

New Phytologist Supporting Information

Article title: **Rhizosphere fungal guild responses to the root economics space in young and old grassland monocultures**

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Tab. S5 Results of additional partial distance-based redundancy analyses (db-RDA) of the fungal communities when accounting for the annotation rate of sequence reads.

Fig. S1 Rarefaction curves showing the accumulation of ASVs with increasing number of sequence reads. Each of the 50 samples is represented by one line.

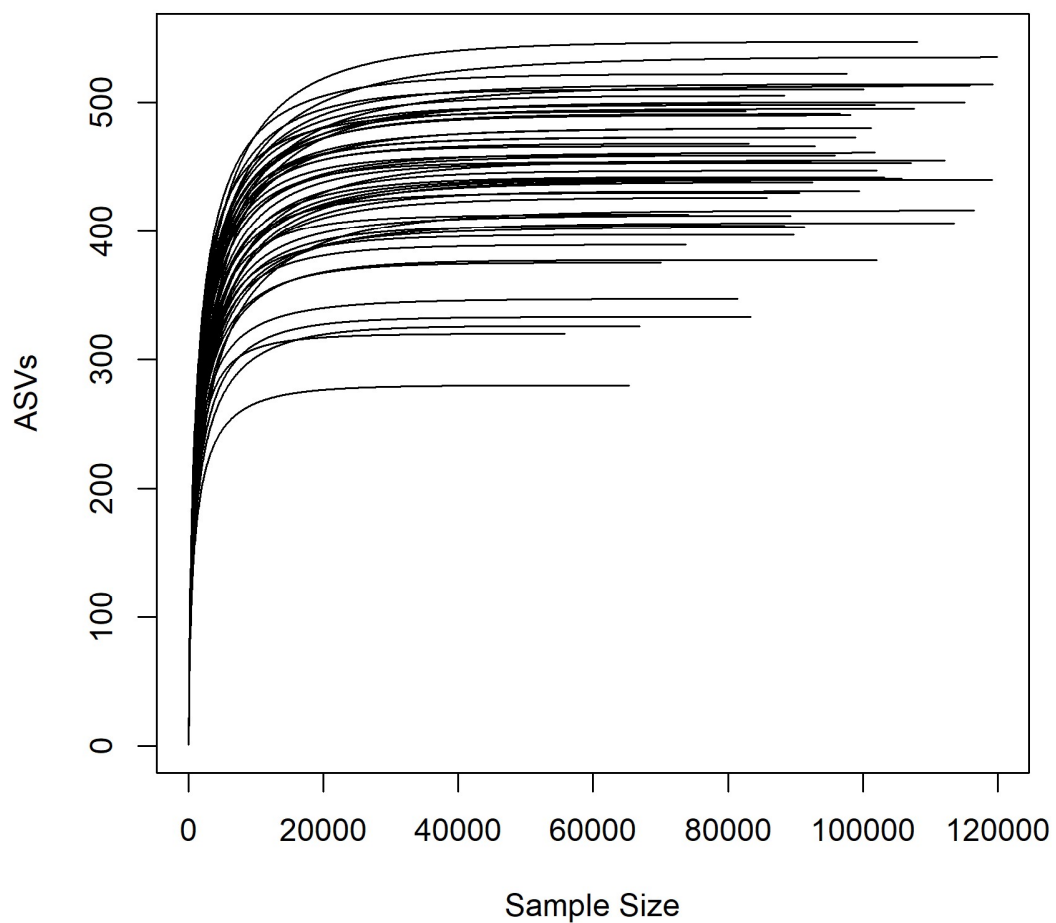


Fig. S2 Principal coordinate analysis (PCoA) ordination plot of fungal communities in old and young plots based on Bray-Curtis dissimilarities.

