

Soil history strengthens plant diversity effects on fungal diversity and community assembly across the plant-soil continuum

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Tables are available in the Supplementary Files section.

**Soil history strengthens plant diversity effects on fungal diversity and community
assembly across the plant-soil continuum**

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Abstract:

The effects of plant species richness on soil microbial diversity and the associated ecosystem functions strengthen over time. However, the mechanisms underlying the plant diversity effects on fungal diversity and community composition along the plant-soil continuum remain largely unknown. Here, we established a plant diversity gradient (species richness level 1, 2, 3 and 6 plant species), factorially crossed with plant community-specific soil- and plant history treatments derived from a long-term field biodiversity experiment, in an Ecotron experiment. We studied the effect of plant species richness on fungal community diversity and composition across different compartments: bulk soil, rhizosphere, and roots. Using ITS amplicon sequencing, we determined fungal diversity, community composition, and assembly processes across the compartments. Fungal diversity increased significantly with plant species diversity, to varying degrees according to the compartments and history treatments. While plant history did not affect fungal community composition, shared soil history markedly increased fungal richness and affected community composition. Notably, the effect of soil and plant history on the relationship between plant species richness and the fungal diversity depended on the studied compartment, with the strongest positive plant diversity effect on fungal diversity in roots and rhizosphere soil in treatments with shared soil history. Key environmental variables were considered for exploring possible mechanisms responsible for plant species richness and history treatment effects on fungal community diversity and composition, with only plant biomass being a significant mediator. Our findings indicate that plant species richness leaves a legacy in soil, and this plant community-specific soil legacy is a significant driver of fungal diversity and community composition, but only in the compartments directly influenced by roots.

1 **Main**

2 The importance of biodiversity for ecosystem functioning is well documented and widely
3 recognized^{1,2}, with higher plant diversity enhancing productivity, nutrient retention, and temporal
4 stability across ecosystems³. Consequently, biodiversity loss is closely linked to declines in
5 ecosystem functioning and integrity^{4,5}, primarily through reductions in the quality, quantity, and
6 heterogeneity of plant-based resources. Such losses disrupt trophic interactions, nutrient cycling,
7 and habitat provisioning across ecological scales^{6,7}. Advancing a better and mechanistic
8 understanding of biodiversity-ecosystem functioning relationships by elucidating ecological
9 processes and feedbacks that sustain ecosystem functioning is therefore essential for guiding
10 conservation, management, and restoration strategies⁸.

11
12 Several mechanisms, including niche partitioning, resource-use complementarity, selection
13 effects, biotic and abiotic facilitation, and pathogen dilution, have been proposed to explain how
14 plant diversity sustains ecosystem functioning^{9,10}. Many of these mechanisms are mediated by
15 interactions between plants and soil microorganisms, which are themselves key drivers of
16 ecosystem processes^{11,12}. The interplay between plant species and the soil microbiome can promote
17 greater microbial diversity and foster the development of more specialized plant-microbe
18 associations^{13,14}. Among soil microbes, fungi play key roles in decomposition, enhanced plant
19 nutrient acquisition^{15,16}, primary productivity^{17,18}, drought tolerance, and pathogen resistance^{19,20}.
20 Fungi interact with plants both directly in the rhizosphere (the soil directly surrounding the roots)
21 and through root associations, including mycorrhiza^{21,22}, and indirectly via organic matter
22 decomposition, nutrient cycling, and pathogen prevalence in the bulk soil and rhizosphere^{23,24}. Soil
23 fungal diversity is positively related to plant diversity^{25,26}, which in turn supports ecosystem

1 functioning by enhancing nutrient uptake and carbon assimilation of the host plants^{27,28}. Previous
2 studies have reported that when plant diversity decreases, fungal communities may change in
3 abundance and distribution of different taxa contained within them^{29,30}, and such compositional
4 changes may in turn modify plant community composition and productivity³¹. Likewise, higher
5 plant diversity is linked to higher diversity and more distinct fungal functional guilds, such as
6 saprotrophic fungi, arbuscular mycorrhizal fungi, pathogenic and parasitic fungi^{30,32}. Moreover,
7 soils with higher plant diversity show increased fungal-to-bacterial ratios as well as greater
8 biomass and activity of arbuscular mycorrhizal fungi³², highlighting the significance of soil fungi
9 in biodiverse grassland communities.

10
11 Long-term biodiversity experiments have shown that the effects of plant species richness on
12 ecosystem functioning strengthen over time³³. Likewise, plant diversity effects on soil microbial
13 communities develop gradually as plant–soil feedbacks accumulate³⁴. Therefore, knowledge of
14 how the soil microbiomes co-assemble with plant communities over time, and how plant diversity
15 loss affects co-assembly processes, is important for elucidating the links between above- and
16 belowground biodiversity and ecosystem functioning. Co-assembly is shaped by soil history, i.e.,
17 the legacy of biotic and abiotic soil parameters shaped by plant-soil interactions³⁵, and plant
18 history, reflecting species-level responses to past biotic and abiotic selection pressures that
19 enhance niche differentiation³⁶. Plant–soil feedback mechanisms can promote co-adaptation
20 between plant hosts and their associated microbiomes, selecting for plant individuals that enhance
21 beneficial interactions and reduce antagonistic ones³⁷.

Over the past decade the role and importance of belowground plant-soil legacy has emerged as a central focus in ecological research. Plants can change both the biotic and abiotic components of the soil ecosystem, resulting in soil legacies that can inhibit or facilitate the growth of subsequent plant communities³⁸. Such legacy effects may arise *via* shifts in the abundance of soil-borne pathogens or mutualistic symbionts^{39,40}, the accumulation of allelochemicals⁴¹, or changes in available resources, such as organic matter dynamics and soil-nitrogen levels dynamics⁴². In recent years, significant progress has been made in understanding microbial assembly patterns and mechanisms along the plant-soil continuum⁴³. Different root niches harbor distinct microbial communities, and assembly patterns of these specific communities are a key mechanism shaping microbial community composition. Microbial community assembly describes the mechanisms and ecological processes that govern community diversity, function, distribution, and biogeography^{44,45}, and are mainly determined by stochastic and deterministic processes⁴⁵. Stochastic processes are mainly driven by factors like dispersal (including homogeneous dispersal), dispersal limitation, ecological drift⁴⁶. These processes underscore the role of random events like dispersal, immigration, speciation, extinction, and colonization in shaping the microbial community composition⁴⁷. In contrast, deterministic processes are governed by either homogeneous selection where consistent biotic and abiotic environmental conditions shape community structure and composition, or heterogeneous selection where diverse environmental factors such as pH, temperature, salinity, and soil moisture drive community composition⁴⁸. Disentangling ecological processes driving community assembly is important to better understand how deterministic and stochastic factors shape fungal communities across the plant-soil continuum.

1 The effect of plant diversity and the legacy of co-assembly processes also has a spatial component:
2 soil is heterogeneous with a continuum of microhabitats from bulk soil to the rhizosphere and root
3 endosphere^{49,50}, where increasing host selection pressure shapes microbial communities^{50,51}. As
4 such, the fungal diversity and community composition vary across these microhabitats⁵², with the
5 diversity generally being lower in roots and the rhizosphere compared to bulk soil⁵³. This
6 compartment-specific filtering leads to increasingly specialized and distinct microbial functional
7 guilds along the soil-root continuum, mainly via niche differentiation, and host plant identity and
8 diversity²⁹. The distribution and abundance of fungal communities in bulk soil are primarily driven
9 by soil physicochemical properties and nutrient availability^{52,54}, while the distribution of
10 rhizosphere and root communities are largely explained by plant traits, species diversity, plant
11 phenology and host specificity^{24,55}.

12
13 Assuming a selective recruitment process from bulk soil to the rhizosphere and root compartments,
14 and differences in substrates, we hypothesized to find a decrease in fungal diversity from bulk soil
15 to rhizosphere and roots (hypothesis 1a). In addition, we expected the root-associated communities
16 to show stronger patterns of deterministic assembly (hypothesis 1b). Further, given the
17 compartment-specific processes that connect plants and fungi, we expected differences in the
18 positive relationships between plant diversity and fungal diversity in bulk soil, the rhizosphere,
19 and roots (hypothesis 2a), with stronger soil legacy effects in the bulk soil and stronger plant legacy
20 effects on the root-associated fungal communities (hypothesis 2b). To test these hypotheses, we
21 used an Ecotron experiment, “the JenaTron Experiment”⁵⁶, where manipulation of plant species
22 richness (1, 2, 3 and 6) was coupled with combinatorial treatments of plant community-specific
23 plant- and soil history. We investigated the treatment effects on fungal communities across three

compartments - bulk soil, rhizosphere, and roots - and explored potential mediation by plant and soil characteristics.

