**Supplementary Information**

Plant species richness and the root economics space drive soil fungal communities

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**Supplementary Table S1: Summary of ANOVA results for the effect of plant species richness on the rotated components of the root trait PCA and on the individual fine root traits.** The scores of each plant community (n = 73) along the first and second rotated component (RC) in the root trait PCA were extracted and used as response in the model. Experimental block was included as a random effect to account for spatial effects in the field site. dferror, error degrees of freedom; SE, standard error.

|  |  |
| --- | --- |
|  | **Effect of plant species richness (log)** |
| **Response** | **dferror** | **Coefficient** | **SE** | ***F*** | ***p*** |
| RC1 (Collaboration gradient) | 68.301 | 0.092 | 0.110 | 0.704 | 0.404 |
| RC2 (Conservation gradient) | 68.416 | -0.246 | 0.106 | 5.412 | **0.023** |
|  |  |  |  |  |  |
| Root diameter | 72.000 | 0.004 | 0.004 | 0.925 | 0.339 |
| Specific root length | 69.180 | -11.678 | 8.121 | 2.068 | 0.155 |
| Root nitrogen  | 71.000 | -0.176 | 0.046 | 14.417 | **<0.001** |
| Root tissue density | 69.328 | 0.003 | 0.003 | 0.8616 | 0.357 |

**Supplementary Table S2: Summary of ANOVA with type 1 sum-of-squares results for fungal diversity and relative abundance of guilds.** The scores of each plant community (n = 73) along the first and second rotated axis in the root trait PCA were extracted and used as fixed effect in the model. Experimental block was included as a random effect to account for spatial effects in the field site. AMF, arbuscular mycorrhizal fungi; dferror, error degrees of freedom; SE, standard error.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Response** | **Guild** | **Predictor** | **df** | **dferror** | ***F*** | ***p*** |
| Shannon diversity | Saprotrophs | Plant species richness (log) | 1 | 66.615 | 11.589 | **0.001** |
| Collaboration gradient (‘outsourcing’) | 1 | 68.995 |  0.013 | 0.908 |
| Conservation gradient (‘fast’) | 1 | 68.798 |  7.231 | **0.009** |
| Plant pathogens | Plant species richness (log) | 1 | 66.236 |  0.003 | 0.960 |
| Collaboration gradient (‘outsourcing’) | 1 | 68.542 |  5.248 | **0.025** |
| Conservation gradient (‘fast’) | 1 | 68.767 |  6.824 | **0.011** |
| AMF | Plant species richness (log) | 1 | 66.533 |  2.079 | 0.154 |
| Collaboration gradient (‘outsourcing’) | 1 | 67.964 |  7.861 | **0.007** |
| Conservation gradient (‘fast’) | 1 | 66.026 |  0.708 | 0.403 |
| Relative abundance | Saprotrophs | Plant species richness (log) | 1 | 66.136 |  0.162 | 0.689 |
| Collaboration gradient (‘outsourcing’) | 1 | 67.629 |  5.832 | **0.018** |
| Conservation gradient (‘fast’) | 1 | 67.830 |  2.323 | 0.132 |
| Plant pathogens | Plant species richness (log) | 1 | 69.000 |  0.917 | 0.342 |
| Collaboration gradient (‘outsourcing’) | 1 | 69.000 |  5.996 | **0.017** |
| Conservation gradient (‘fast’) | 1 | 69.000 |  2.001 | 0.162 |
| AMF | Plant species richness (log) | 1 | 66.209 |  0.402 | 0.528 |
| Collaboration gradient (‘outsourcing’) | 1 | 68.333 |  0.981 | 0.326 |
| Conservation gradient (‘fast’) | 1 | 68.572 |  0.000 | 0.995 |



**Supplementary Fig. S1: Rarefaction curves of the ITS2 sequence data.** Each line represents one of the 73 samples, showing increasing number of amplicon sequence variants (ASVs) with the number of reads until saturation is reached.

 ** Supplementary Fig. S2: Unrotated PCA of the community root traits.** Each point represents a plant community (*n* = 73). Points are color-coded for plant species richness of the plot. RC, rotated component.

