences at the leaf level cascade via litter into soil microbial communities (Wardle, 2004; de Vries *et al.*, 2012; Bardgett, 2017). Litter from 'slow' leaves is known to decompose slower because of the low relative nitrogen and high lignin content, whereas litter of plant species at the 'fast' end of the spectrum decomposes faster (Wardle, 2002, 2004). This 'fast – slow' framework inspired many studies to reveal effects of leaf traits on soilborne fungal communities (de Vries *et al.*, 2012; Semchenko *et al.*, 2018; Buzzard *et al.*, 2019). Despite these efforts, little focus has been on the effect of root traits on soil-borne fungal communities. However, there is evidence that root traits are more important drivers of soil fungal communities than aboveground traits (Orwin *et al.*, 2010; Cantarel *et al.*, 2015).

Recent advances in trait-based ecology have shown that belowground resource strategies are not always following aboveground resource strategies (Weemstra *et al.*, 2016). Bergmann *et al.* (2020) proposed the first comprehensive framework for fine-root traits: the root economics space (RES). It consists of two orthogonal axes of root trait variation: The 'fast – slow' axis (i.e. conservation gradient) with species with high relative root nitrogen content (RN) at the 'fast' side and high root tissue density (RTD) at the 'slow' side (Fig. 1). However, there is another, even more important axis of trait variation in roots, the 'collaboration gradient' with a 'do-it-yourself' ('DIY') strategy of

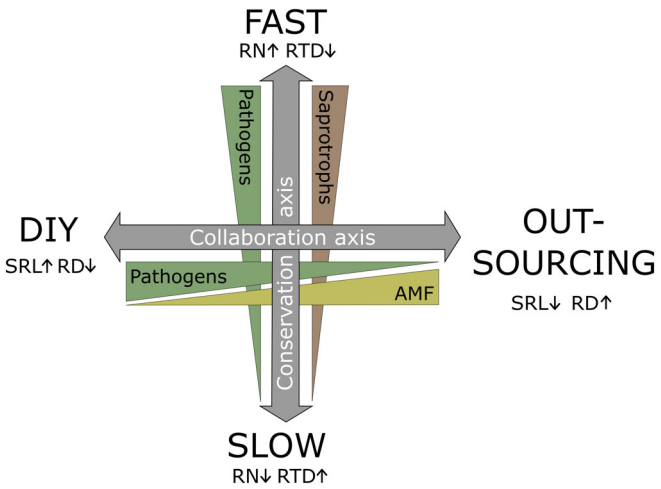


Fig. 1 Hypothesized changes in fungal guild relative abundance along the root economics space (RES). The RES is depicted as the two main axes of root trait variation (gray arrows). The collaboration gradient ranges from an 'outsourcing' to a 'DIY' approach of nutrient uptake; the conservation gradient distinguishes 'fast' and 'slow' plant and root strategies (Bergmann *et al*., 2020). Colored arrows depict the hypothesized correlation and relative abundance of fungal guilds to the axes of the RES. AMF, arbuscular mycorrhizal fungi; RD, root diameter; RN, root nitrogen; RTD, root tissue density; SRL, specific root length.

plants that invest in high specific root length (SRL) to an 'outsourcing' strategy with thick roots (high RD) that trade their carbon for resource acquisition through symbiosis with arbuscular mycorrhizal fungi (Bergmann *et al*., 2020; Weigelt *et al*., 2021). The proposal of the RES has triggered research on its correlation with other plant traits and processes, for example investigations of how the collaboration axis relates to root respiration (Han & Zhu, 2021), root exudation (Han *et al*., 2022; Williams *et al*., 2022) or plant–soil feedbacks (Semchenko *et al*., 2022; Rutten & Allan, 2023). Specifically, the latter study opens an avenue to relate root trait variation to soilborne fungi. We expect that due to the close link of roots and soil microbial communities, the collaboration gradient will have strong effects on soil fungal communities.

Soil fungi are important drivers of many ecosystem functions, such as decomposition (Bani *et al*., 2018), plant nutrient acquisition, primary production (Mommer *et al*., 2018), drought resistance (Francioli *et al*., 2020) and physicochemical soil properties (Duchicela *et al*., 2013; Muneer *et al*., 2020). There is a variety of mechanisms how plants affect the soil fungal community, including root exudates (Bais *et al*., 2006; Hu *et al*., 2018), as well as litter quantity and quality (Wardle, 2004; Veen *et al*., 2019). Furthermore, plants and fungal communities are symbiotically linked through mycorrhizal associations (Smith & Read, 2008) or endophytic symbioses (van der Heijden *et al*., 2017). More than 80% of terrestrial plants form symbioses with mycorrhizal fungi (Brundrett, 2002). Since many mechanisms underlying plant–fungal interactions are closely linked to roots and their traits, linking soil fungi and root traits is a major frontier in ecology (Laliberté, 2017; Semchenko *et al*., 2022).

Based on their strategy and functional role, fungal taxa are often classified into guilds. The major guilds in the soil include saprotrophic, mycorrhizal and pathogenic fungi (Rayner, 1926; Ainsworth, 1976; Zanne *et al*., 2020).

Saprotrophic fungi mainly decompose organic matter and are critical to ecosystem functioning due to their ability to degrade complex carbon compounds, such as cellulose and lignin (Dighton, 2007). In addition, saprotrophs also take up more easily degradable carbon compounds, such as exudates of plant roots (de Vries & Caruso, 2016). While studies on decomposition and turnover of plant litter in the soil mostly focused on aboveground litter, the turnover of roots is increasingly recognized (Mommer *et al*., 2015; Liu *et al*., 2019; Su & Xu, 2021) since large parts of plant-derived carbon, especially in grasslands, are allocated belowground (Mokany *et al*., 2006; Poorter *et al*., 2012). Plants at the 'fast' end of the conservation gradient are generally associated with faster rates of production and decomposition, suggesting a more rapid breakdown of 'fast' above- and belowground plant litter compared with 'slow' species (Wardle, 2004). Decomposition of root material has been shown to increase with high relative root nitrogen content (Vivanco & Austin, 2006; Zhang *et al*., 2008; Prieto *et al*., 2016), while it decreases with high lignin content and therefore higher C : N ratio (Silver & Miya, 2001; Freschet *et al*., 2012; Smith *et al*., 2014). However, studies that investigated the correlation of root decomposition with individual traits of the collaboration gradient (SRL or RD) have been inconclusive so far (Hobbie *et al*., 2010; Guerrero-Ramírez *et al*., 2016; Jo *et al*., 2016; Prieto *et al*., 2016; Jiang *et al*., 2021). Rhizodeposition, which includes root exudates and all other root-derived carbon (Oburger & Jones, 2018), is an additional key carbon source for saprotrophic fungi in the rhizosphere (Nguyen, 2003; Jones *et al*., 2009). Compared with overall plant litter, rhizodeposits are largely easier to digest and therefore a more easily available source of carbon for rhizosphere fungi (Paterson, 2003). Previous research suggests that rhizodeposition increases in plants with a 'fast' acquisitive strategy (e.g. Guyonnet *et al*., 2018), but its link with the collaboration axis is less clear (Wen *et al*., 2019; Meier *et al*., 2020; Han *et al*., 2022; Williams *et al*., 2022). Taken together, if the RES is a major driver of fungal community composition, we expect saprotrophic fungi to be most abundant in the rhizosphere of plants with 'fast' compared with 'slow' roots due to the effects of above- and belowground litter quantity and quality, and potentially increased rhizodeposition. Because the change in litter quality along the conservation axis requires fungal species with different decay capabilities, it is likely that the saprotroph fungal community composition is linked to the conservation axis (Francioli *et al*., 2021). We further expect that the ratio of opportunistic Ascomycota to more specialized lignin-decomposing Basidiomycota (Dix & Webster, 1995) increases in the rhizosphere of plants with a more acquisitive strategy along the conservation axis due to lower complexity of the litter.

Following the idea of the collaboration gradient, we expect that arbuscular mycorrhizal fungi (AMF) are less abundant in the rhizosphere of plants with thin ('DIY') compared with thicker 'outsourcing' roots. Arbuscular mycorrhizal colonization rate is correlated with a high root diameter, because thicker roots have fractionally more cortical tissue where AMF are located (Brundrett, 2002; Kong *et al*., 2014). Taken together, we expect higher

relative AMF abundance toward the 'outsourcing' side of the collaboration axis. Accordingly, the community composition of AMF should be correlated with the collaboration, rather than with the conservation axis.

Plant pathogens include fungal species that derive their carbon from living plants and cause negative impacts on the host plant. Pathogenic fungi in the rhizosphere interfere with primary plant defense and employ a variety of strategies for invading plant roots (Agrios, 2005; Doehlemann *et al.*, 2017). Plant defense mechanisms against pathogenic fungi include reduced palatability, for example through increased root toughness and reduced relative nitrogen content, as well as production of secondary compounds (Freschet *et al.*, 2021). According to the growth–defense trade-off, plants with a 'fast' growth strategy should be less defended against herbivores and pathogens (Coley *et al.*, 1985). This suggests that plants with root traits on the 'fast' end of the conservation gradient could have a higher abundance of pathogenic fungi in the rhizosphere. However, this has rarely been tested belowground and is often focused on (arthropod) herbivory rather than pathogens (but see Lemmermeyer *et al.*, 2015; Semchenko *et al.*, 2018). Furthermore, it is unclear whether tissue density has similar effects on pathogen infection as it does on herbivory (Sattler & Funnell-Harris, 2013). On the contrary, mycorrhizal colonization by AMF was shown to provide some protection against pathogens and improve plant disease resistance (Dehne, 1982; Smith, 1988; Hooker *et al.*, 1994; Newsham *et al.*, 1995b; Whipps, 2004; Powell *et al.*, 2009). As an example, AMF are known to increase the jasmonic acid pathway, enabling plants to respond rapidly to pathogen attacks (Pozo & Azcón-Aguilar, 2007; Jung *et al.*, 2012; Cameron *et al.*, 2013). While the mechanisms of AMF protection against antagonists are not completely resolved, we expect the previously demonstrated protective function to lead to lower relative pathogen abundance and diversity toward the 'outsourcing' end of the collaboration axis, where AMF should be more abundant. Detailed descriptions and references of the expected relationship between RES axes and relative abundance of fungal guilds can be found in Table 1. Although we have no strong prediction for the species richness within guilds, we expect that increasing relative abundance of a guild may also lead to an increase in the number of species of the respective guild.