Results

Fungal community diversity, composition, and assembly differ among bulk soil, rhizosphere, and root endosphere

The high-quality fungal amplicon sequencing variants, pooled at plant community level and clustered at approximately species level yielded a rarefied dataset of 1,981 operational taxonomic units (OTUs). Overall, 13 fungal phyla were identified (Dataset S3); fungal communities were highly dominated by Ascomycota (~75%), Basidiomycota (~13%), Mortierellomycota (~3.5%), and Glomeromycota (~2%) that accounted for 95% of the sequences. Fungal diversity within a plant community (alpha-diversity) decreased from bulk soil to roots (Figure 1a), while beta-diversity (taxonomic variations across experimental units, plant community, soil- and plant history) showed the opposite trend (Figure 1c). In particular, fungal alpha-diversity was significantly higher in bulk soil compared to the roots ($p < 0.05$), independent of the type of the alpha-diversity measure (Figure 1a; Extended Data Figure 1 & Dataset S4 & S6). Bulk and rhizosphere soil communities showed similar levels of alpha-diversity ($p > 0.05$; Figure 1a). Fungal OTU richness showed a positive correlation between compartments, with stronger relationships observed between the rhizosphere and roots ($R^2 = 0.54$; $p < 0.001$), compared to bulk soil and roots ($R^2 = 0.38$; $p < 0.001$) (Figure S3). Similarly, the differences between fungal communities were largest when comparing bulk soil and roots (PERMANOVA, $F = 72.49$; $R^2 = 0.28$; $p < 0.001$), and rhizosphere and roots (PERMANOVA, $F = 66.02$; $R^2 = 0.26$; $p < 0.001$) (Figure 1b; Dataset S7), while the differences between bulk soil and rhizosphere communities were smaller (PERMANOVA, $F = 17.63$; $R^2 = 0.08$; $p < 0.001$) (Figure 1b & Dataset S7).

1
2 The differences in fungal community composition (Extended results 1, Figure 1d, Extended
3 Dataset S9 & S10) were reflected by differences in predicted ecologically relevant fungal
4 functional guilds. Compared to roots, fungal taxa inhabiting the bulk soil and rhizosphere showed
5 a higher proportion of saprotrophs ($25.2 \pm 5.7\%$ vs $16.6 \pm 7.8\%$) and ambiguous fungi (11.7 ± 1.6
6 $\%$ vs $4.7 \pm 2.6\%$) (Kruskal–Wallis, $p < 0.05$) (Figure 1e & Dataset S11). In contrast, root samples
7 were enriched (Kruskal–Wallis, $p < 0.05$) for symbiotrophs ($13.1 \pm 9.8\%$ vs $2.3 \pm 1.1\%$),
8 pathotrophs ($5.2 \pm 7.2\%$ vs $4.4 \pm 2.8\%$), and unidentified fungi ($42 \pm 13\%$ vs $28.5 \pm 8.0\%$).
9 Likewise, the functional potential was largely similar between bulk soil and the rhizosphere
10 (Kruskal–Wallis $p > 0.05$).

11
12 The differences between root and soil compartments likely reflect specific ecological filtering,
13 where bulk soil and rhizosphere environments provide diverse and nutrient-rich habitats that favor
14 saprotrophic and competitive fungal life-styles. Across the compartments, ecological drift, a
15 stochastic process, was dominant and played a major role ($92.61 \pm 2.56\%$; mean $\pm$ s.d), while
16 homogeneous selection, a deterministic process, had a relatively small role in controlling the
17 spatial turnover of fungal communities in bulk soil, rhizosphere, and roots (0.52 ± 0.73 ; mean $\pm$ s.d,
18 Extended Dataset S12). Dispersal limitation was the second most influential ($4.05 \pm 0.74\%$; Figure
19 2a; Extended Dataset S12) process. Overall, drift dominated the community turnover and showed
20 the strongest relative influence in bulk soil (95.42%), followed by root (92.04%) and rhizosphere
21 (90.38%) (Figure 2a; Dataset S12; Extended results 2). These findings were corroborated by
22 differences in the Dispersal-Niche Continuum Index⁵⁷ (Dataset S13) and analysis of the neutral
23 community model⁴⁷ (Figure 2 b & c). The lower migration rates in roots ($m = 0.006$) than in the

rhizosphere ($m = 0.033$) and bulk soil ($m = 0.067$) (Extended Data Figure 2 & Dataset 14) imply a dispersal limitation in the root compartment.

Shared soil history drives responses of soil and root fungal community diversity and functional groups to plant diversity

Given the differences in fungal community assembly, diversity, taxonomic composition, and functional guilds among the compartments, we assessed compartment-specific plant diversity effects and their potential mechanisms. Across all three compartments, fungal OTU richness increased significantly with plant species richness, with more variance explained in the rhizosphere ($F = 12.28$; $p = 0.003$) and bulk soil ($F = 10.64$; $p = 0.005$) compared to roots ($F = 8.76$; $p = 0.009$) (Figure 3 & Table 1). Alpha-diversity measures that account for evenness (Shannon diversity index and Simpson's diversity) of fungal OTU relative abundances displayed weaker, but generally consistent trends (Extended results 3 & Extended Data Figure 3 a & b; & Table 1). Plant diversity also had significant but marginal and compartment-specific effects on fungal community composition in roots (variation = 5.4%; PERMANOVA, $F = 1.1$; $R^2 = 0.024$; $p = 0.125$), the rhizosphere (variation = 4.7%; $F = 1.0$; $R^2 = 0.015$; $p = 0.029$), and bulk soil (variation = 4.5%; $F = 0.9$; $R^2 = 0.014$; $p = 0.142$) (Figure 4, Table 2 & Extended Dataset Figure 4 & Table 2).

Next, we investigated compartment-specific effects of shared history of plants and soils on the plant-fungal richness relationship. Overall, fungal communities in the soils that had previously harboured grassland plant communities with the same species compositions in the field differed from previously bareground soils, with similarly strong positive effects (i.e., soil history effects) on fungal richness in bulk soil ($F = 35.02$, $p < 0.001$), rhizosphere ($F = 34.65$, $p < 0.001$), and roots

($F = 42.763$, $p < 0.001$) (Figure 3, Table 1). Similarly, soil history had a strong and significant effect on fungal community composition in bulk soil ($F = 22.8$, $R^2 = 0.178$, $p < 0.001$), the rhizosphere ($F = 23.5$, $R^2 = 0.181$, $p < 0.001$), and roots ($F = 14.5$, $R^2 = 0.104$, $p < 0.001$) (Figure 4, Table 2). In contrast, common plant history showed no significant effect on fungal richness (all $p > 0.05$) (Figure 3 & Table 1) or fungal community composition (all $p > 0.218$, $R^2 < 1\%$) (Figure 4, Table 2) in either compartment.

Notably, the three compartments displayed different and unexpected interaction effects of common soil- or plant history on the relationship between plant species richness and fungal richness. In bulk soil, the positive relationship between plant species richness and fungal richness remained constant, regardless of soil or plant history ($F = 0.05$ & 0.03 , $p = 0.827$ & 0.869), respectively (Figure 3 & SR:SH interaction in Table 1). In the rhizosphere, plant history enhanced the effect of plant species richness on fungal richness ($F = 10.26$, $p = 0.002$; Figure 3 & Table 1), while in roots, soil history strengthened this positive relationship ($F = 6.446$, $p = 0.013$; Figure 3 & SR: SH interaction in Table 1). Furthermore, the fungal richness and diversity were not affected by any three-way interaction (SR x SH x PH interaction) in either of the compartments (all $p > 0.46$) (Table 1).