**Supplementary Methods S1: Nitrogen measurement using near-infrared spectroscopy (NIR)**

Milled root samples were freeze-dried again to measure NIR in the range of 9090–4000cm−1 at 8cm−1 resolution in transmission mode (Multi-Purpose FT-NIR-Analyzer, Bruker Corporation, Billerica, USA). For each sample, five independent measurements were averaged. We converted transmission to absorbance as log10(1/Transmission) and used it in combination with a bootstrapped CARS-PLSR procedure(Richter & Bassi 2023) to predict nitrogen content. These 26 samples were distributed among plots with 1, 2, 4, 8, 16 and 60 species plots with 2, 1, 3, 9, 8 and 3 samples, respectively.

**Supplementary Methods S2: Amplicon sequencing and description of ITS2 dataset**

After PCR, three positive PCR products were pooled, purified with AMPure XP beads (Beckman Coulter), and indexed with the Nextera XT Illumina Index Kit (Illumina Inc., San Diego, USA). The samples were then pooled to equal molarity and the paired-end sequencing of 2 × 300 bp was performed using a MiSeq Reagent Kit v3 on an Illumina MiSeq System at the Department of Soil Ecology of the Helmholtz-Centre for Environmental Research (UFZ, Halle/Saale, Germany). To overcome the amplification bias against AMF with ITS primers (Tedersoo *et al.* 2015), we used the sequence data from Albracht *et al.* (2024). In short, the SSU of AMF was amplified using a nested PCR with primer pair Glomer 1536 / WT0 for the first PCR and NS31 / AML2 for the second PCR according to Wahdan *et al.* (2021) and the sequencing was performed as for the ITS2 sequencing. For a detailed description of the sequencing of AMF communities we refer to Albracht *et al.*(2024)*.*

To summarize, *cutadapt*(Martin 2011) was used to trim the primer sequences from raw reads. Quality trimming was performed with a minimum length of 70 bp for ITS and 260 bp (fwd) / 210 bp (rvs) for AMF, truncation of reads at positions with a PHRED score below 15 for ITS, and exclusion of reads with an expected error higher than 2. The identification of exact sequence variants (Amplicon Sequence Variants, ASVs) included merging read pairs with a minimum overlap of 15 bp (ITS) and 12 bp (AMF) and a maximum of three (ITS) and zero (AMF) mismatches. Chimeras were filtered using DADA2's 'consensus' algorithm. Taxonomic classification was conducted using the *mother* (Schloss *et al.* 2009) implementation of the Bayesian classifier against the UNITE v8.2 database (Nilsson *et al.* 2019) (ITS) and SILVA v138 SSUref database (Quast *et al.* 2013) for AMF. Non-fungal ASVs (for ITS sequences) and non-Glomeromycotinian ASVs (for AMF sequences) were discarded. For ITS data, ASVs were then assigned to putative fungal guilds based on their taxonomic annotation and the FungalTraits database (Põlme *et al.* 2021). Further, all Glomeromycotinian ASVs in the ITS data were assigned to be arbuscular mycorrhizal. For the AMF data, ASVs were blasted against the MaarjAM database (Öpik *et al.* 2010) and assigned to virtual taxa (VTX). ASVs without VTX assignment were extracted, singletons removed, and used for the construction of a maximum likelihood phylogenetic tree based on a general time-reversible, discrete gamma (GTR+G) model using raxML (Stamatakis 2014) and FasttreeMP (Price *et al.* 2010). Consequently, these ASVs were associated with custom virtual taxa (VTC) characterized by cophenetic distances below 0.03.

Our filtered ITS2 sequence dataset of 73 samples consisted of 3,251,726 fungal sequence reads, with a mean of 44,544 reads per sample (min. 26,494; max. 61,786 reads per sample). These were grouped into 5,276 ASVs, of which 4,472 were taxonomically assigned to the phylum level. Of these, Ascomycota (73.80%) had the most sequence reads, followed by Basidiomycota (12.07%), Mortierellomycota (11.43%) and Chytridiomycota (2.12%). A total of 2,309 ASVs (43.76%, corresponding to 64.09% of reads) could be assigned to at least one guild in the FungalTraits database (Põlme *et al.* 2021). Of these, saprotrophs (which include soil saprotrophs, litter saprotrophs, wood saprotrophs, and unspecified saprotrophs) made up 58.34%, plant pathogens 25.82%, and arbuscular mycorrhiza 0.30% of reads.

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