Previous studies that linked plant traits in general with the abundance of fungal guilds, such as saprotrophs, pathogens and AMF, have found mixed results (Eissenstat *et al.*, 2015; Leff *et al.*, 2018; Semchenko *et al.*, 2018). Lozano *et al.* (2021) have shown that root traits could explain shifts in fungal communities in response to drought. To our knowledge, the only study that specifically assessed the relationships between the root traits of the RES on fungal communities was performed in a pot experiment of individual seedlings and has found strong effects (Sweeney *et al.*, 2021). The authors could show that the proportion and diversity of most guilds, as well as their community composition, were affected by root traits. Previous studies suggested that soil microorganisms respond to changes in community composition with a time lag (Habekost *et al.*, 2008; Eisenhauer *et al.*, 2012). We therefore expect that relationship between root trait axes and the fungal guilds will strengthen over time.

Expanding on these previous findings, in this study we test the links of the two axes of the RES to the main rhizosphere fungal guilds in a Central European grassland experiment. We sequenced soilborne fungal communities of rhizosphere soil in monocultures of 25 plant species of two different ages (4 and 18 yr) of the Jena Experiment. In addition, we measured root traits on individual plants. In order to link the RES to soil fungal communities, we investigated three fungal measures: (1) the relative abundance of fungal guilds (i.e. changes in guild proportion), (2) the fungal diversity within guilds and (3) the fungal community composition, among and within guilds. The difference in plot age further allows us to evaluate whether the relationship between root trait axes and fungal community changed over time.

Materials and Methods

Study site

The study was conducted at the Jena Experiment. This large-scale long-term biodiversity experiment is located on a former arable field in the floodplain of the river Saale 51°N, 11°E, 135 m above sea level (Roscher *et al.*, 2004). We used monoculture plots established in 2002. The plots are not fertilized and are mown twice a year with the plant material being removed. The initial size of the plots was 3.5 × 3.5 m but was reduced to 1 × 1 m in 2009. To maintain the sown species composition, the communities are weeded manually two to three times per year in spring, summer and autumn (Weisser *et al.*, 2017). In addition to the plots established in 2002, a new set of 1 × 1 m monocultures was sown in 2016, using the seeds from the same seed supplier (Vogel *et al.*, 2019). These plots contain soil from the same origin as was used in 2002, but with an additional legacy of 14 yr of agricultural use and excluding soil and plant history of the old plots. Due to these differences, we do not aim to compare the differences between the old and young plots, but investigate whether RES patterns are present in the two groups. All plots (old and young) are located in a single field site, grouped in four experimental blocks parallel to the Saale River.

Sampling design and soil collection

Between 18 May and 5 June 2020, we took soil cores (5 cm diameter, 10 cm depth) of three to five plant individuals of 25 plant species in old and young monoculture plots of the Jena Experiment. The aboveground plant was cut at 1 cm from ground level, and the soil core was taken with the remaining plant stump in the middle of the core. The full list of plant species can be found in the Supporting Information Table S1. The soil cores were kept at 4°C after sampling until further preparation (maximum 24 h after sampling). The soil cores were shaken by hand and the loose soil discarded to remove the nonroot-associated soil. After this, the soil cores consisted of the root system and attached soil, of which a pooled sample at plot level

Table 1 Expected effects and hypothesized or demonstrated mechanisms of the RES axes on the abundance of main rhizosphere fungal guilds.

	(+)/(−)	Expected strength of effect	Rationale	Selected references
Saprotroph relative abundance				
Collaboration				
'Outsourcing' (high RD, low SRL)	(+)/(−)	Low	Changes in exudation/rhizodeposition rate (both directions previously reported)	Ryan *et al*. (2012); Wen *et al*. (2019); Williams *et al*. (2022)
Conservation				
'Fast' (high RN, low RTD)	(+)	High	Increased litter production due to short lifespan of roots and higher productivity	Jackson *et al*. (1996); Ryser (1996); McCormack *et al*. (2015)
			Lower complexity of litter	Coley *et al*. (1985); Wardle (2004); Bardgett (2017)
			Higher exudation/rhizodeposition rate	Kaštovská *et al*. (2015); Guyonnet *et al*. (2018); Henneron *et al*. (2020b); Sun *et al*. (2021); Williams *et al*. (2022); Wen *et al*. (2022)
AMF relative abundance				
Collaboration				
'Outsourcing' (high RD, low SRL)	(+)	High	Larger parenchyma cortex increases root colonization rate that should also increase AMF abundance in the rhizosphere	Ryan *et al*. (2012); Wen *et al*. (2019); Williams *et al*. (2022)
Conservation				
'Fast' (high RN, low RTD)	(−)	Low	Shorter lifespan of roots could reduce chances of colonization	Semchenko *et al*. (2018)
Pathogen relative abundance				
Collaboration				
'Outsourcing' (high RD, low SRL)	(−)	High	Direct or indirect plant protection by AMF, e.g. through induction of defense	Dehne (1982); Smith (1988); Hooker *et al*. (1994); Newsham *et al*. (1995a,b); Whipps (2004); Pozo & Azcón-Aguilar (2007); Powell *et al*. (2009); Jung *et al*. (2012); Cameron *et al*. (2013)
			Increased nutritional status could reduce susceptibility to pathogen attacks	Linderman (1994); Azcón-Aguilar & Barea (1996)
			Fewer infection sites in the root (due to lower relative surface area) and competition for space with AMF inside the root	Linderman (1994); Traquair (1995); Cordier *et al*. (1996); Smith & Read (2008); Laliberté *et al*. (2015)
Conservation				
'Fast' (high RN, low RTD)	(+)	Medium	Lower investments in defense mechanisms and compounds ('growth–defense trade-off'), decreased mechanical resistance, decreased root lifespan	Coley *et al*. (1985); Simms & Rausher (1987); Herms & Mattson (1992); Ryser (1996); Miedes *et al*. (2014); Semchenko *et al*. (2018)

RD, root diameter; RN, root nitrogen; RTD, root tissue density; SRL, specific root length.

was collected for the DNA extraction. Following previous studies, we considered this as rhizosphere soil (e.g. Smiley, 1974; Courchesne & Gobran, 1997; Ding *et al*., 2019). This soil was frozen to −20°C immediately.

Root trait measurements

After the removal of some soil by shaking the core, the root system with the remaining attached soil was soaked in water for *c*. 15 min and then repeatedly rinsed with tap water over a sieve and cleaned with forceps until it was free of soil particles and debris. The roots attached to the remaining stump of the core plant were carefully separated and pooled for all individuals per plot, and roots with a diameter larger than 2 mm were discarded. Remaining fine roots were scanned and measured using an Epson Expression 11000XL (Epson, Tokyo, Japan) flatbed scanner at 600 dpi and the software WinRhizo (Regent Instruments Inc., Quebec City, QC, Canada) to determine root length and root diameter. The scanned roots were weighed, dried (48 h at 70°C) and weighed for dry mass. We calculated specific root length (SRL, root length : dry mass) and root dry matter content (RDMC, root dry mass : root fresh mass). RDMC was used as a proxy for RTD, as previous studies have shown a close correlation (Birouste *et al*., 2014). To quantify relative root nitrogen content, a subset of roots was freeze-dried and ground using a zirconium kit in a ball mill (MM400; Retsch, Haan, Germany). After milling, the samples were freeze-dried again, and we measured near-infrared spectra (NIR) in the range of 12 489–3594 cm^{-1} (809–2782 nm) at 8 cm^{-1} resolution in transmission mode (Multi-Purpose FT-NIR-Analyzer; Bruker Corp. Billerica, MA, USA). Transmission was converted to absorbance as $\log_{10}(1/\text{Transmission})$. We measured five replicates per sample

and shook the sample in between to account for spectral variability within a sample. For 75% of the samples, we quantified relative nitrogen content using an elemental analyzer (Elementar vario el II, Hanau, Germany). Mean trait values per plant functional group can be found in Table S2. To quantify the mycorrhizal colonization rate, we washed a subset of roots of each sample, incubated the roots at 95°C in 10% KOH for 10 min and washed again. We used acidic ink solution (5% acetic acid, 5% pelican blue ink) for staining and 20% acetic acid for destaining for 15 min at 75°C (Vierheilig *et al.*, 1998). This process was repeated twice, and the roots were put on microscopy slides in glycerin. We then assessed the mycorrhizal colonization rate with a digital microscope at ×200 magnification (Keyence VHX, Osaka, Japan) using the scoring system described by Trouvelot *et al.* (1986) on 73–140 images.

Fungal amplicon sequencing

Genomic DNA was extracted from 0.4 g of thawed and homogenized rhizosphere soil using DNeasy PowerSoil Kit (Qiagen) following the manufacturer's instructions. DNA content was measured with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany), and the extracted DNA was stored at −20°C. The internal transcribed spacer (ITS) region 2 was amplified using ITS4 and ITS7:ITS7o primers (White *et al.*, 1990; Ihrmark *et al.*, 2012; Kohout *et al.*, 2014). Doing this, not all fungi are covered equally well and there is some amplification bias against AMF (Tedersoo *et al.*, 2015). However, previous studies have shown that the AMF richness and community composition data from ITS2 sequencing can yield similar responses as the more AMF-specific 18S approach (Berruti *et al.*, 2017; Lekberg *et al.*, 2018) and that it is therefore appropriate to compare AMF communities within one dataset from similar locations.