Fungal community composition was also affected by a significant two-way interaction between plant diversity and soil history in roots ($F = 1.77$, $R^2 = 0.013$, $p = 0.048$). A similar trend was observed in the rhizosphere ($F = 1.77$, $R^2 = 0.013$, $p = 0.06$) and bulk soil ($F = 1.8$, $R^2 = 0.014$, $p = 0.06$) but these effects were marginally significant (SR:SH interaction in Table 2), indicating that plant diversity effects on root communities depended on soil history. We found no additional

significant interaction effects of plant species richness and history treatments on fungal community composition (all $p > 0.05$), in either of the compartments (SR:PH, SH:PH & SR:SH:PH interactions in Table 2).

To better understand the differences in community composition in terms of the potential interaction between fungal and plant communities, we tested plant diversity and history treatment effects and their interactions on the relative abundance of broad fungal functional guilds (for example: pathotrophs, saprotrophs, or symbiotrophs in terms of broad trophic guilds), in the three compartments. In agreement with the effects on fungal taxonomic community composition, soil history affected the relative abundances of all three guilds in bulk and rhizosphere soils, with proportions of saprotroph and symbiotroph fungi increasing with soil history, and the relative abundance of pathotroph fungi decreasing (Figure 5, Table 3). Additionally, in bulk soil, the positive plant species richness effect on the relative abundance of saprotrophs was conditional on soil history. Similarly, the interaction between plant diversity and soil history had significant positive effects on fungal symbiotrophs in the rhizosphere. Notably, shared soil history had a negative effect on the relative abundances of pathotrophs in all compartments, but shared plant history led to an increase in pathotroph proportions at higher plant species richness in bulk soil only, highlighting the differences in functional and spatial effects of plant diversity on fungal communities.

Potential mediating role of aboveground biomass in driving fungal diversity and community composition

To identify the possible mechanisms responsible for plant diversity and history treatment effects on soil, rhizosphere, and root fungal communities, various environmental factors were fitted as covariates⁵⁸. Plant biomass was positively correlated with fungal OTU richness more strongly in roots ($F = 10.67$, $p = 0.001$) and the rhizosphere ($F = 6.58$, $p = 0.012$) as compared to bulk soil ($F = 4.79$, $p = 0.03$) (Extended Dataset Table 3). Further CN ratio was positively with fungal OTU richness more strongly in bulk soil ($F = 10.238$, $p = 0.002$), and in roots ($F = 10.110$, $p = 0.002$), compared to rhizosphere ($F = 4.254$, $p = 0.043$) (Extended Dataset Table 4). However, fitting plant biomass and carbon-to-nitrogen ratio (C:N) ratio first in sequential models⁵⁸ did not fully eliminate the significant positive effects of plant species richness and soil history on observed OTU richness (Figure 3 & Table 1). This indicates that plant diversity and soil history effects are more important and have significant effects on fungal diversity across bulk soil, rhizosphere and root compartments. In contrast, no significant effects of other measured covariates were observed on fungal diversity and community composition across the compartments (Supplementary Data Table 1-7) (all $p > 0.05$).

Discussion

To sustain the integrity of ecosystems despite global environmental changes and biodiversity loss^{59,60}, it is important to understand the conditions and mechanisms linking biodiversity and ecosystem functioning. While the importance of fungal diversity has already been convincingly supported by biodiversity experiments⁶¹⁻⁶³, this study delivers the first differential and factorial analysis of fungal communities among a plant diversity gradient based on the mode, localization, and timing of the interaction with plant and soil community history. The fact that microbial communities in roots differ from rhizosphere and bulk soil is well documented⁶⁴, and this is mainly

1 attributed to the selective root environment, host-mediated selection processes⁶⁵, and differences
2 in soil properties driving niche differentiation and recruitment from bulk soil^{66–68}. In addition to
3 environmental differences, the general contrast between these compartments is also an important
4 cause of difference in fungal diversity and community composition⁶⁷. Fungal observed OTU
5 richness, Chao1, Shannon's diversity, and Simpson's index in roots were generally lower than in
6 rhizosphere and bulk soil, supporting our first hypothesis, that plant microbiomes are recruited
7 from bulk soil and follow a spatial gradient from the bulk soil, across the rhizosphere to the roots
8 ^{55–57}. This, in turn, highlights the strong selective effects of root systems, which has some general
9 consequences on plant microbiome assemblages¹². While fungal alpha-diversity decreased, beta-
10 diversity (taxonomic variations across experimental units, plant community species richness, soil
11 legacy, and plant legacy) increased from bulk soil to the rhizosphere and roots. These findings
12 further support the notion that root community selection contributed to the reduced diversity in
13 roots^{69,70}. The enrichment and depletion of certain taxa and lower contribution of ecological drift
14 to community assembly processes in the rhizosphere and root compartments suggest selective
15 effects of the rhizosphere and dispersal limitations^{71–73}. The relative enrichment of different OTUs
16 in the root endosphere supports a recruitment model, where the nutrients and signalling compounds
17 produced by the plants selectively attract taxa that can colonize the root ^{74,75}. Furthermore, several
18 fungal taxa (for example; Ascomycota, Basidiomycota, Chytridiomycota,
19 Entomophthoromycota, Rozellomycota and Zoopagomycota) and their associated fungal guilds
20 (mostly pathotrophs) that were depleted in the roots were also consistently depleted in the
21 rhizosphere (Dataset 8), suggesting that selectivity for root colonization occurs primarily in the
22 rhizosphere, indicating that the rhizosphere plays an important gating role in limiting microbial
23 entry into roots⁷⁴.

1
2 Since fungal communities' functions drive pathogen prevalence, carbon cycling, nutrient
3 mobilization, and decomposition^{76,77}, we also inferred the functional guilds in the fungal
4 communities along the soil-plant continuum, and our analysis revealed distinct compartment-
5 specific patterns. Saprotrophs were found significantly enriched in bulk soil and the rhizosphere.
6 This is consistent with the notion that bulk and rhizosphere soils are the primary sites of
7 decomposition^{24,78,79}. Likewise, the higher abundance of symbiotrophs in roots reflect the host-
8 driven preference for beneficial symbionts⁸⁰ and the availability of nutrients creating an
9 environment favorable for the growth, proliferation, and survival of fungal pathogens^{68,77}.

10
11 The differences in fungal community composition and functional guilds across the compartments
12 were also reflected by fungal community assembly processes. In this study, both deterministic and
13 stochastic processes were concurrently present and determined the fungal community assembly in
14 bulk soil, the rhizosphere, and roots. However, contrary to our expectations, the composition and
15 assembly of fungal communities in the bulk soil, rhizosphere, and root compartments were mainly
16 governed by stochastic processes, with ecological drift contributing the most. This supports the
17 notion that during the early stages of plant growth, fungi tend to be rare and dominated by
18 stochastic processes. The dominance of stochastic processes suggests that under certain ecological
19 conditions, such as a nutrient-rich or highly managed environments, neutral mechanisms may
20 override deterministic selection^{81,82}, and as such result in dominance of stochastic process-driven
21 fungal assembly in the bulk soil, rhizosphere, and roots. Moreover, the relatively smaller role of
22 deterministic processes in controlling spatial turnover of the fungal community may be attributed
23 to limited host selection. In addition, the root-associated microbial communities tend to exhibit

1 delayed responses to environmental variations, largely due to the secretory activity of roots (i.e.,
2 the release of root exudates)⁸³, thus resulting in a stronger dispersal limitation on fungal
3 communities colonizing the rhizocompartments⁸⁴. In the roots, the release of large amounts of
4 exudates can influence the fungal community by selective recruitment^{85,86}, and as such the root
5 fungal community is modulated by the combined effects of host selection and environmental
6 filtering from surrounding bulk soils. Overall, these results suggest that the investigated
7 compartments bulk soil, rhizosphere, and roots emerged as key determinants of fungal functional
8 group distributions, highlighting strong ecological filtering at the plant-soil interface^{77,87}.