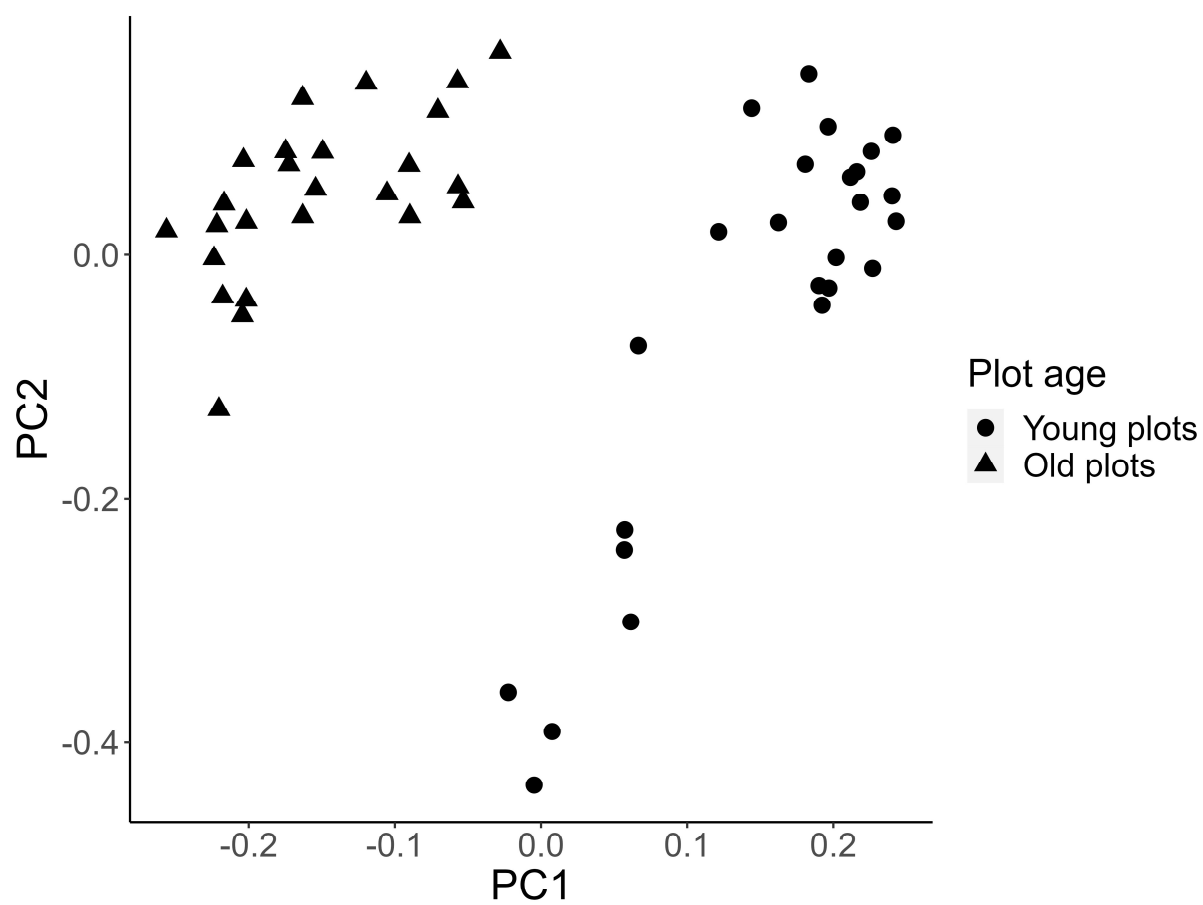


Fig. S3 Principal coordinate analysis (PCoA) ordination plots of fungal communities in old and young plots based on Bray-Curtis dissimilarities. Each point represents the fungal community of one sample, color coded for the score of the varimax-rotated PCA of root traits, indicating the plants position along the collaboration and conservation axis. Higher values along the collaboration axis indicate an ‘outsourcing’ root strategy, higher values along the conservation axis indicate a ‘fast’ root strategy.