Each sample was amplified using 7.5 µl KAPA HiFi HotStart ReadyMix Polymerase (Roche) and 0.3 µl of each primer and 1 µl of template in 15 µl reaction. The template contained DNA extract with an adjusted concentration of 10 ng μl^{-1}. PCR conditions were as follows: 3 min at 95°C, followed by 30 cycles of 95°C for 20 s, 56°C for 20 s and 72°C for 20 s, with a final extension of 72°C for 5 min. Amplification of each reaction was checked by electrophoresis in a 1.5% agarose gel with ethidium bromide staining and three positive PCR products were pooled. Thirty microliter amplicons were purified with AMPure XP beads (Beckman Coulter, Krefeld, Germany) and the Illumina index and sequencing adapters were added by PCR using a Nextera XT Illumina Index Kit (Illumina Inc., San Diego, CA, USA). Finally, library quantification was done using a Nanodrop spectrophotometer and samples were pooled to equal molarity. The paired-end sequencing of 2 × 300 bp was performed using a MiSeq Reagent Kit v.3 on an Illumina MiSeq System at the Department of Soil Ecology of the Helmholtz-Centre for Environmental Research (UFZ, Halle/Saale, Germany).

Sequence read processing was performed using the snakemake implementation *dadasnake* (Weißbecker *et al.*, 2020) of the DADA2 pipeline (Callahan *et al.*, 2016). In short, sequences of the primers were trimmed from the raw reads using CUTADAPT (Martin, 2011). The reads were quality-trimmed with a minimum length of 40 bp, truncation of reads at positions with a PHRED score below 15, and discarding reads with a higher expected error than 2 (Callahan *et al.*, 2016). Exact sequence variants (Amplicon Sequence Variants, ASVs) were determined, read pairs with a minimum overlap of 20 bp and a maximum of two mismatches were merged, and filtered for chimeras using DADA2's 'consensus' algorithm. The MOTHUR (Schloss *et al.*, 2009) implementation of the Bayesian classifier was used for taxonomic classification against the UNITE v.8.2 database, followed by BLASTN against the NCBI ITS sequence database in case of missing classification from MOTHUR, and nonfungal ASVs, as well as singletons, were discarded. Rarefaction curves showed sufficient coverage of ASV richness across samples (Fig. S1). We then assigned ASVs to putative fungal guilds based on their taxonomic annotation and the FUNGALTRAITS database (Põlme *et al.*, 2021). For analyses of fungal alpha-diversity, we rarefied the dataset to account for any possible effect of sequencing depth on ASV richness.

Statistical analysis

Data analyses were performed using R v.4.2.2 (R Core Team, 2022) and appropriate packages. We performed a principal component analysis (PCA) based on the root traits RD, SRL, RN and RDMC with varimax rotation using the package PSYCH (Revelle, 2022). The rotation was used to increase interpretability by aligning variables and components. The rotated components (RC) 1 and 2 of the varimax-rotated PCA clearly reflected the main axes of the RES (Fig. 2), namely the collaboration and conservation axes. As we aimed for a holistic view on the species and there was no distinct differentiation of species in the RES depending on plot age, we did not separate PCAs. Therefore, in subsequent analyses, the scores of the first and second axis were used to test the effects of the collaboration and conservation axis on the fungal communities.

Fungal abundance and taxonomy, as well as trait data, were kept in a PHYLOSEQ object (McMurdie & Holmes, 2013) and filtered for ASVs that are annotated with only a single lifestyle in FUNGALTRAITS to exclude ASVs that can switch between guilds or have multiple guild annotations on genus level. Exceptions to this were saprotrophic fungal ASVs that had multiple categorizations with all of them being a type of saprotrophic lifestyle and plant pathogens being secondary classified as 'root-associated'. We classified all sequences belonging to the phylum Glomeromycota as AMF. The exclusion of ASV with multiple guild annotations reduces the number of sequences that our conclusions are based on but increases the robustness of our analyses and can be regarded as a conservative approach to classification. For analyses of saprotroph diversity and relative abundance, we summed up read numbers of ASVs of the guilds litter saprotroph, soil saprotroph, wood saprotroph and unspecified saprotroph as given by the functional classification based on the FUNGALTRAITS database. While these saprotrophic guilds differ in their ecology, we analyze them together as we expect similar effects on all subgroups, as

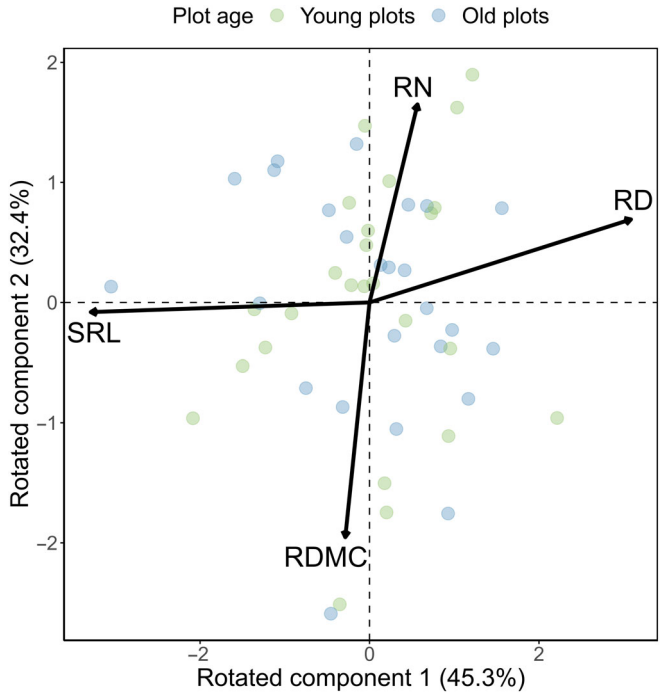


Fig. 2 Varimax-rotated principal component analysis biplot of root traits measured in monocultures of our study. The collaboration gradient loads on the first axis and explains most variation, while the conservation gradient loads mostly on the second axis. Points are color-coded for plot age. RD, root diameter; RDMC, root dry matter content; RN, root nitrogen; SRL, specific root length. RDMC was used as a proxy for root tissue density.

their main substrates are all likely to change in quality (and quantity) along, especially the conservation axis.

We calculated Shannon diversity across all guilds and separately within the three relevant fungal guilds (saprotrophs, AMF and plant pathogens). We tested the effect of the two rotated components on fungal Shannon diversity using a linear mixed-effect model with the RC1 and RC2 as the fixed effect and the experimental block as random term to account for spatial effects of the field site. Separate models were used for young and old plots because they differ in conditioning time for the plant communities and therefore possibly also in directions or strength of root trait effects on the fungal community. The mixed models were built using package LME4 (Bates *et al.*, 2015) and tested for significance using ANOVA with type III sum-of-squares as implemented in LMERTEST (Kuznetsova *et al.*, 2017). For analyses of guild abundance, we converted the absolute number of reads per guild per sample to the abundances relative to the total number of reads that could be assigned to a single guild in the sample. This relative abundance was then used as a dependent variable in a mixed model with the same structure of fixed and random terms as described above. To meet the model assumption of normal distribution of residuals, relative abundance values were log-transformed.

To analyze how well the PCA axes predict fungal community composition, we Hellinger-transformed ASV read numbers (Legendre & Gallagher, 2001), and calculated pairwise Bray–Curtis dissimilarities. To test how fungal community composition is influenced by the two PCA axes, we used partial distance-based redundancy analysis (db-RDA) on the dissimilarity matrix as implemented in the *capscale* function of the package VEGAN (Oksanen *et al.*, 2022). Effects of experimental block were controlled for and the RDA then constrained by the RC scores for the collaboration axis and the conservation axis of root traits. This was done separately for young and old plots, as community composition differed between plot ages (Fig. S2), and for the overall fungal community and individual subsets of only saprotrophic, arbuscular mycorrhizal and plant pathogenic ASVs. We tested the significance of the RDA's RC1 and RC2 using the *anova.cca* command with 9999 permutations and type III sum of squares. For visualization, we fitted only the significant root trait axes on the ordination plots using a generalized additive model as implemented in the *ordisurf* function in VEGAN (Oksanen *et al.*, 2022). Unconstrained PCoA ordination plots can be found in Fig. S3.

Because the taxonomic and functional annotation rate and therefore the subset of the fungal community that we analyze differs between samples, we ran additional analyses to test whether the correlations between root traits and the fungal communities differ when controlling for the annotation rate. For this, we included the annotation rate as a random term in the linear mixed models of fungal diversity and relative abundance and included it as a second conditioning variable in the db-RDA.

To identify which fungal genera responded most strongly to the root trait gradients, we tested for differential abundance using DESEQ2 (Love *et al.*, 2014) with the ZINB-WaVE extension (Risso *et al.*, 2018). The zero-inflated negative binomial model accounts for the high number of zeros in the abundance matrix. Abundances of ASVs belonging to a single guild (using the same criteria as described above) were aggregated at genus level and genera with a prevalence of < 20% (within one plot age) were excluded from the differential abundance analysis. For Glomeromycota that could not be identified at genus level but are considered arbuscular mycorrhizal, higher taxonomic level assignments were included in the analyses. DESEQ2 was run using Wald tests and the Benjamini–Hochberg adjustment for *P*-values. Genera with adjusted *P*-values below 0.05 were considered significant. Analyses were run separately for old and young plots.

It is important to note that our statistical analyses are focused on testing our hypotheses on the effect of the two gradients of the RES, and we deliberately chose not to include other explaining biotic or abiotic variables, such as soil texture, nutrient content or plant functional group, for which the effect on fungal communities has been tested before (e.g. Lange *et al.*, 2014; Francioli *et al.*, 2021). Furthermore, our approach is based on using root traits as the predictor of soil fungal communities, but this relationship is not necessarily unidirectional as the fungal community can also affect plant traits. As an example, root system architecture can be modified by interaction with AMF or pathogenic fungi (Hodge *et al.*, 2009).

Results

Root traits

The varimax-rotated PCA of the four root traits (RD, SRL, RN and RDMC) of 25 plant species growing in monoculture showed that the first axis explained 45% and the second axis explained 32% of the total variation in root traits. Along the first axis, RD and SRL were negatively correlated, while RN and RDMC were negatively correlated along the second axis (Fig. 2). Mycorrhizal colonization rate was significantly correlated with the first axis in young plots monocultures (Pearson's $r_{(23)} = 0.52$, $P = 0.008$), but not in old plots (Pearson's $r_{(23)} = 0.30$, $P = 0.15$; Fig. S4). The root traits of the RES, however, did not differ significantly between young and old plots (Fig. S5).