9
10 In this study, we hypothesized that plant species richness would differentially affect fungal
11 richness and community composition across bulk soil, rhizosphere, and root compartments. Our
12 findings that plant diversity increases fungal richness and alters community composition are in
13 accordance with the reports of plant diversity effects on fungal diversity in bulk soil^{23,87},
14 rhizosphere⁵⁵, and the roots⁸⁸. This may be due to the ability of more diverse plant communities to
15 provide different resources, ecological niches, and to alter biotic and abiotic conditions in ways
16 that facilitate the coexistence of multiple fungal guilds⁸⁹. We found compartment-specific effects
17 of plant diversity on fungal community composition, with the effects in the root compartment
18 being much more pronounced than in the rhizosphere and bulk soil. This aligns with previous
19 findings of plant diversity effects on fungal community composition (Dataset S3)^{90,91}. Overall,
20 these findings suggest that bulk soil and rhizosphere fungal communities are less strongly
21 influenced by plant species richness than root microbial communities, which can be attributed to
22 the strong plant defense and influence of root exudation, and growth, all of which facilitate
23 microbial selection from the rhizosphere and bulk soil^{30,83,92}, thus potentially resulting in

1 compartment-specific differences in the relationship between fungal diversity and plant species
2 richness. In addition, plant species richness can significantly influence the composition of the
3 rhizosphere microbiome, by releasing different metabolites and signalling molecules⁸³, that
4 potentializes the microbial selection by the rhizosphere. For example, increased plant diversity
5 was shown to change rhizosphere microbial communities, with more plant species recruiting more
6 fungal groups⁹³.

7
8
9 In addition to the strong main effects of plant diversity on fungal richness and community
10 composition in bulk soil, rhizosphere, and root compartments, interactions with soil history and
11 plant history were generally weak and compartment specific (Figure 4). This may be because the
12 influence of soil legacy effects is diluted or masked in the presence of strong plant diversity
13 effects⁹⁴. As suggested by Hannula *et al.*⁹⁵, legacy effects often persist predominantly inside roots,
14 reflecting the stronger host filtering by the roots compared to the rhizosphere and bulk soil. Soil
15 history suggests that physicochemical properties and fungal pools change over time, driving the
16 structure, composition, and assembly processes of subsequent microbial communities of the root
17 endosphere⁹⁶. As expected, soil history had stronger effects on fungal richness and community
18 composition across the investigated compartments. The findings of this study are consistent with
19 previous findings in the Jena Experiment and other grassland ecosystems^{23,61,96}, showing stronger
20 soil-legacy effects on structuring and shaping fungal richness and community composition. Further
21 away from the plant roots, the soil microbiome may change more slowly than the rhizosphere
22 microbiome, so that different results are apparent across the compartments²³.

1 In contrast to the strong effect of soil history, effects of plant history on fungal richness and
2 community composition were not detected. This result was unexpected and may suggest that plant
3 history effects on fungal communities may need more time to develop or are too subtle to be
4 influential (both in terms of genotype selection and course of the experiment)²³. Overall, our
5 findings suggest that soil history is a dominant factor for building up plant diversity effects on
6 fungal richness and community composition across bulk soil, rhizosphere, and root compartments.

7
8 Fungal diversity, through the diversity of different functional guilds such as saprotrophic,
9 pathotrophic, and symbiotrophic fungi, is known to regulate the plant–soil feedbacks in grassland
10 ecosystems⁹⁷. Both plant species richness and soil history have stronger linkages with fungal
11 functional guilds than with fungal richness alone²³. Fungi support their host plants directly by
12 supplying water and nutrients and indirectly by improving soil properties for example soil
13 aggregation, water retention, and nutrient cycling^{98,99}. We found a positive relationship between
14 soil history and fungal functional guilds, and to a lesser extent, the interactive effects of plant
15 diversity and soil and plant community history. Notably soil history significantly influenced fungal
16 diversity (Figure 3, Table 1) and composition across compartments (Figure 4, Table 2), providing
17 additional support to the idea that soil history plays a key role in shaping fungal functional guilds.
18 The significant effects of soil history on the relative abundance of fungal functional guilds may be
19 attributed to changes in nutrient dynamics, organic matter, and soil structure^{100,101}, promoting the
20 establishment of specific fungal communities and ecological niches, each adapted to the unique
21 resources and conditions^{101–103}. Overall, our results indicate that significant plant diversity effects
22 on root and soil fungal communities are mostly due to significant soil history effects, highlighting
23 an important mechanism of long-term plant diversity effects on ecosystem functioning^{9,56}. It is

likely that plant species have specific effects on root and soil microbial communities; the present study did not investigate the effects of plant identity and traits on fungal diversity and community composition. Future research may focus on investigating the effects of certain plant species and functional groups on fungal diversity and community composition across the bulk soil, rhizosphere, and root compartments.

Potential mediating role of aboveground biomass in plant richness effects on fungal diversity

Previous studies have suggested that plant diversity affects fungal community diversity and composition mainly through increased plant productivity^{26,58}. We hypothesized that a range of environmental variables lead to stronger plant diversity and history treatments effects on fungal diversity and composition. As such, we tested the effects of different environmental variables on fungal diversity and a separate analysis was performed by fitting these variables as covariates in sequential models. Although, aboveground plant biomass and carbon-to-nitrogen ratio (C: N) were significantly positively correlated with fungal richness across the compartments; however, in contrast to our expectation, these covariates did not diminish the effects of plant diversity and soil history. Therefore, the results support the conclusion of previous findings^{58,83} that the quality, quantity (here: plant biomass), and diversity of rhizodeposits influence microorganisms in soil and root compartments. Moreover, the observed positive correlation between carbon-to-nitrogen ratio and plant species richness can be attributed to the fact that higher plant biomass drives higher CN ratio^{104,105}. Variations in other potential mediators such as soil microbial biomass C, bacterial biomass, and fungal biomass, soil basal respiration, microbial respiratory quotient, soil moisture, and soil fauna diversity could also not explain significant plant diversity effects on fungal diversity and community composition across the compartments.

Here, we found that the fungal richness and composition across bulk soil, rhizosphere, and root compartments was significantly influenced by plant species richness and soil history. Notably, weak but compartment-specific interactions among plant species richness, soil history, and plant history further modulated fungal diversity and community composition across compartments, particularly enhancing fungal diversity in the rhizosphere. Differences in community assembly processes among compartments highlight the dominant role of stochastic forces, especially ecological drift, in structuring fungal communities, with bulk soil and rhizosphere being more strongly influenced than roots. Overall, our findings suggest that soil microbial communities are mainly influenced by plant diversity, and that long-term plant diversity effects on soil microbial communities^{23,106,107} are mostly due to plant community-specific soil history. The study further indicates feedback loops between plant diversity loss has effects on soil fungal diversity and community composition and subsequent ecosystem functioning.

Methods

Study area and experimental design

This study was conducted at the iDiv Ecotron^{108,109} platform, an indoor experimental mesocosm facility located at the Research Station of the Helmholtz-Centre for Environmental Research (UFZ) in Bad Lauchstädt, Germany. This indoor research facility houses 24 identical experimental units – the EcoUnits, each containing four independent near-natural and controlled ecosystems. Ninety-six ecosystems within 24 EcoUnits were prepared with monocultures ($n = 6 \times 4 = 24$) or mixed plant communities (2-species mixes; $n = 9 \times 4 = 36$; 3-species mixes; $n = 7 \times 4 = 28$; and 6-species mixes; $n = 2 \times 4 = 8$) (see Dataset S1, for more details on plant species composition and

replicates for each plant diversity level), with every subunit of an EcoUnit containing one combination of history treatments: soil monoliths (each with a depth of 0.8 m and diameter: 0.5 m) from a field site of the Jena Experiment (a long-term grassland biodiversity experiment in Jena, Thuringia, Germany, 50°55' N, 11°35' E, 130 m above sea level) (see <https://the-jena-experiment.de/>, for a more detailed description about the experiment) with the same plant community (shared soil history “+ SH”) or from a bare-soil plot (“- SH”) in combination with plants grown from seeds of the field site with the same plant community (common plant history “+ PH”) or freshly acquired seeds (“- PH”) (Figure S1). The soil of the field site is rich in nutrients and is classified as Eutric Fluvisol, developed from loamy fluvial river sediments. The soil exhibits a homogeneous texture, with a composition of 16% of sand, 59% of silt, and 25% of clay¹¹⁰. The pH of the soil ranges from 7.2-7.4. Furthermore, the concentrations of key elements are as follows: Carbon (C) is $20.9 \pm 2.6 \text{ g kg}^{-1}$, nitrogen (N) is $2.2 \pm 0.2 \text{ g kg}^{-1}$ and the C/N ratio 9.4 ± 0.4 ¹¹⁰. Additionally, each EcoUnit is equipped with a programmable multi-color light system, an irrigation system with deionized water, and a centrally maintained ambient temperature system^{108,109}. During the experimental phase, a day-night period of 16h/180c day and 8h/120c night were established to be consistent with regional natural daylight conditions¹¹¹. Irrigation is tailored to plant needs, initially set at 450 mL every 12 hours and increased to 750 mL every 12 hours as required, to ensure optimal soil moisture and plant water demands, while maintaining a moderate humidity level of 60% -70% relative humidity (RH)¹¹¹. This fully factorial experimental design allows to disentangle the effects of common plant and soil history on the relationship between plant species richness and fungal community diversity and composition. A detailed description of the experimental setup of the JenaTron can be found at Madj *et al.*,¹¹¹.