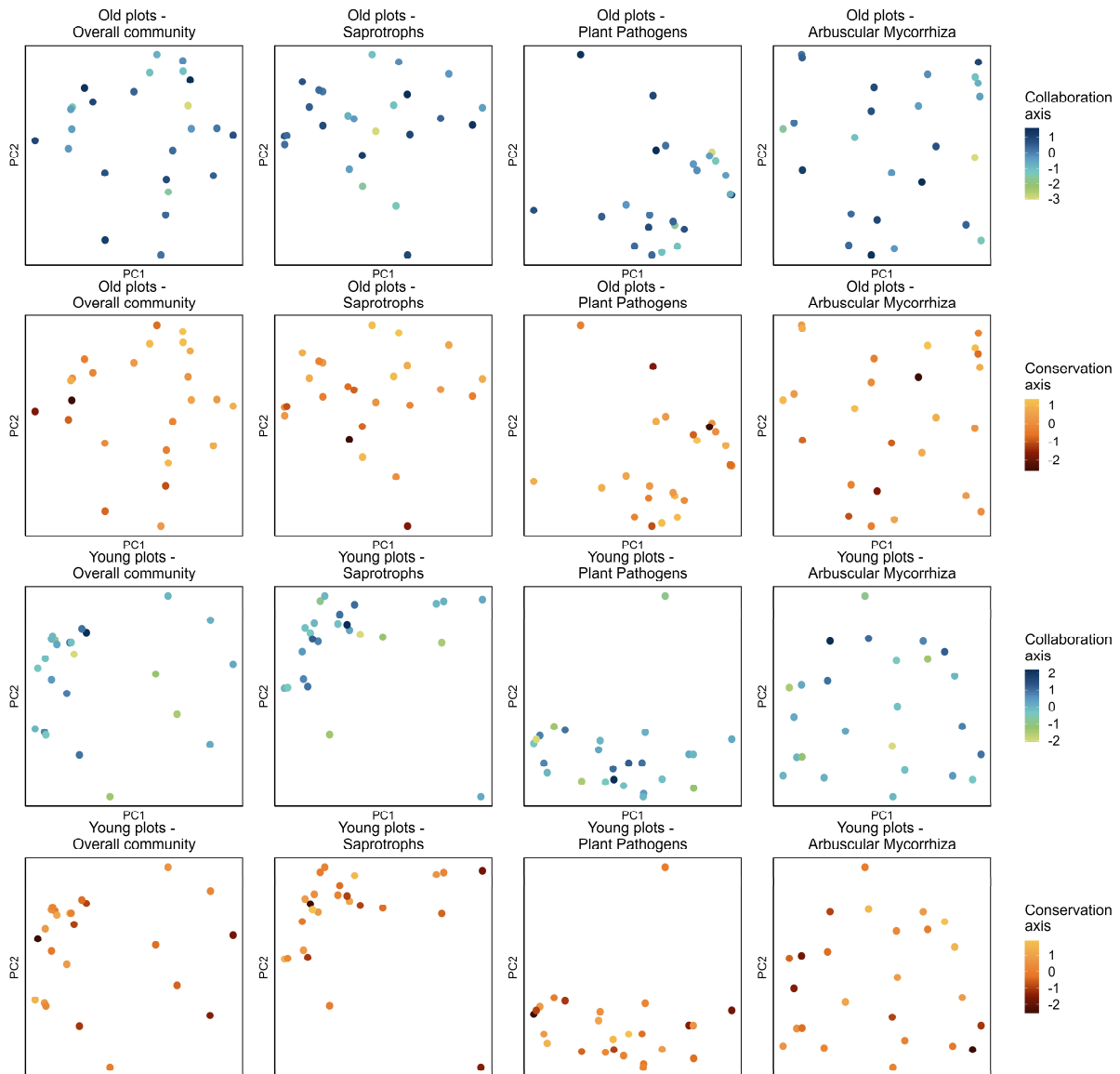


Fig. S4 Correlation between mycorrhizal colonization rate, as determined by staining and microscopic image analysis, and the two axes of the varimax-rotated PCA of root traits. R , Pearson correlation coefficient. The grey areas depict the 95% confidence interval.