Fungal community

Our quality-filtered dataset consisted of 4741 028 fungal sequence reads, with a mean of 94 821 reads per sample (minimum 55 826; maximum 119 915 reads per sample), that were grouped into 5466 ASVs. Of all sequence reads that could be assigned to a phylum, most belonged in the Ascomycota (63.1%), followed by Basidiomycota (14.0%), Mortierellomycota (10.5%), Chytridiomycota (2.6%) and Glomeromycota (1.8%). 2283 ASVs (41.8%, corresponding to 54.2% of reads) could be assigned to at least one guild from the FungalTraits database (Põlme *et al.*, 2021). 1620 ASVs (29.6%, corresponding to 30.8% (±7.2% SD) of reads) are listed as belonging to only a single guild (according to our classification described above), which were then analyzed in more detail. Of these three guilds, saprotrophs (which include soil saprotrophs, litter saprotrophs, wood saprotrophs and unspecified saprotrophs) accounted for the majority of reads (76.4%), followed by arbuscular mycorrhiza (6.3%) and plant pathogens (2.1%).

Fungal guild richness and relative abundance of fungal guilds

Neither in old nor young monocultures, the Shannon diversity of total fungi or individual fungal guilds was significantly associated with axes of the RES (Fig. S6; Table S3), except for the Shannon diversity of fungal saprotrophs in young monocultures, which increased significantly with 'fast' root traits along the conservation gradient ($P = 0.037$). Overall, fungal Shannon diversity was significantly higher in old compared with young monocultures (t-test $P = 0.001$; Fig. S7).

We found no significant effect of the collaboration and conservation axis on the relative abundance of saprotrophic and plant pathogenic fungi (Fig. S8; Table S3). AMF relative abundance tended to decrease with 'fast' root traits in old plots ($P = 0.070$) but was unaffected in young plots (Table S3). Our additional analyses that included the taxonomic and functional annotation rate showed no major changes in the strength or direction of trait–fungal relationships (Table S4).

Fungal community composition

The overall fungal community composition in old monocultures was significantly affected by the conservation gradient of the RES ($R^2 = 0.078$, $P = 0.005$, Table 2). When analyzing communities of the separate fungal guilds, we found that the conservation axis is strongly associated with the saprotrophic fungal community composition ($R^2 = 0.087$, $P = 0.007$) and marginally significantly associated with the AMF community composition ($R^2 = 0.066$, $P = 0.100$) in old monocultures. The plant pathogenic community was, however, affected by the collaboration gradient ($R^2 = 0.113$, $P = 0.031$) in old monocultures (Fig. 3). By contrast, we could not find any significant relationship between root trait gradients and fungal community composition in young monocultures (Table 2). Furthermore, the Basidiomycota :

Table 2 Results of the partial distance-based redundancy analysis of the overall fungal community and individual subsets of fungal saprotrophs, AMF and plant pathogens constrained by the scores of the collaboration axis and the conservation axis of the varimax-rotated principal component analysis of root traits.

		Old monocultures			Young monocultures		
Predictor	df	R^2	F-value	P-value	R^2	F-value	P-value
Overall community							
Collaboration gradient	1	0.051	0.976	0.557	0.060	1.144	0.228
Conservation gradient	1	**0.078**	**1.475**	**0.005**	0.052	0.985	0.489
Saprotroph community							
Collaboration gradient	1	0.046	0.882	0.692	0.061	1.155	0.265
Conservation gradient	1	**0.087**	**1.648**	**0.007**	0.043	0.808	0.722
AMF community							
Collaboration gradient	1	0.043	0.816	0.831	0.063	1.198	0.160
Conservation gradient	1	0.066	1.249	0.100	0.048	0.903	0.675
Pathogen community							
Collaboration gradient	1	**0.113**	**2.139**	**0.031**	0.053	1.008	0.431
Conservation gradient	1	0.05	0.957	0.472	0.053	1.013	0.418

Analyses within a guild only include ASVs that are assigned to a single guild. We controlled for the effects of experimental block. The overall community analysis includes all ASVs, independent of their guild or taxonomic classification. Statistically significant values are indicated in bold text. ASV, amplicon sequence variants; df, degrees of freedom.

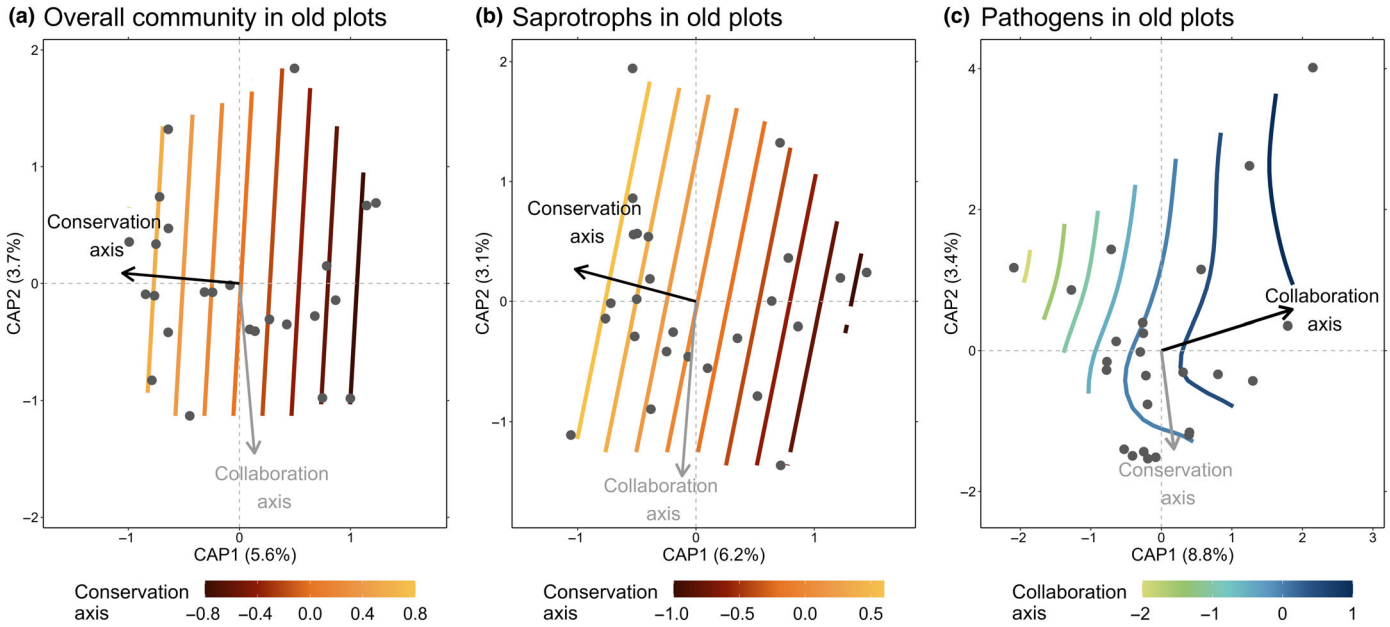


Fig. 3 Ordinations using partial distance-based redundancy analysis of the overall fungal community (a) and individual subsets of saprotrophs (b) and plant pathogens (c) constrained by the scores of the collaboration axis and the conservation axis of the varimax-rotated principal component analysis of root traits. Black arrows indicate significant, gray arrows indicate nonsignificant variables. Each point represents the fungal community (or the fungal community within a guild) of one plot. Colored lines show fitted values for the collaboration or conservation axis from generalized additive models. Note the different scales of the axes.

Ascomycota ratio was not significantly correlated with the RES axes (Fig. S9). The additional analyses that included the taxonomic and functional annotation rate as a conditioning variable showed similar results for relationships between saprotrophs and pathogens with root trait gradients. Effects on overall fungal community composition were weaker and not significant when controlling for the annotation rate (Table S5).