Sample collection

Samples from all 96 ecosystems (subunits) were collected after a 5-month growth period (June-October), during a sampling campaign conducted from October 23 to October 29, 2022. Bulk soil was sampled as composite samples (3 cores of 2 cm diameter) and the collected soil samples were frozen in plastic bags at -20 °C for DNA analysis. For rhizosphere and root samples, three plant individuals per plant species were excavated using a shovel. To collect rhizosphere soil, soil clods were gently cracked to expose the roots of the target species. Loose soil was discarded, and soil adhering to the fine roots of the target species was collected using sterile spoons and tweezers. Rhizosphere soil collected from three individuals of each species within a community was pooled into a single composite sample. To clean the roots, each of the three samples were separately soaked in water for 15 min to loosen up the attached soil. The roots were repeatedly washed to thoroughly remove all the remaining soil from the root systems. Using a stereo microscope and sterile tweezers, any remaining dirt and roots from non-target species were carefully removed. Bulkied from all three individuals, fine roots were taken and frozen at -20 °C for subsequent DNA extraction.

DNA extraction

Total DNA was extracted from 0.3 -0.4 g of thawed and homogenized bulk soil or rhizospheric soils and 0.1-0.2 g of root samples by using the Quick DNA Fecal/Soil Microbe Miniprep Kit by Zymo Research (Germany), according to the manufacturer's protocol. The quality and concentration of the extracted metagenomic DNA was checked by using a NanoDrop 2000 spectrophotometer and stored at -20 °C for further downstream analysis.

ITS marker amplicon sequencing

The fungal community profiling of bulk soil, rhizosphere and root samples was carried out by sequencing the ITS2 (internal transcribed spacer 2) region of the ribosomal DNA. ITS2 amplification was performed using the widely applied barcoded primer set ITS4¹¹², and a 1:1 mixture of fITS7 and fITS7o^{113,114}. PCR reaction mixtures were made using 7.5 µl KAPA HiFi HotStart ReadyMix (Roche), 0.3 µl of each primer, and 20ngl of template DNA in 15 µl reaction volume. PCR amplification was carried with an initial denaturation temperature of 3 min 95 °C, followed by 30 cycles of 20 s at 98°C, 20 s at 56°C and 20 s at 72 °C, and with a final extension of 5 min at 72 °C. The negative controls for PCR operations were run under the same conditions. Finally, the quality of the amplified PCR products was determined by 1.5% agarose gel electrophoresis. The amplified PCR products of three reactions were pooled and further processed for the library preparation using the Nextera XT v2 Illumina Index Kit (Illumina Inc., San Diego, CA, USA). The quantified libraries were pooled and sequenced (paired-end sequencing: 2 × 300 bp) on the Illumina MiSeq platform at the Department of Soil Ecology (Helmholtz Centre for Environmental Research – UFZ, Halle (Saale), Germany).

Sequencing data processing

The Illumina-sequencing generated paired-end, demultiplexed FASTQ files containing raw sequences were processed using the DADA2-based (version 1.2.6) dada2^{115,116}. In the first place, primer and barcode sequences were removed using the Cutadapt program. Next, quality filtering and length trimming of Illumina generated paired-end raw reads were conducted using DADA2 functions to remove ambiguous bases, truncate at low-quality positions (Phred scores Q < 16) and remove low-quality reads (maxEE 3). After quality filtering, pseudo-pooling of

sequencing reads from the same soil compartment within each subunit of the Ecotron was performed by concatenating a down sampled fraction of the reads to reach the same number of 38,000 reads per pseudo-pooled (i.e.; subunit-level) sample. The reads were clustered to ASVs in pooled mode. Forward and reverse ASVs were merged with a minimum overlap of 14 nt (up to 2 mismatches) and chimeras were removed using the consensus algorithm in DADA2. The detailed read information is given in the Supplementary data (Dataset S2).

For taxonomic assignment and diversity analysis, amplicon sequence variants were clustered at 97% similarity level into OTUs using vsearch (v 2.22.1). Taxonomic annotation of the 97% OTUs was performed against the fungal ITS2 sequences in the UNITE database (v. 8.2)¹¹² database using the mothur (v 1.41) implementation of the naïve Bayes classifier¹¹⁷. Two different databases, the FungalTraits¹¹⁸ and FUNGuild¹¹⁹ were used to obtain the functional predictions (ecological guilds) of fungal communities. These databases were used to extrapolate and associate fungal taxa with their putative ecophysiological roles. The predicted annotated functional guilds include “saprotrophs”, “symbiotrophs”, “pathotrophs” as the primary ecophysiological guilds.

Data analysis: alpha- and beta-diversity analysis and compartment-specific OTUs

Rarefaction curves for all the samples from bulk soil, rhizosphere and root endosphere compartments reached plateau, indicating good coverage and sufficient sequencing depth to cover the majority of the fungal diversity (Figure S2). Good’s coverage of the fungal communities ranged from 98.3 to 1 across the individual samples, with the good’s index of all the samples combined reaching 1 (Dataset S3). The microeco v1.15.0 package was used to study fungal communities by generating a micro table object in R version 4.2.2, integrating an OTU abundance table, taxonomic

assignment table, phylogenetic tree and sample metadata descriptions. The relative abundance of fungal taxa at phylum level were calculated using the `trans_abund` function of the `microeco` package¹²⁰.

Alpha-diversity comparisons between the bulk soil, rhizosphere and root compartments were performed in terms of observed OTU richness, Chao1, Shannon-Wiener diversity and Simpson's evenness for each compartment. All these indices were performed on rarified data by using the `trans_alpha` function of the `microeco` package¹²⁰, and compared using Kruskal–Wallis test followed by pairwise comparisons by Dunn's test at a p -value < 0.05 . To explore differences in fungal β -diversity across different ecounits and treatments (plant species richness and plant history and soil history), pairwise comparisons within each compartment were calculated using Aitchison's distance as implemented in the `robCompositions` package¹²¹ and visualized in terms of principal component analysis (PCA). Differences were assessed based on filtered and rarefied abundance matrices after replacing the zeros with a pseudo-count of 1. To cross-check the results using ecological distance metrics, weighted and unweighted Unifrac, Bray Curtis and Jaccard distance metrics were calculated from the sum-normalized OTU tables for each compartment. Effects of the compartments bulk soil, rhizosphere and root on fungal community composition were estimated by PERMANOVA¹²² using the “`adonis2`” function of the “`vegan`” package.

Differential abundance analysis was performed by using the `trans_diff` function and the OTUs were extracted by applying the standard Aldex2 (Wilcox) method implemented in R package “`microeco`”¹²⁰, additionally used indicator species analysis to identify specific taxa that characterize each compartment by using the “`multipatt`” function from the `indicspecies` package

and tested indval¹²³. The statistical significance of the association was performed with 9,999 permutations to elucidate the OTUs that were highly associated with bulk soil, rhizosphere and roots. We used a generalized linear model (GLM) from EdgeR¹²⁴ to detect the differentially abundant (enriched and depleted) OTUs in the rhizosphere and root endosphere compared to bulk soil⁷⁴. Enriched and depleted OTUs were defined on the basis of log fold changes (FC) as: OTUs with FC value greater than 1 and p value less than 0.05 were considered as enriched OTUs, while OTUs having FC value less than 1 and a p value less than 0.05 were considered as depleted OTUs^{74,122}.