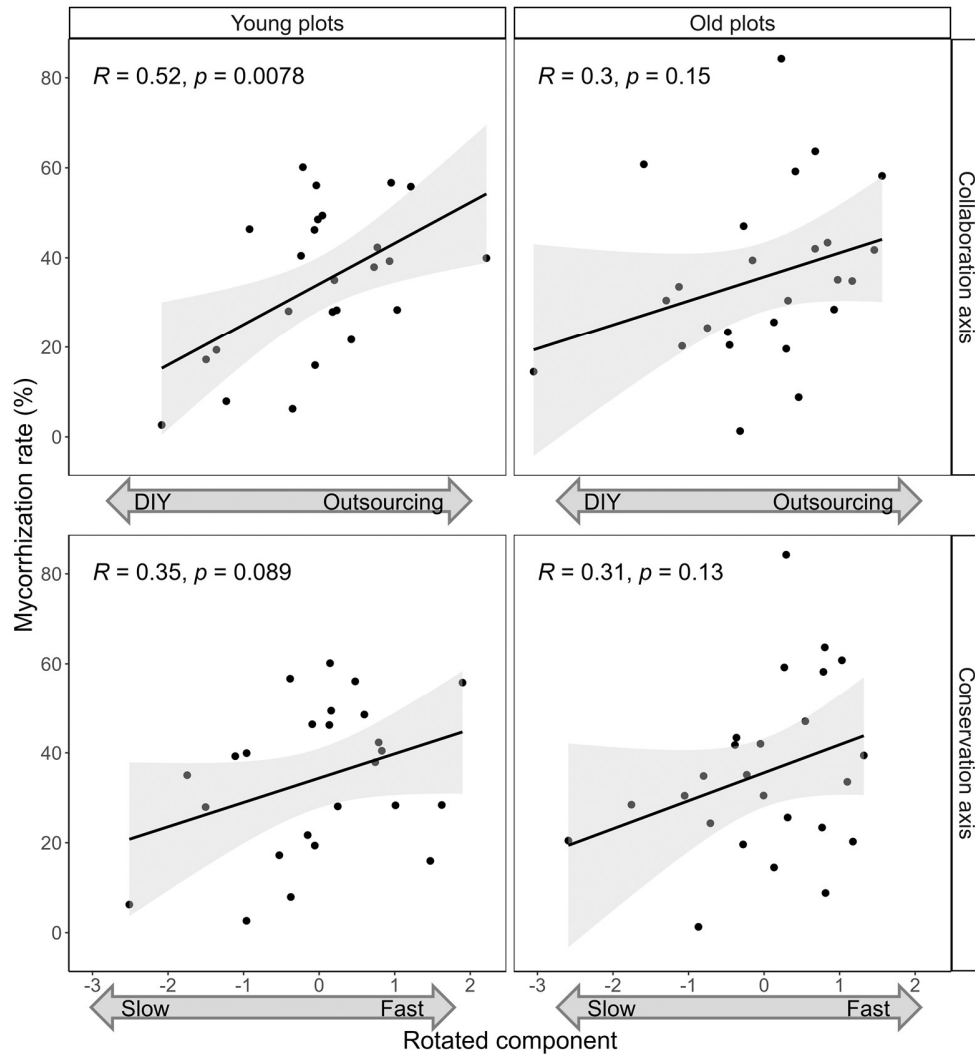


Fig. S5 Differences between root traits of the 25 plant species in the young (4-year-old) and old (18-year-old) monocultures. SRL, specific root length; RDMC, root dry matter content. Lower and upper hinges correspond to the first and third quartiles; bold horizontal line represents the median. Whiskers extent to the lowest and largest value, no further than 1.5* inter-quartile range.