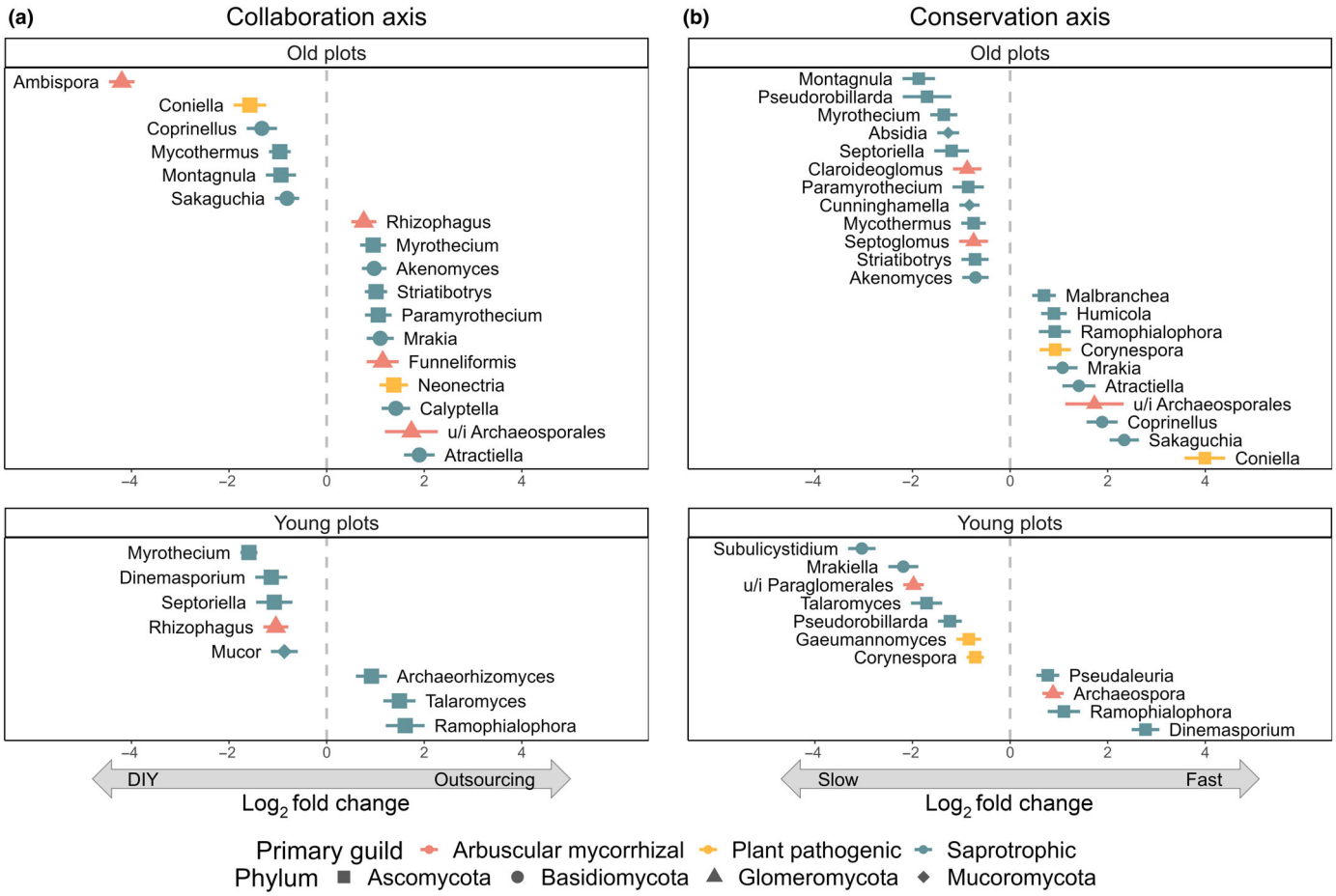
The differential abundance analyses showed that in old monocultures, 17 and 22 fungal genera differed significantly in abundance along the collaboration axis and conservation axis, respectively (Fig. 4). In young monocultures, there were only 8 and 11 genera differing along the collaboration axis and conservation axis, respectively. Overall, there was no clear trend for genera of the same fungal guild to respond in the same direction: along the 'outsourcing' strategy, for example in old monocultures, some arbuscular mycorrhizal genera decreased (*Ambispora*) or increased (*Funneliformis* and *Rhizophagus*), while the plant pathogenic genus *Coniella* decreased and *Neonectria* increased in abundance (Fig. 4a). Along the 'fast' strategy in old plots, the pathogenic genera *Coniella* and *Corynespora* increased significantly (Fig. 4b). Saprotrophic fungal genera were significantly affected in both plot ages and along both trait axes but the highest proportion of significantly abundant genera was found along the conservation axis in old monocultures (Fig. 4b,c).

Discussion

We proposed and tested a conceptual framework that links the two orthogonal RES axes to soil fungal communities. We showed that the root trait variation in 25 grassland plant species growing for several years in monoculture was consistent with the RES (Bergmann *et al.*, 2020). The RES did not strongly affect fungal diversity and relative guild abundance but had a significant effect on fungal community composition.

Trait relationships with fungal saprotrophs

While we hypothesized that the relative abundance and diversity of fungal saprotrophs increases in the rhizosphere of plants with trait expression toward the 'fast' end of the conservation gradient, we could not confirm this hypothesis, as the expected relationship was only found for the Shannon diversity in young plots. We did find that saprotrophic fungal community composition is affected by the conservation axis. Two of the main pathways in which plants affect saprotrophic fungal communities are the production of litter and rhizodeposition, providing quality and quantity of the carbon input into the soil. In grasslands, primary production and thus litter quantity (Mokany *et al.*, 2008; Lavorel & Grigulis, 2012), and rhizodeposition (Henneron *et al.*, 2020a) is expected to be higher in fast-growing species compared with slow-growing species (Wardle, 2004). In addition, litter quality also increases from slow to fast, because the short-lived and 'cheaper' plant structures lead to easily decomposable litter with lower C : N ratio and lower tissue density (Wardle, 2002, 2004; Bardgett, 2017). The weak association of the conservation gradient with saprotroph abundance and diversity might be related to the diverging effects of litter amount and quality: while 'fast' species produce more litter, 'slow' species produce more complex litter, which takes longer to break down (Wardle, 2004). Therefore, divergent plant strategies might lead to equal effects on saprotroph abundance and diversity and thus ultimately no significant effect along the conservation trait gradient.



(c)

Old plots				Young plots		
Taxa	Differentially abundant taxa		Guild	Taxa	Differentially abundant taxa	
	Collaboration axis	Conservation axis			Collaboration axis	Conservation axis
63	17.5%	27.0%	Saprotrophs	62	11.3%	11.3%
15	26.7%	20.0%	AMF	12	8.3%	16.7%
6	33.3%	33.3%	Pathogens	6	0%	33.3%

Fig. 4 Fungal genera that significantly changed in abundance along the root trait gradients. Log_2 fold change (±SE) was calculated using DESEQ2 based on a zero-inflated negative binomial distribution. A positive log_2 fold change indicates an increased abundance of the genus in the rhizosphere of plants with an 'outsourcing' strategy along the collaboration axis (a) or plants with a 'fast' strategy along the conservation axis of root traits (b). Proportion of differentially abundant taxa of the total number of taxa per guild varies between plot ages and the root trait axes (c). Glomeromycota that were not identified at the genus level are included with the note 'u/i' (unidentified) at the order level. All significantly differently abundant genera with a minimum prevalence of 20% were included in the analyses.

The community composition showed a significant change in the saprotrophic fungal community along the conservation gradient. This suggests that saprotrophic ASVs respond differently to more conservative or more acquisitive root traits, resulting in species turnover of saprotrophs along the conservation gradient. We expected to see this also in the ratio of more opportunistic (Ascomycota) to more specialized lignin decomposers (Basidiomycota) but found that this was not a driver of changes in saprotrophic fungal community composition along the conservation gradient. On genus level, however, we found varying responses to the trait axes, with a higher proportion of genera affected by the conservation axis than the collaboration axis. Interestingly, genera within the phylum Basidiomycota were overall less affected than Ascomycota in young plots compared with old plots. This could be related to higher lignification rates with age of some roots, which could lead to a larger range of litter quality in old compared with young plots.

Trait relationships with arbuscular mycorrhizal fungi

We expected a strong correlation of the collaboration gradient with AMF diversity, relative abundance and community composition, with higher richness and abundance at the 'outsourcing' trait expressions. While we, as well as previous studies

(Brundrett, 2002; Ma *et al.*, 2018), could show that the mycorrhizal colonization rate is correlated with root traits representing the collaboration gradient, we could not show this for AMF relative abundance and diversity from our sequence analyses in the rhizosphere samples. This could potentially be due to the fact that mycorrhizal colonization (and DNA) in roots does not linearly scale to (hyphal) DNA content in the soil (Hart & Reader, 2002; Olsson *et al.*, 2010). Additionally, the weak results for AMF may also arise from a lack of accurate diversity detection due to our of primer selection (Lekberg *et al.*, 2018). In contrast to our results, Sweeney *et al.* (2021) found a strong positive correlation between relative AMF abundance in the rhizosphere and root diameter in temperate grassland plant species growing under controlled conditions for 12 wk. Spitzer *et al.* (2021), however, could also not show any relationships between root diameter and relative AMF abundance. Instead, they found that root carbon content (corresponding to a 'slow' plant strategy) strongly increased AMF abundance in the soil of tundra plants in a glasshouse experiment over 12 wk. These differential findings between root traits and AMF abundance in the rhizosphere might be related to the different time scales and ecosystems of the studies and warrant further research. Overall, our results suggest that the effects of the collaboration gradient on the rhizosphere AMF community are not straightforward and that the root mycorrhizal colonization is not a strong predictor of the AMF community in the rhizosphere.

Trait relationships with fungal pathogens

We hypothesized that plant pathogenic fungi respond to the growth–defense trade-off along the conservation axis but more strongly to antagonistic effects of AMF and should therefore also link to the collaboration axis. Specifically, we expected lower relative abundance and diversity of pathogens in the rhizosphere of plants at the 'outsourcing' end of the gradient. However, we could not find this change in relative abundance and diversity of pathogens in our sequences. Instead, we found the community composition of pathogens to be affected by the collaboration axis. As we could not find any significant changes in AMF communities in the rhizosphere, the change in the pathogenic community composition is unlikely to be caused by effects of AMF in the soil but could potentially still be related to effects of mycorrhizal colonization or AMF communities in the root. This speculation should be tested in follow-up work. The same was found in the differential abundance analyses, showing that especially in old plots certain pathogenic taxa are affected by the collaboration gradient, but also conservation strategies, and are replaced by other pathogens. Interestingly, some saprotrophic and pathogenic genera show differential abundance along both trait axes, for example the genus *Coniella* that increases in abundance with 'DIY' and 'fast' root traits. Variation in the abundance of fungal genera in the rhizosphere of plants with functional strategies at the extreme ends of both trait gradients, that is sample points in the far end corners of the PCA (Fig. 2), likely underlies the significant relationships of fungal abundance with both root trait axes. For pathogenic fungi, these results are in line with our expectation that 'DIY' and 'fast' plant strategies show the same direction of effects. For saprotrophic fungi, the number of genera that are affected not only by the conservation axis but also the collaboration axis is surprising and warrants further research. The importance of the fungal guild community composition for the individual plant cannot be assessed with our data. Linking the correlation of root traits and the pathogenic community with plant functioning in future studies would help in exploring their ecological significance in natural communities.

As discussed in the previous section, the rhizosphere AMF community did not follow the collaboration axis as hypothesized. In spite of this, interactions between AMF and plant pathogens in the roots are still plausible, as we found that mycorrhizal colonization rate increased in roots of 'outsourcing' species. Proposed mechanisms of AMF protection against pathogens include improved nutrient acquisition of the plant leading to higher resistance, competition between AMF and pathogens for photoassimilates of the plant, competition for space and colonization sites, alterations in anatomical and morphological root traits, and stimulation of soil biota that are antagonistic to pathogens (Smith, 1988; Azcón-Aguilar & Barea, 1996; Borowicz, 2001; Sikes, 2010; Frew *et al.*, 2022). The fact that we did not find changes in diversity and relative abundance of pathogens suggests that interactions between AMF in the root and pathogens in the rhizosphere are either not very strong or the effect is not as universal as expected. Another explanation might be that protection against pathogens is potentially more dependent on the mycorrhizal colonization within the root rather than the AMF community in the rhizosphere, and collaboration with AMF could therefore still reduce pathogen abundance in the root. However, this hypothesis needs to be studied in the future. Furthermore, while the richness and abundance of plant pathogens in our samples is similar to the results of other studies (Sweeney *et al.*, 2021), they are low compared with the other guilds and effects therefore not as likely to be detected.