Data analysis: fungal community assembly

We employed a phylogeny-based null model detailed in Stegen *et al.*⁸² to classify different ecological processes underlying the drivers of fungal community assembly. The processes are characterized as either: i) stochastic including dispersal limitations, ecological drift, and homogeneous dispersal or ii) deterministic including homogeneous and heterogeneous selection⁸². For this, first, we quantified the phylogenetic turnover by calculating the β -nearest taxon index (β NTI) and the Raup–Crick metric based on Bray–Curtis dissimilarity (RC-bray) using the trans_nullmodel class from microeco package version v1.15.0 R package¹²⁰. A significant deviation in β NTI values i.e., ($|\beta\text{NTI}| > 2$) and $|\beta\text{NTI}| < -2$ indicate greater than or lesser phylogenetic turnover than expected representing heterogeneous and homogeneous selection processes, respectively^{125,126}. Subsequently, β NTI and RCbray value of $|\beta\text{NTI}| < 2$ and RCbray $> +0.95$ and $|\beta\text{NTI}| < 2$ and RCbray < -0.95 estimates the influence of dispersal limitation and homogenizing dispersal, respectively. Similarly, the fractions of pairwise comparisons with $|\beta\text{NTI}| < 2$ and RCbray < -0.95 estimates the relative influence of ecological drift acting alone on

community assembly. The relative contribution of different ecological processes was further quantified using the iCAMP^{127,128} function from the microeco v1.15.0 R package¹²⁰.

To further evaluate whether dispersal or niche-based processes drive the community assembly processes, calculations were performed by dispersal niche continuum index (DNCI) package⁵⁹.

The modified stochastic ratio (MST) was used to determine the relative importance of stochastic (MST > 0.5) and deterministic (MST < 0.5) processes in driving fungal community assembly using the NST R package¹²⁸. Additionally, we applied a neutral community model (NCM) to determine the potential role of stochastic processes in fungal community assembly¹²⁹. The model predicts the relationship between the detection frequency of each microbial taxa and its relative abundance within the broader metacommunity. In the NCM analysis, R^2 represents the overall fitness of the model fit, NM estimates the dispersal between the communities and m is the immigration rate.

For calculations, the datasets were separately used from the bulk soil, rhizosphere, and root compartments, and neutral community model (NCM) was used to estimate the niche breadth values as detailed in Sun *et al.*¹³⁰. To test model fit and parsimony, adjusted R^2 and Akaike information criterion (AIC) were used (Dataset S4). To elucidate the patterns of dispersal limitation and species sorting and their influence on fungal communities in bulk soil, the rhizosphere and root compartments, we calculated the Levins' niche breadth (Bcom) using the “niche width” function in R package spaa¹³¹.

Data analysis: plant species richness effects on fungal community diversity and composition

We used linear mixed-effects models to test the effects of plant species richness, soil history, and plant history on fungal alpha diversity metrics; calculated separately for bulk soil, the rhizosphere and root compartments. Alpha-diversity was assessed using three indices: observed OTU richness, Shannon diversity, and Simpson diversity (all calculated after rarefaction). Each model included log₂-transformed plant species richness (logSR) as a continuous fixed effect, and soil history (with vs. without: SH (+) and SH (-)) and plant history (with vs. without: PH (+) and PH (-)) as categorical fixed effects. All two-way and three-way interactions between plant richness and the history treatments were included, yielding the model formula:

Diversity metric ~ logSR × soil history × plant history.

To account for the experimental design, we included random intercepts for block (hall_block) and units nested within blocks. Models were fitted using the “lme” function from the nlme R package (v3.1-167), with restricted maximum likelihood (REML) estimation. The significance of fixed effects was assessed via Type I ANOVA.

To test the effects of plant species richness, soil history, plant history, and their two-way and three-way interactions on fungal community composition, we performed permutational multivariate analysis of variance (PERMANOVA) using the “adonis2” function from the vegan R package (v2.6-10)¹²². The response variable was a matrix of pairwise Aitchison distances, calculated separately for each compartment (bulk soil, the rhizosphere, and root). The full model included plant species richness (SR), soil history (SH), plant history (PH), and their two-way interactions (SR × SH, SR × PH and SH × PH) and three-way interactions (SR × SH × PH) as fixed effects. To account for the experimental design, permutations were constrained to between eco-unit permutations for SR and within eco-unit permutation for SH and PH and their interactions¹³².

Models were evaluated using 999 permutations and tested by terms (by = "terms"). R^2 values represent the proportion of variation explained by each term in the model.

To test the effects of plant diversity, soil history, plant history and their two and three-way interactions on the relative abundance of fungal functional guilds, we extracted different trophic modes from the FUNGuild database¹¹⁹. The final model was the same as in alpha-diversity, except for the response variable which included the relative abundance of each identified trophic guild (saprotrophs, symbiotrophs and pathotrophs). The unidentified trophic modes were excluded from the model analysis.

Data analysis: environmental covariates

To identify the mechanisms responsible for plant diversity and history treatments effects on fungal diversity and composition across compartments, key biotic, abiotic, and edaphic variables known to affect soil microbial community diversity and composition, were fitted as covariates. The covariates were always fitted first, followed by plant species richness (logSR) and history treatments (soil history and plant history), and include aboveground biomass (biomass), soil microbial biomass C (Cmic), basal respiration (BR), carbon-to-nitrogen (CN) ratio, soil moisture (SM), soil fauna diversity (SF), and microbial respiratory quotient (RQ), bacterial biomass (BB), and fungal biomass (FB). These covariates were tested, given their impacts on soil microbial communities^{23,61,133}. The tested model's formula was: Diversity metric ~ covariate + logSR × soil history × plant history.

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Author contributions:

M. A. M., N. E. and A. H. B. contributed to the conceptualization of the project. Y. H. contributed to visualization. A. M. M., C. A., L. B., P. M. B., Y. P., B. S., M. D. S., M. B., F. B., C. R., M. S., S. S., A. W. contributed to methodology and investigation. The writing was led by M.A.M, with reviewing and editing contributed by N.E. and A. H.B.

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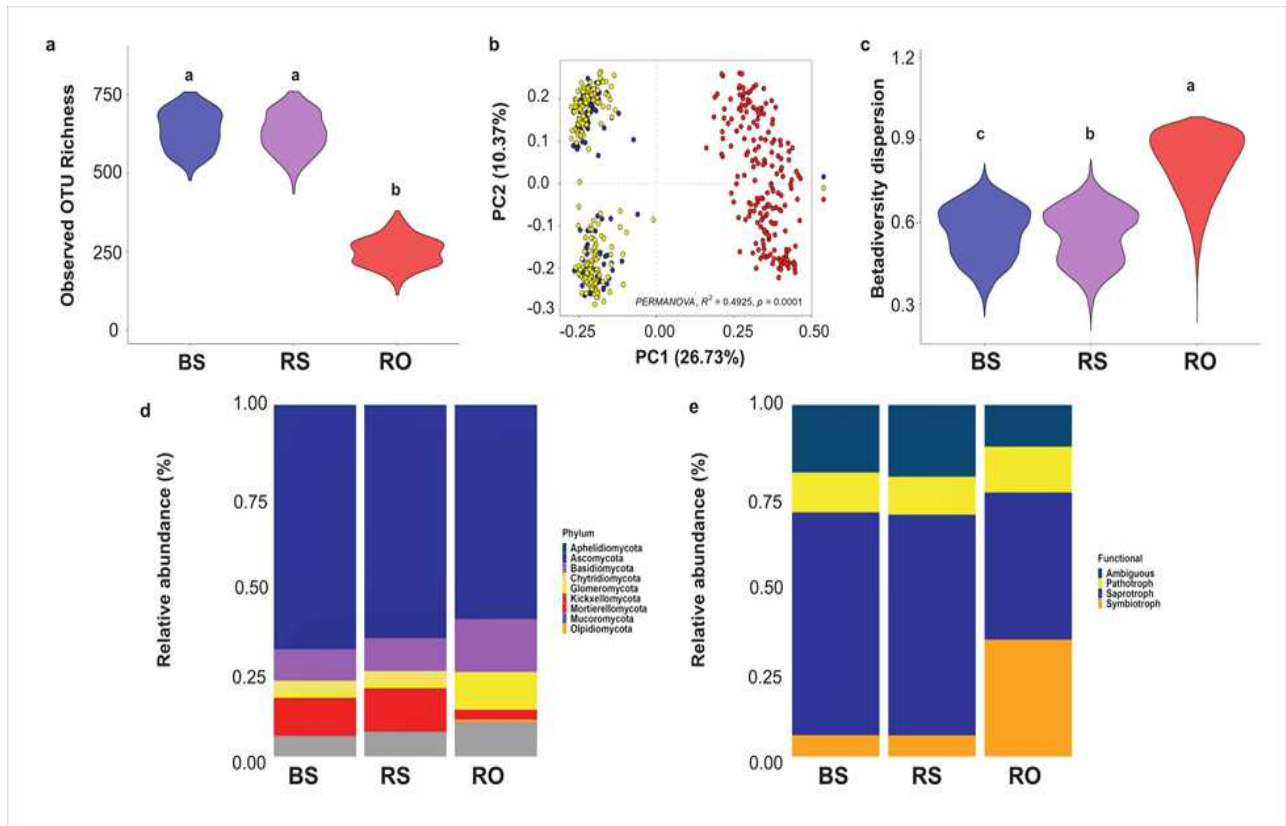