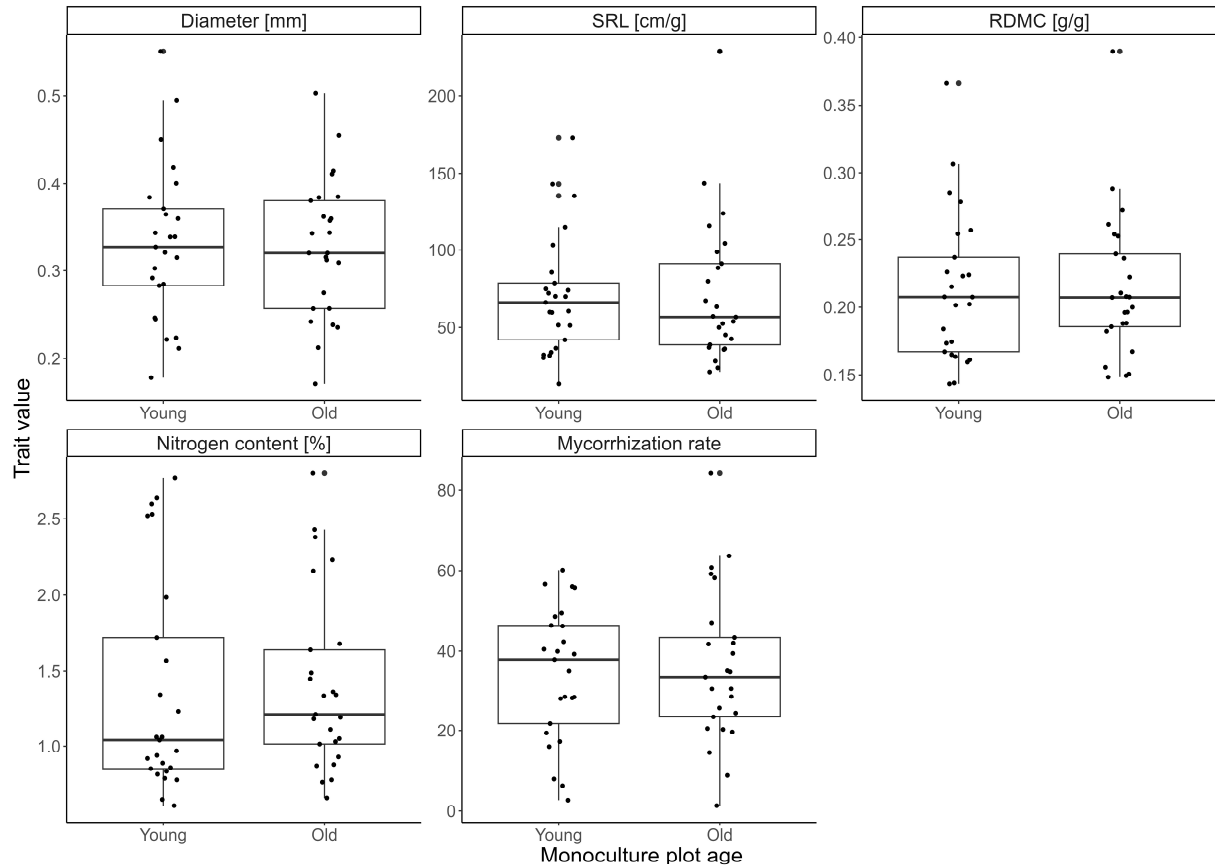


Fig. S6 Relationships between the collaboration axis and the conservation axis of the root economics space and the Shannon diversity of the overall fungal community, saprotrophs, arbuscular mycorrhizal fungi, and plant pathogens. Values for the collaboration and conservation axis are varimax-rotated scores from the principal component analysis of the root traits. The lines show model predictions from linear mixed effect models. Note the different scales on the axes. Dashed lines show non-significant relationships. The blue and green areas depict the 95% confidence interval. For detailed model results, see Supporting Information Table S2.