Given that we could not find trait effects on relative fungal guild abundance, it is not surprising that richness within fungal guilds was also not strongly affected. Since previous research has shown that plant communities in general can be an important driver of fungal diversity (e.g. Dassen *et al.*, 2017), we expect that root trait–fungal richness patterns on community level should be stronger in mixed plant communities compared with monocultures. We could only show root trait–fungi patterns in the 18-yr-old monocultures, but not in the 4-yr-old monocultures. Previous studies have shown a delayed response of soil microorganisms to changes in plant community composition (Bartelt-Ryser *et al.*, 2005; Habekost *et al.*, 2008; Eisenhauer *et al.*, 2010, 2012). We here show the same for trait–fungal relationships (that potentially also underlie some of the abovementioned relationships). Other studies – working with much younger plants, that is 3 months (Spitzer *et al.*, 2021; Sweeney *et al.*, 2021) did find effects of root traits on relative fungal guild abundance and diversity. However, these studies investigated plants in pots in a glasshouse with stable abiotic conditions. In the field site of our experiment, the high variability in abiotic conditions between experimental plots is known to also affect fungal communities (de Vries *et al.*, 2012; Lange *et al.*, 2014; Dassen

et al., 2017), which might outweigh any effects of root traits in our younger plots.

Methodological considerations

In our exploratory investigations in monocultures, we used fungal guilds as classified by the FungalTraits database (Põlme *et al.*, 2021). While the categorization of fungi in functional guilds at genus level allows for a broad assessment of fungal community composition (Zanne *et al.*, 2020), it also has some weaknesses. It is known that some fungal taxa can switch their resource use and thus change the guild they belong to, or species of the same genus can have very different functional roles (Selosse *et al.*, 2018). As an example, the differentially abundant genus *Septoriella* is listed as a saprotroph in FungalTraits and is therefore considered as such in our analyses, but the genus nonetheless contains pathogenic species (e.g. *Septoriella hirta*; Sprague, 1950). To address such issues, further improvements in the quality of functional databases are critical (Selosse *et al.*, 2018). Our approach to quantify relative guild abundance from sequence reads requires all guilds to be sequenced together using the same primer set. One of the drawbacks of the use of relative abundances is the compositional nature of the data and the lack of absolute measurements. While we could not show the hypothesized shifts in the relative abundances of guilds along the RES axes, quantitative measurements of absolute fungal (guild) abundance should be considered in future studies on the role of root traits and the RES on fungal communities.

Preliminary evaluation of framework

Our proposed framework aims to apply the RES to better understand how plants and their traits shape soil fungal communities and ultimately affect soil biodiversity and ecosystem functioning. Using a case study of long-term monocultures, we could show that in 18-yr-old plots, the community composition of the overall fungal community, of the saprotrophic fungal community and in parts also of the arbuscular mycorrhizal community respond to the conservation ('fast – slow') gradient, while the pathogenic fungal community composition in the rhizosphere was affected by the collaboration gradient. These effects were not found in young 4-yr-old monocultures of the same plant species. However, our study provides little support for effects of the collaboration and conservation gradient on the relative abundance and diversity of the soil fungal guilds so far. We are convinced that further applications of our framework – including absolute abundances of fungal guilds and spanning larger environmental gradients – will help to improve our understanding of plant–fungal interactions in the soil and ultimately shed more light on the effects of plant traits on soil functioning.

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Competing interests

None declared.

Author contributions

JH, JB, NE, CAG, AH-B, TWK, ML, LM and AW designed the study. LB carried out the soil and root sampling. JH and LB measured root traits. CA measured mycorrhizal colonization rate. MDS performed DNA extraction. JH and CA prepared and conducted the sequencing. JH and AH-B processed the sequence data. JH performed the data analysis and wrote the manuscript. All authors contributed to the interpretation of the data and commented on the manuscript.

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Data availability

Root trait and fungal data produced in this study are available online in the Jena Experiment database (doi: 10.25829/XJJM-3D69; doi: 10.25829/2NG9-H416; doi: 10.25829/208Z-PN38; doi: 10.25829/7ASR-PZ67). The Illumina sequence data generated in this study are available in the NCBI Sequence Read Archive (BioProject: PRJNA918982).

References

Agrios GN. 2005. *Plant pathology*. Amsterdam, the Netherlands: Elsevier.

Ainsworth GC. 1976. *Introduction to the history of mycology*. Cambridge, UK: Cambridge University Press.

Azcón-Aguilar C, Barea JM. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. *Mycorrhiza* **6**: 457–464.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* **57**: 233–266.

Bani A, Pioli S, Ventura M, Panzacchi P, Borruso L, Tognetti R, Tonon G, Brusetti L. 2018. The role of microbial community in the decomposition of leaf litter and deadwood. *Applied Soil Ecology* **126**: 75–84.

Bardgett RD. 2017. Plant trait-based approaches for interrogating belowground function. *Biology and Environment: Proceedings of the Royal Irish Academy* **117B**: 1–13.

Bartelt-Ryser J, Joshi J, Schmid B, Brandl H, Balser T. 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspectives in Plant Ecology, Evolution and Systematics* **7**: 27–49.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using LME4. *Journal of Statistical Software* **67**: 1–48.

Bergmann J, Weigelt A, van der Plas F, Laughlin DC, Kuyper TW, Guerrero-Ramirez N, Valverde-Barrantes OJ, Bruelheide H, Freschet GT, Iversen CM *et al.* 2020. The fungal collaboration gradient dominates the root economics space in plants. *Science Advances* **6**: eaba3756.

Berruti A, Desirò A, Visentin S, Zecca O, Bonfante P. 2017. ITS fungal barcoding primers versus 18S AMF-specific primers reveal similar AMF-based diversity patterns in roots and soils of three mountain vineyards. *Environmental Microbiology Reports* **9**: 658–667.

Birouste M, Zamora-Ledezma E, Bossard C, Pérez-Ramos IM, Roumet C. 2014. Measurement of fine root tissue density: a comparison of three methods reveals the potential of root dry matter content. *Plant and Soil* **374**: 299–313.

Borowicz VA. 2001. Do arbuscular mycorrhizal fungi alter plant–pathogen relations? *Ecology* **82**: 3057–3068.

Brundrett MC. 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* **154**: 275–304.

Buzzard V, Michaletz ST, Deng Y, He Z, Ning D, Shen L, Tu Q, Van Nostrand JD, Voordeckers JW, Wang J *et al.* 2019. Continental scale structuring of forest and soil diversity via functional traits. *Nature Ecology & Evolution* **3**: 1298–1308.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Cameron DD, Neal AL, van Wees SCM, Ton J. 2013. Mycorrhiza-induced resistance: more than the sum of its parts? *Trends in Plant Science* **18**: 539–545.

Cantarel AAM, Pommier T, Desclos-Theveniau M, Diquélou S, Dumont M, Grassein F, Kastl E-M, Grigulis K, Laîné P, Lavorel S *et al.* 2015. Using plant traits to explain plant–microbe relationships involved in nitrogen acquisition. *Ecology* **96**: 788–799.

Coley PD, Bryant JP, Chapin FS. 1985. Resource availability and plant antiherbivore defense. *Science* **230**: 895–899.

Cordier C, Gianinazzi S, Gianinazzi-Pearson V. 1996. Colonisation patterns of root tissues by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato. *Plant and Soil* **185**: 223–232.

Courchesne F, Gobran GR. 1997. Mineralogical variations of bulk and rhizosphere soils from a Norway spruce stand. *Soil Science Society of America Journal* **61**: 1245–1249.

Dassen S, Cortois R, Martens H, de Hollander M, Kowalchuk GA, van der Putten WH, De Deyn GB. 2017. Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. *Molecular Ecology* **26**: 4085–4098.

Dehne HW. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* **72**: 1115–1119.

Dighton J. 2007. Nutrient cycling by saprotrophic fungi in terrestrial habitats. In: Kubicek CP, Druzhinina IS, eds. *Environmental and microbial relationships*. Berlin, Heidelberg, Germany: Springer, 287–300.

Ding Z, Tang M, Chen X, Yin L, Gui H, Zhu B. 2019. Measuring rhizosphere effects of two tree species in a temperate forest: a comprehensive method comparison. *Rhizosphere* **10**: 100153.

Dix NJ, Webster J. 1995. *Fungal ecology*. Dordrecht, the Netherlands: Springer.

Doehlemann G, Ökmen B, Zhu W, Sharon A. 2017. Plant pathogenic fungi. *Microbiology Spectrum* **5**: 1–23.

Duchicela J, Sullivan TS, Bontti E, Bever JD. 2013. Soil aggregate stability increase is strongly related to fungal community succession along an abandoned agricultural field chronosequence in the Bolivian Altiplano. *Journal of Applied Ecology* **50**: 1266–1273.

Eisenhauer N, Beßler H, Engels C, Gleixner G, Habekost M, Milcu A, Partsch S, Sabais ACW, Scherber C, Steinbeiss S *et al.* 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* **91**: 485–496.

Eisenhauer N, Reich PB, Scheu S. 2012. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic and Applied Ecology* **13**: 571–578.

Eissenstat DM, Kucharski JM, Zadworny M, Adams TS, Koide RT. 2015. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. *New Phytologist* **208**: 114–124.

Francioli D, van Rijssel SQ, van Ruijven J, Termorshuizen AJ, Cotton TEA, Dumbrell AJ, Raaijmakers JM, Weigelt A, Mommer L. 2021. Plant functional group drives the community structure of saprophytic fungi in a grassland biodiversity experiment. *Plant and Soil* **461**: 91–105.

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.

Freschet GT, Aerts R, Cornelissen JHC. 2012. A plant economics spectrum of litter decomposability. *Functional Ecology* **26**: 56–65.