Figure 1: Fungal community diversity and community composition in the different compartments, bulk soil (BS), rhizosphere (RS), and roots (RO). (a) alpha-diversity estimated based on observed OTUs. (b) beta-diversity visualized using principal component analysis (PCA) of the Aitchison distance between fungal communities with PERMANOVA result of the compartment effect. (c) beta-diversity variation within compartments based on Aitchison pairwise distance. Relative abundance profiles of the main fungal (d) phyla and (e) fungal functional guilds. The proportions in the bars represent the average abundance of each taxon or fungal guild across all samples of the respective compartment. a & c: One-way ANOVA was used to assess differences across compartments, and the means were separated using Tukey's post-hoc test, and groups with the same letter denote no significant differences ($P > 0.05$).

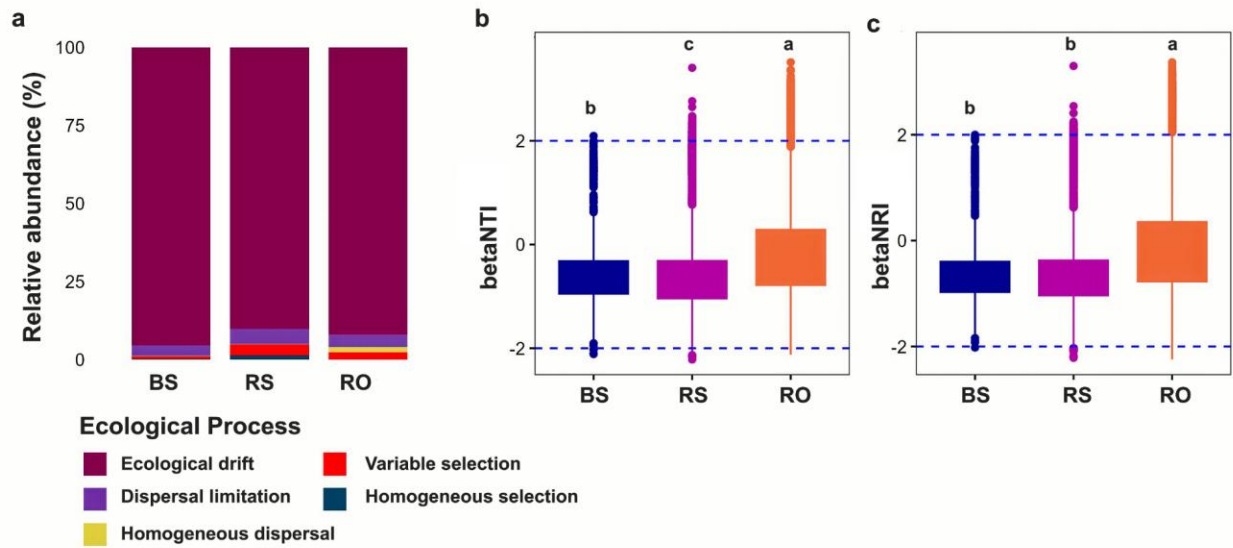


Figure 2: Determinism and stochasticity of fungal community assembly across different compartments: bulk soil (BS), rhizosphere (RS), and roots (RO). (a) The relative contribution of different assembly processes, analysed based on beta nearest taxon index (β NTI) and Bray-Curtis-based Raup-Crick index (RCbray). The ecological processes are categorized as: homogeneous selection (β NTI < -2), heterogeneous (variable) selection (β NTI $> +2$), homogenizing dispersal ($|\beta$ NTI| < 2 and RCbray < -0.95), dispersal limitation ($|\beta$ NTI| < 2 and RCbray $> +0.95$) and ecological drift or drift alone ($|\beta$ NTI| < 2 and $|\text{RCbray}| < 0.95$), respectively.

The positive and negative values of (b) β NTI and (c) β NRI reflect the greater ($>$) and lesser ($<$) than expected turnover of phylogenetic composition, respectively. The dashed horizontal lines indicate the 95% confidence intervals, with values below $+2$ and above -2 considered statistically significant, marking the threshold for neutral assembly processes. The relative proportions of deterministic ($|\beta$ NTI| > 2) and stochastic ($|\beta$ NTI| < 2) processes are indicated by percentage numbers. Statistical significance between compartments is denoted by different letters above the

- 1 boxes. The horizontal line inside each boxplot represents the median, while the upper and lower
- 2 box edges represent the 25th percentile (Q1) and 75th percentile (Q3), respectively, and the
- 3 whiskers extend to the smallest and largest values within 1.5 times the interquartile range.

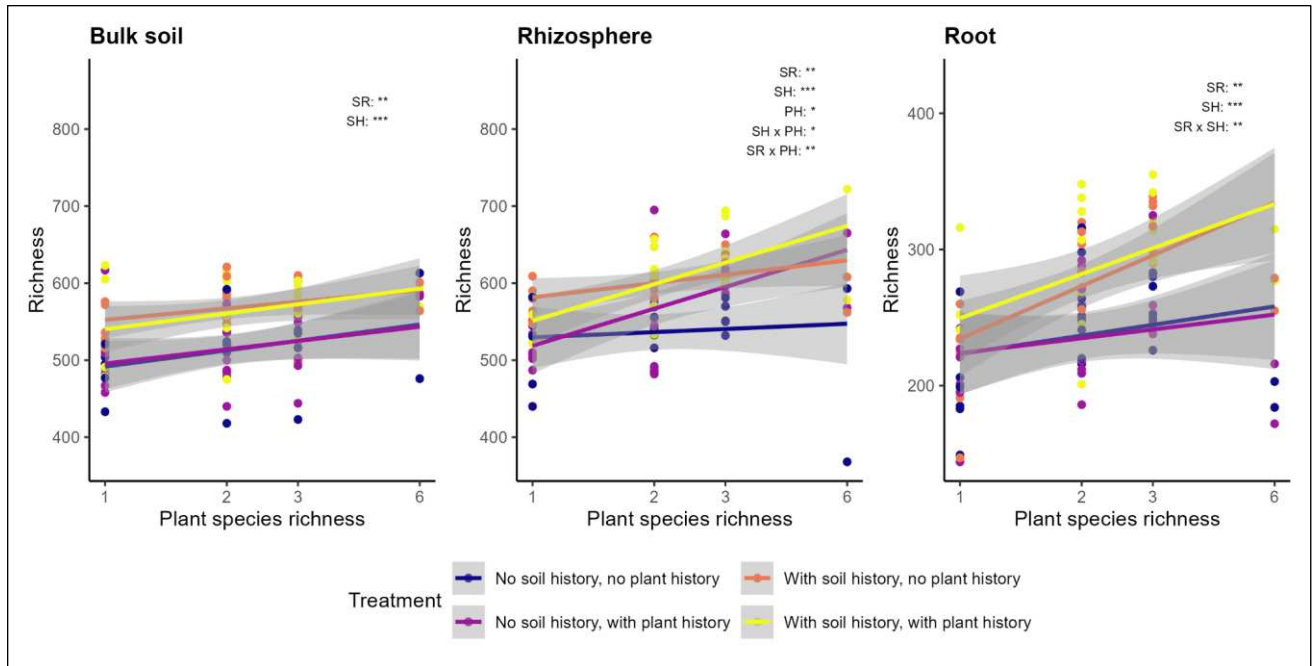


Figure 3: Relationships between plant species richness (log-linear) and fungal diversity in the compartments: bulk soil (BS), rhizosphere (RS), and roots (RO). Fungal observed OTU richness is plotted against log₂-transformed plant species richness (x-axis labels back-transformed for clarity) and linear models with 95% confidence intervals are shown, with colours indicating different treatment levels. Asterisks denote significance levels for fixed effects from linear mixed models: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and n.s. = not significant. Mixed models include random effects for block and unit. Non-significant ($p > 0.05$, for all the variables) two-way (SR:SH, SR:PH, and SH:PH) and three-way (SR:SH:PH) interactions are not shown.