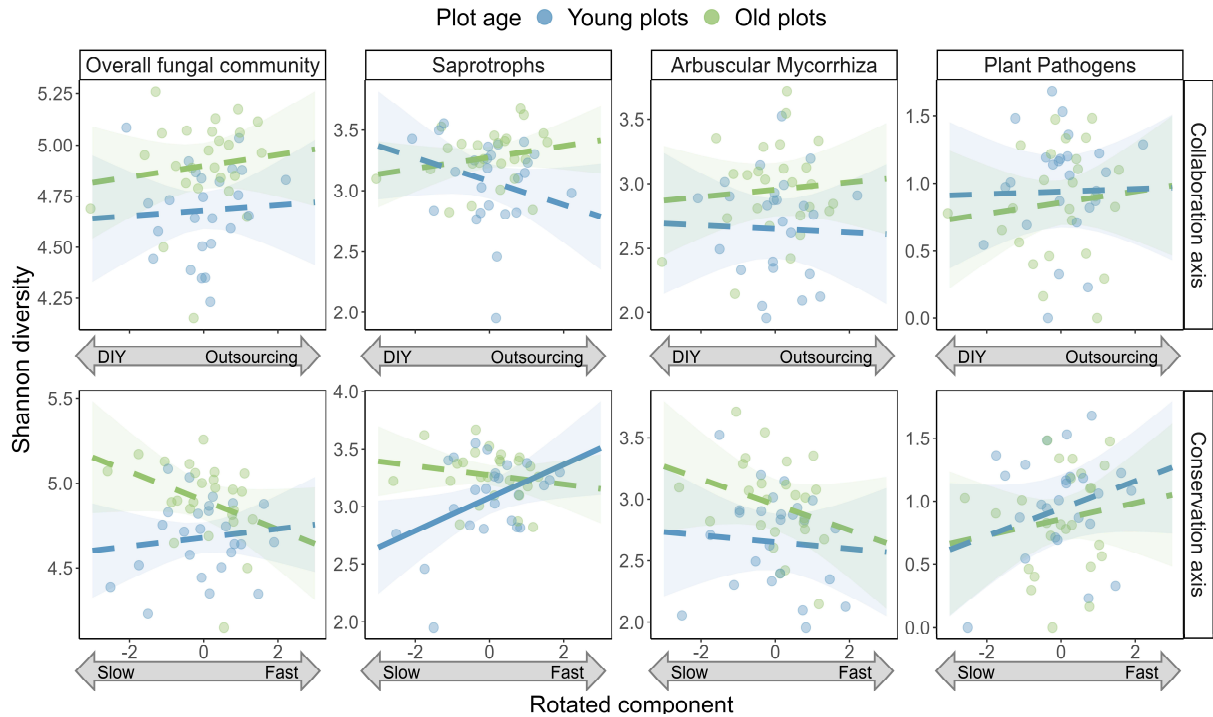


Fig. S7 Inverse Simpson, observed number of ASVs and Shannon diversity of the fungal community in old and young monocultures after rarefaction. Lower and upper hinges correspond to the first and third quartiles; bold horizontal line represents the median. Whiskers extent to the lowest and largest value, no further than 1.5* inter-quartile range.

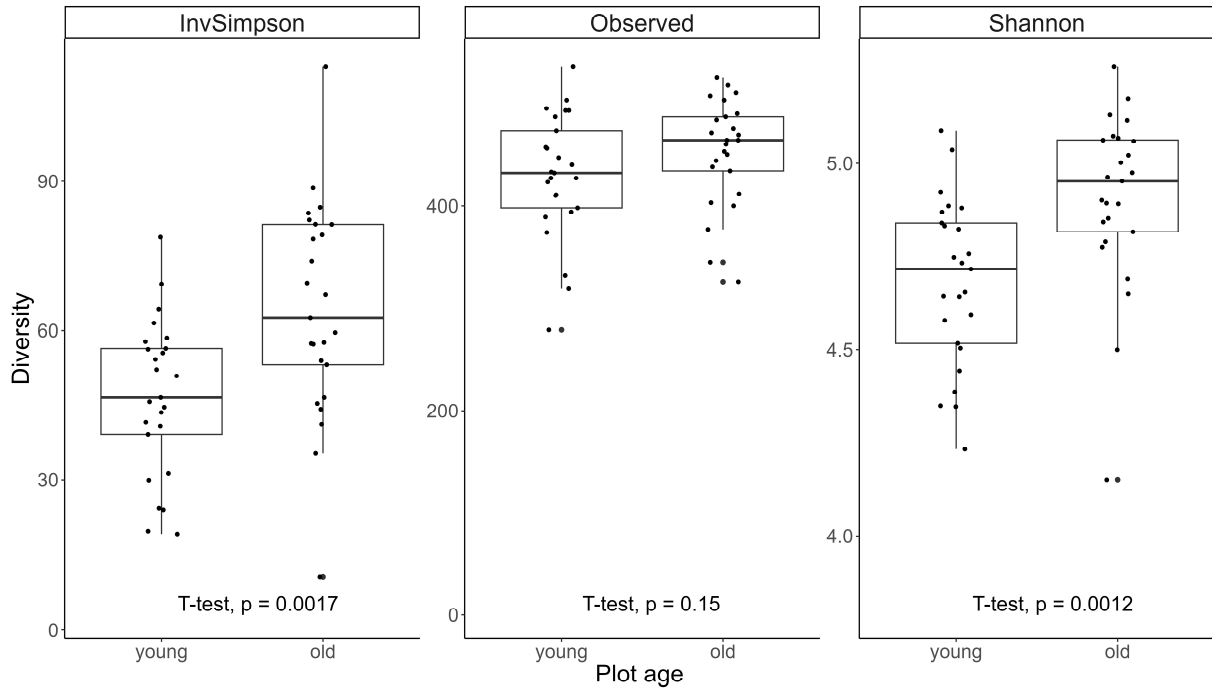


Fig. S8 Relationship between the collaboration axis and the conservation axis of the Root Economics Space and the relative abundance of saprotrophs, arbuscular mycorrhiza and plant pathogens. Values for the collaboration and conservation axis are varimax-rotated scores from the principal component analysis of the root traits. The lines show model predictions from linear mixed effect models. Note the different scales on the axes. Dashed lines show non-significant relationships. The blue and green areas depict the 95% confidence interval. For detailed model results, see Supporting Information, Table S2.