Freschet GT, Roumet C, Comas LH, Weemstra M, Bengough AG, Rewald B, Bardgett RD, De Deyn GB, Johnson D, Klimešová J *et al.* 2021. Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytologist* **232**: 1123–1158.

Frew A, Antunes PM, Cameron DD, Hartley SE, Johnson SN, Rillig MC, Bennett AE. 2022. Plant herbivore protection by arbuscular mycorrhizas: a role for fungal diversity? *New Phytologist* **233**: 1022–1031.

Guerrero-Ramírez NR, Craven D, Messier C, Potvin C, Turner BL, Handa IT. 2016. Root quality and decomposition environment, but not tree species richness, drive root decomposition in tropical forests. *Plant and Soil* **404**: 125–139.

Guyonnet JP, Cantarel AAM, Simon L, Haichar FZ. 2018. Root exudation rate as functional trait involved in plant nutrient-use strategy classification. *Ecology and Evolution* **8**: 8573–8581.

Habekost M, Eisenhauer N, Scheu S, Steinbeiss S, Weigelt A, Gleixner G. 2008. Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. *Soil Biology and Biochemistry* **40**: 2588–2595.

Han M, Chen Y, Li R, Yu M, Fu L, Li S, Su J, Zhu B. 2022. Root phosphatase activity aligns with the collaboration gradient of the root economics space. *New Phytologist* **234**: 837–849.

Han M, Zhu B. 2021. Linking root respiration to chemistry and morphology across species. *Global Change Biology* **27**: 190–201.

Hart MM, Reader RJ. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist* **153**: 335–344.

van der Heijden MGA, Dombrowski N, Schlaeppi K. 2017. Continuum of root–fungal symbioses for plant nutrition. *Proceedings of the National Academy of Sciences, USA* **114**: 11574–11576.

Henneron L, Cros C, Picon-Cochard C, Rahimian V, Fontaine S. 2020a. Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition. *Journal of Ecology* **108**: 528–545.

Henneron L, Kardol P, Wardle DA, Cros C, Fontaine S. 2020b. Rhizosphere control of soil nitrogen cycling: a key component of plant economic strategies. *New Phytologist* **228**: 1269–1282.

Herms DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. *The Quarterly Review of Biology* **67**: 283–335.

Hobbie SE, Oleksyn J, Eissenstat DM, Reich PB. 2010. Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. *Oecologia* **162**: 505–513.

Hodge A, Berta G, Doussan C, Merchan F, Crespi M. 2009. Plant root growth, architecture and function. *Plant and Soil* **321**: 153–187.

Hooker JE, Jaizme-Vega M, Atkinson D. 1994. Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In: Gianinazzi S, Schüepp H, eds. *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems.* Basel, Switzerland: Birkhäuser, 191–200.

Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger T, van der Heijden MGA *et al.* 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* **9**: 2738.

Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid J, Brandström-Durling M, Clemmensen KE *et al.* 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* **82**: 666–677.

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* **108**: 389–411.

Jiang L, Wang H, Li S, Fu X, Dai X, Yan H, Kou L. 2021. Mycorrhizal and environmental controls over root trait–decomposition linkage of woody trees. *New Phytologist* **229**: 284–295.

Jo I, Fridley JD, Frank DA. 2016. More of the same? *In situ* leaf and root decomposition rates do not vary between 80 native and nonnative deciduous forest species. *New Phytologist* **209**: 115–122.

Jones DL, Nguyen C, Finlay RD. 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soil* **321**: 5–33.

Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ. 2012. Mycorrhiza-induced resistance and priming of plant defenses. *Journal of Chemical Ecology* **38**: 651–664.

Kaštovská E, Edwards K, Picek T, Šantrůčková H. 2015. A larger investment into exudation by competitive versus conservative plants is connected to more coupled plant–microbe N cycling. *Biogeochemistry* **122**: 47–59.

Kohout P, Sudová R, Janoušková M, Čtvrtlíková M, Hejda M, Pánková H, Slavíková R, Štajerová K, Vosátka M, Sýkorová Z. 2014. Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: is there a universal solution? *Soil Biology and Biochemistry* **68**: 482–493.

Kong D, Ma C, Zhang Q, Li L, Chen X, Zeng H, Guo D. 2014. Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytologist* **203**: 863–872.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. LMERTEST package: tests in linear mixed effects models. *Journal of Statistical Software* **82**: 1–26.

Laliberté E. 2017. Below-ground frontiers in trait-based plant ecology. *New Phytologist* **213**: 1597–1603.

Laliberté E, Lambers H, Burgess TI, Wright SJ. 2015. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytologist* **206**: 507–521.

Lange M, Habekost M, Eisenhauer N, Roscher C, Bessler H, Engels C, Oelmann Y, Scheu S, Wilcke W, Schulze E-D *et al.* 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. *PLoS ONE* **9**: e96182.

Lavorel S, Grigulis K. 2012. How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. *Journal of Ecology* **100**: 128–140.

Leff JW, Bardgett RD, Wilkinson A, Jackson BG, Pritchard WJ, De Long JR, Oakley S, Mason KE, Ostle NJ, Johnson D *et al.* 2018. Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *The ISME Journal* **12**: 1794–1805.

Legendre P, Gallagher ED. 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* **129**: 271–280.

Lekberg Y, Vasar M, Bullington LS, Sepp S-K, Antunes PM, Bunn R, Larkin BG, Öpik M. 2018. More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers? *New Phytologist* **220**: 971–976.

Lemmermeyer S, Lörcher L, van Kleunen M, Dawson W. 2015. Testing the plant growth-defense hypothesis belowground: do faster-growing herbaceous plant species suffer more negative effects from soil biota than slower-growing ones? *The American Naturalist* **186**: 264–271.

Linderman RG. 1994. Role of VAM fungi in biocontrol. In: Pfleger FL, Linderman RG, eds. *Mycorrhizae and plant health.* St Paul, MN, USA: APS, 1–26.

Liu X, Lin TC, Vadeboncoeur MA, Yang Z, Chen S, Xiong D, Xu C, Li Y, Yang Y. 2019. Root litter inputs exert greater influence over soil C than does aboveground litter in a subtropical natural forest. *Plant and Soil* **444**: 489–499.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESEQ2. *Genome Biology* **15**: 550.

Lozano YM, Aguilar-Trigueros CA, Roy J, Rillig MC. 2021. Drought induces shifts in soil fungal communities that can be linked to root traits across 24 plant species. *New Phytologist* **232**: 1917–1929.

Ma Z, Guo D, Xu X, Lu M, Bardgett RD, Eissenstat DM, McCormack ML, Hedin LO. 2018. Evolutionary history resolves global organization of root functional traits. *Nature* **555**: 94–97.

Martin M. 2011. CUTADAPT removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17**: 10–12.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari H, Hobbie EA, Iversen CM, Jackson RB *et al.* 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* **207**: 505–518.

McMurdie PJ, Holmes S. 2013. PHYLOSEQ: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**: e61217.

Meier IC, Tückmantel T, Heitkötter J, Müller K, Preusser S, Wrobel TJ, Kandeler E, Marschner B, Leuschner C. 2020. Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity. *New Phytologist* **226**: 583–594.

Miedes E, Vanholme R, Boerjan W, Molina A. 2014. The role of the secondary cell wall in plant resistance to pathogens. *Frontiers in Plant Science* **5**: 358.

Mokany K, Ash J, Roxburgh S. 2008. Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. *Journal of Ecology* **96**: 884–893.

Mokany K, Raison RJ, Prokushkin AS. 2006. Critical analysis of root:shoot ratios in terrestrial biomes. *Global Change Biology* **12**: 84–96.

Mommer L, Cotton TEA, Raaijmakers JM, Termorshuizen AJ, van Ruijven J, Hendriks M, van Rijssel SQ, van de Mortel JE, van der Paauw JW, Schijlen EGWM *et al.* 2018. Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. *New Phytologist* **218**: 542–553.

Mommer L, Padilla FM, van Ruijven J, de Caluwe H, Smit-Tiekstra A, Berendse F, de Kroon H. 2015. Diversity effects on root length production and loss in an experimental grassland community. *Functional Ecology* **29**: 1560–1568.

Muneer MA, Wang P, Zhang J, Li Y, Munir MZ, Ji B. 2020. Formation of common mycorrhizal networks significantly affects plant biomass and soil properties of the neighboring plants under various nitrogen levels. *Microorganisms* **8**: 230.

Newsham KK, Fitter AH, Watkinson AR. 1995a. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology & Evolution* **10**: 407–411.

Newsham KK, Fitter AH, Watkinson AR. 1995b. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology* **83**: 991–1000.

Nguyen C. 2003. Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie* **23**: 375–396.

Oburger E, Jones DL. 2018. Sampling root exudates – mission impossible? *Rhizosphere* **6**: 116–133.

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E *et al.* 2022. *VEGAN: community ecology package.* [WWW document] URL https://CRAN.R-project.org/package=vegan [accessed 5 May 2023].

Olsson PA, Rahm J, Aliasgharzad N. 2010. Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiology Ecology* **72**: 125–131.

Orwin KH, Buckland SM, Johnson D, Turner BL, Smart S, Oakley S, Bardgett RD. 2010. Linkages of plant traits to soil properties and the functioning of temperate grassland. *Journal of Ecology* **98**: 1074–1083.

Paterson E. 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. *European Journal of Soil Science* **54**: 741–750.

Põlme S, Abarenkov K, Henrik Nilsson R, Lindahl BD, Clemmensen KE, Kauserud H, Nguyen N, Kjøller R, Bates ST, Baldrian P *et al.* 2021. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity* **105**: 1–16.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**: 30–50.

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H. 2009. Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *Proceedings of the Royal Society B: Biological Sciences* **276**: 4237–4245.

Pozo MJ, Azcón-Aguilar C. 2007. Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* **10**: 393–398.

Prieto I, Stokes A, Roumet C. 2016. Root functional parameters predict fine root decomposability at the community level. *Journal of Ecology* **104**: 725–733.

R Core Team. 2022. *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL https://www.R-project.org/ [accessed 5 May 2023].