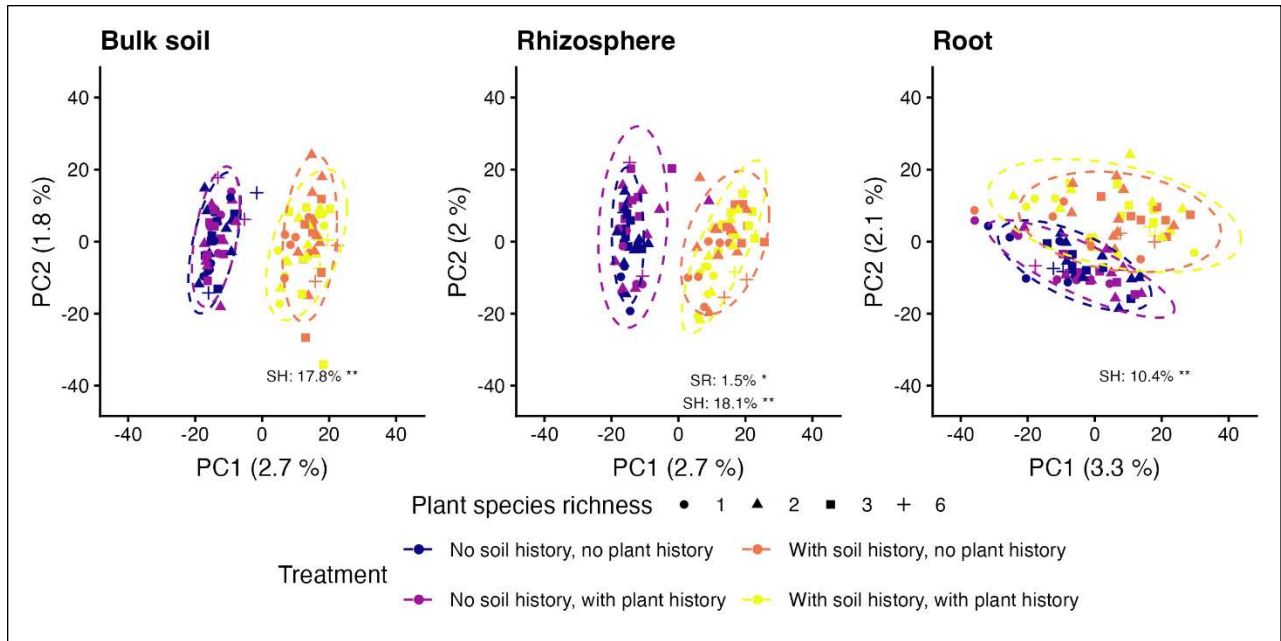


Figure 4: Principal component analysis (PCA) of fungal community composition based on Aitchison distance in (a) bulk soil (BS), (b) rhizosphere (RS), and (c) root (RO) compartments. Points represent fungal communities under different combinations of plant species richness (symbols: 1, 2, 3, and 6 species) and history treatments: soil history (SH; with: wSH, without: noSH), plant history (PH; with: wPH, without: noPH). Ellipses show 95% confidence intervals around treatment group centroids. Percentages on axes indicate the variance explained by the first two principal components. Text annotations indicate the proportion of variation explained (R^2) from PERMANOVA ($n = 999$ permutations), testing the effects of plant species richness (SR), soil history (SH), plant history (PH), and their two-way and (SR:SH, SR:PH, and SH:PH) and three-way (SR:SH:PH) interactions, along with significance levels: $p < 0.05$ (*), $p < 0.01$ (**), n.s. = not significant.

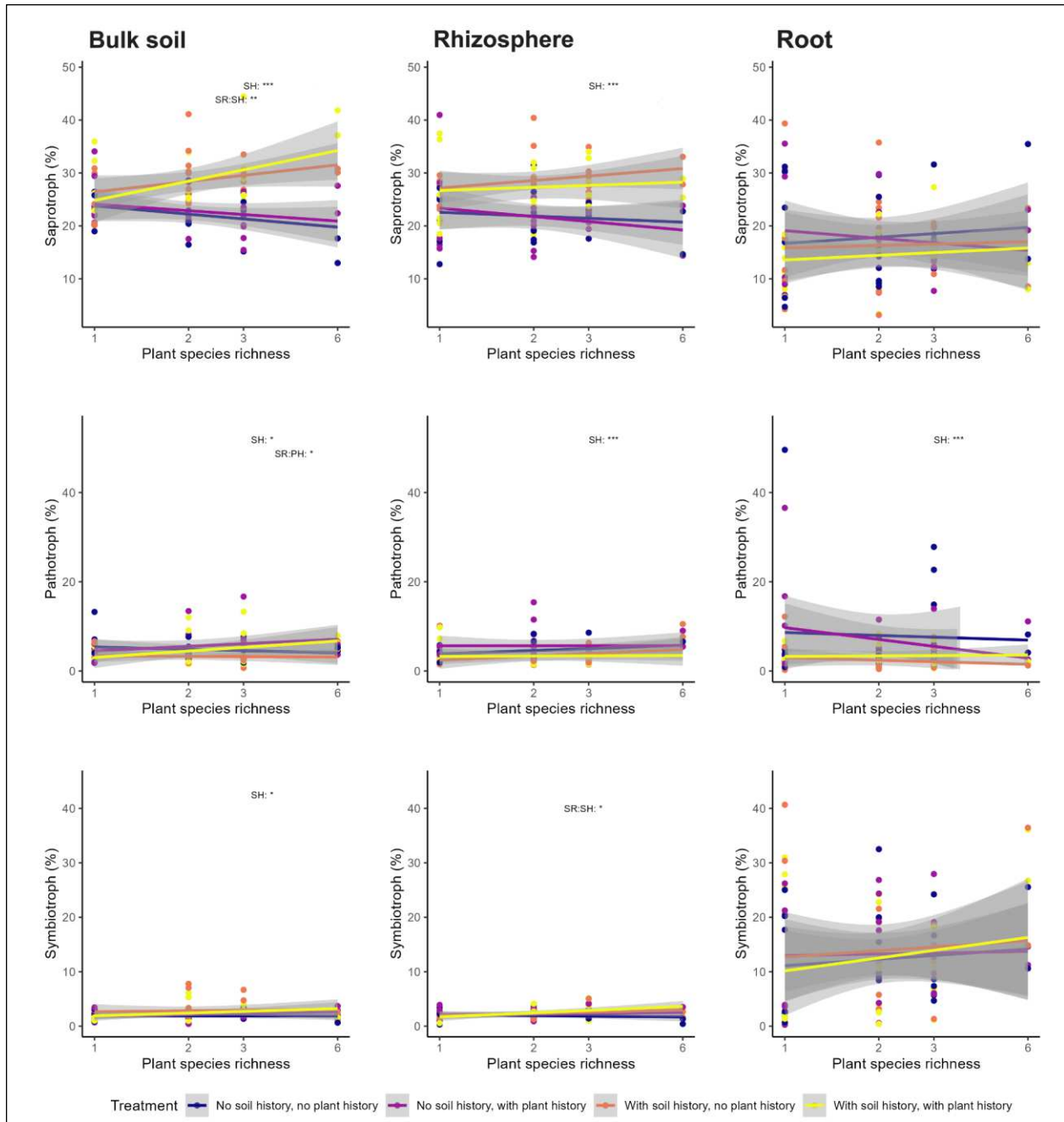


Figure 5: Log-linear relationship between sown plant species richness and relative abundances of fungal ecophysiological guilds. The relative abundance of trophic modes is plotted against log₂-transformed species richness (x-axis labels back-transformed for clarity). Colours indicate treatment levels. Linear models with 95% confidence intervals are shown. Asterisks denote significance levels for fixed effects from linear mixed models: p < 0.05 (*), p < 0.01 (**), p < 0.001

- 1 (***) , and n.s. = not significant. Mixed models include random effects for block and unit. Non-
- 2 significant ($p > 0.05$) effects of individual variables and their two-way (SR:SH, SR:PH and
- 3 SH:PH) and three-way (SR:SH:PH) interactions are not shown.

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