Rayner MC. 1926. Mycorrhiza. *New Phytologist* **25**: 248–263.

Reich PB. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology* **102**: 275–301.

Revelle W. 2022. *psych: procedures for psychological, psychometric, and personality research.* [WWW document] URL https://CRAN.R-project.org/package=psych [accessed 5 May 2023].

Risso D, Perraudeau F, Gribkova S, Dudoit S, Vert J-P. 2018. A general and flexible method for signal extraction from single-cell RNA-seq data. *Nature Communications* **9**: 284.

Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze E-D. 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* **5**: 107–121.

Rutten G, Allan E. 2023. Using root economics traits to predict biotic plant soil-feedbacks. *Plant and Soil* **485**: 71–89.

Ryan MH, Tibbett M, Edmonds-Tibbett T, Suriyagoda LDB, Lambers H, Cawthray GR, Pang J. 2012. Carbon trading for phosphorus gain: the balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition. *Plant, Cell & Environment* **35**: 2170–2180.

Ryser P. 1996. The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. *Functional Ecology* **10**: 717–723.

Sattler S, Funnell-Harris D. 2013. Modifying lignin to improve bioenergy feedstocks: strengthening the barrier against pathogens? *Frontiers in Plant Science* **4**: 70.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ *et al.* 2009. Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Selosse M-A, Schneider-Maunoury L, Martos F. 2018. Time to re-think fungal ecology? Fungal ecological niches are often prejudged. *New Phytologist* **217**: 968–972.

Semchenko M, Barry KE, de Vries FT, Mommer L, Moora M, Maciá-Vicente JG. 2022. Deciphering the role of specialist and generalist plant–microbial interactions as drivers of plant-soil feedback. *New Phytologist* **234**: 1929–1944.

Semchenko M, Leff JW, Lozano YM, Saar S, Davison J, Wilkinson A, Jackson BG, Pritchard WJ, De Long JR, Oakley S *et al.* 2018. Fungal diversity regulates plant-soil feedbacks in temperate grassland. *Science Advances* **4**: eaau4578.

Sikes BA. 2010. When do arbuscular mycorrhizal fungi protect plant roots from pathogens? *Plant Signaling & Behavior* **5**: 763–765.

Silver WL, Miya RK. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. *Oecologia* **129**: 407–419.

Simms EL, Rausher MD. 1987. Costs and benefits of plant resistance to herbivory. *The American Naturalist* **130**: 570–581.

Smiley RW. 1974. Rhizosphere pH as influenced by plants, soils, and nitrogen fertilizers. *Soil Science Society of America Journal* **38**: 795–799.

Smith GS. 1988. The role of phosphorous nutrition in interaction of vesicular-arbuscular mycorrhizal fungi with soilborne nematodes and fungi. *Phytopathology* **78**: 371–374.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis.* Amsterdam, the Netherlands; Boston, MA, USA: Academic Press.

Smith SW, Woodin SJ, Pakeman RJ, Johnson D, Wal R. 2014. Root traits predict decomposition across a landscape-scale grazing experiment. *New Phytologist* **203**: 851–862.

Spitzer CM, Lindahl B, Wardle DA, Sundqvist MK, Gundale MJ, Fanin N, Kardol P. 2021. Root trait–microbial relationships across tundra plant species. *New Phytologist* **229**: 1508–1520.

Sprague R. 1950. *Diseases of cereals and grasses in North America.* New York, NY, USA: The Ronald Press Company.

Su J, Xu F. 2021. Root, not aboveground litter, controls soil carbon storage under grazing exclusion across grasslands worldwide. *Land Degradation & Development* **32**: 3326–3337.

Sun L, Ataka M, Han M, Han Y, Gan D, Xu T, Guo Y, Zhu B. 2021. Root exudation as a major competitive fine-root functional trait of 18 coexisting species in a subtropical forest. *New Phytologist* **229**: 259–271.

Sweeney CJ, de Vries FT, Dongen BE, Bardgett RD. 2021. Root traits explain rhizosphere fungal community composition among temperate grassland plant species. *New Phytologist* **229**: 1492–1507.

Tedersoo L, Anslan S, Bahram M, Põlme S, Riit T, Liiv I, Kõljalg U, Kisand V, Nilsson RH, Hildebrand F *et al.* 2015. Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MycoKeys* **10**: 1–43.

Traquair JA. 1995. Fungal biocontrol of root diseases: endomycorrhizal suppression of cylindrocarpon root rot. *Canadian Journal of Botany* **73**: 89–95.

Trouvelot A, Kough JL, Gianinazzi-Pearson V. 1986. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthode d'estimation ayant une signification fonctionnelle. In: *Physiological and genetical aspects of mycorrhizae: proceedings of the 1st European symposium on mycorrhizae, Dijon, 1–5 July 1985.* Paris, France: INRA Press, 217–222.

Veen GF, Snoek BL, Bakx-Schotman T, Wardle DA, Putten WH. 2019. Relationships between fungal community composition in decomposing leaf litter and home-field advantage effects. *Functional Ecology* **33**: 1524–1535.

Vierheilig H, Coughlan AP, Wyss U, Piché Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* **64**: 5004–5007.

Vivanco L, Austin AT. 2006. Intrinsic effects of species on leaf litter and root decomposition: a comparison of temperate grasses from North and South America. *Oecologia* **150**: 97–107.

Vogel A, Ebeling A, Gleixner G, Roscher C, Scheu S, Ciobanu M, Koller-France E, Lange M, Lochner A, Meyer ST *et al.* 2019. A new experimental approach to test why biodiversity effects strengthen as ecosystems age. In: Eisenhauer N, Bohan DA, Dubrell AJ, eds. *Advances in ecological research.* Amsterdam, the Netherlands: Elsevier, 221–264.

de Vries FT, Caruso T. 2016. Eating from the same plate? Revisiting the role of labile carbon inputs in the soil food web. *Soil Biology and Biochemistry* **102**: 4–9.

de Vries FT, Manning P, Tallowin JRB, Mortimer SR, Pilgrim ES, Harrison KA, Hobbs PJ, Quirk H, Shipley B, Cornelissen JHC *et al.* 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters* **15**: 1230–1239.

Wardle DA. 2002. *Communities and ecosystems: linking the aboveground and belowground components.* Princeton, NJ, USA: Princeton University Press.

Wardle DA. 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629–1633.

Weemstra M, Mommer L, Visser EJW, van Ruijven J, Kuyper TW, Mohren GMJ, Sterck FJ. 2016. Towards a multidimensional root trait framework: a tree root review. *New Phytologist* **211**: 1159–1169.

Weigelt A, Mommer L, Andraczek K, Iversen CM, Bergmann J, Bruelheide H, Fan Y, Freschet GT, Guerrero-Ramírez NR, Kattge J *et al.* 2021. An

integrated framework of plant form and function: the belowground perspective. *New Phytologist* **232**: 42–59.

Weißbecker C, Schnabel B, Heintz-Buschart A. 2020. Dadasnake, a Snakemake implementation of DADA2 to process amplicon sequencing data for microbial ecology. *GigaScience* **9**: 1–8.

Weisser WW, Roscher C, Meyer ST, Ebeling A, Luo G, Allan E, Beßler H, Barnard RL, Buchmann N, Buscot F *et al.* 2017. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: patterns, mechanisms, and open questions. *Basic and Applied Ecology* **23**: 1–73.

Wen Z, Li H, Shen Q, Tang X, Xiong C, Li H, Pang J, Ryan MH, Lambers H, Shen J. 2019. Tradeoffs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. *New Phytologist* **223**: 882–895.

Wen Z, White PJ, Shen J, Lambers H. 2022. Linking root exudation to belowground economic traits for resource acquisition. *New Phytologist* **233**: 1620–1635.

Whipps JM. 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany* **82**: 1198–1227.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols*. San Diego, CA, USA: Elsevier, 315–322.

Williams A, Langridge H, Straathof AL, Muhamadali H, Hollywood KA, Goodacre R, de Vries FT. 2022. Root functional traits explain root exudation rate and composition across a range of grassland species. *Journal of Ecology* **110**: 21–33.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M *et al.* 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

Zanne AE, Abarenkov K, Afkhami ME, Aguilar-Trigueros CA, Bates S, Bhatnagar JM, Busby PE, Christian N, Cornwell WK, Crowther TW *et al.* 2020. Fungal functional ecology: bringing a trait-based approach to plant-associated fungi. *Biological Reviews* **95**: 409–433.

Zhang D, Hui D, Luo Y, Zhou G. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology* **1**: 85–93.

Supporting Information

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

Fig. S1 Rarefaction curves.

Fig. S2 Principal coordinate analysis ordination plot of fungal communities in old and young monocultures based on Bray–Curtis dissimilarities.

Fig. S3 Principal coordinate analysis ordination plots of fungal communities of each guild in old and young monocultures, color-coded with the plant species root trait strategy in the root economics space.

Fig. S4 Correlation between mycorrhizal colonization rate and the two axes of the root economics space.

Fig. S5 Differences in root traits between old and young monocultures.

Fig. S6 Relationship between the collaboration axis and the conservation axis of the root economics space and the Shannon diversity of the total fungal community, saprotrophs, arbuscular mycorrhiza and plant pathogens.

Fig. S7 Inverse Simpson, observed number of ASVs and Shannon diversity of the fungal community in old and young monocultures.

Fig. S8 Relationship between the collaboration axis and the conservation axis of the root economics space and the relative abundance of saprotrophs, arbuscular mycorrhiza and plant pathogens.

Fig. S9 Correlation of Basidiomycota : Ascomycota ratio and the two axes of the root trait PCA.

Table S1 List of plant species of which roots and soil were sampled.

Table S2 Mean root traits per plant functional group.

Table S3 Results of linear mixed-effect models relating the Shannon diversity and relative abundance of fungal ASVs within the three guilds saprotrophs, AMF and plant pathogens to the conservation and collaboration gradient of root traits.

Table S4 Results of additional linear mixed-effect models for Shannon diversity and relative abundance of fungal ASVs when accounting for the annotation rate of sequence reads.

Table S5 Results of additional partial distance-based redundancy analyses of the fungal communities when accounting for the annotation rate of sequence reads.

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