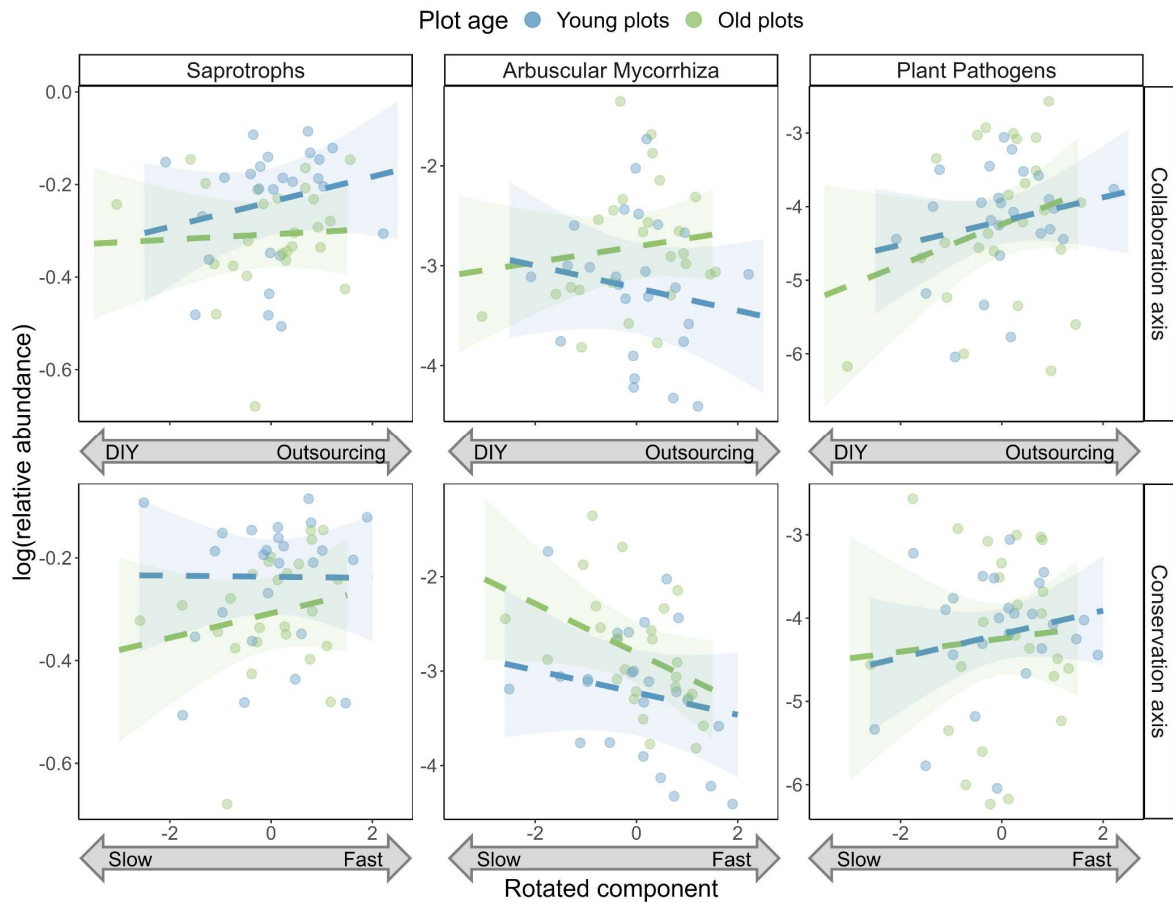


Fig. S9 Correlation of Basidiomycota : Ascomycota ratio and the two axes of the root trait PCA. R , Pearson correlation coefficient. The grey areas depict the 95% confidence interval.

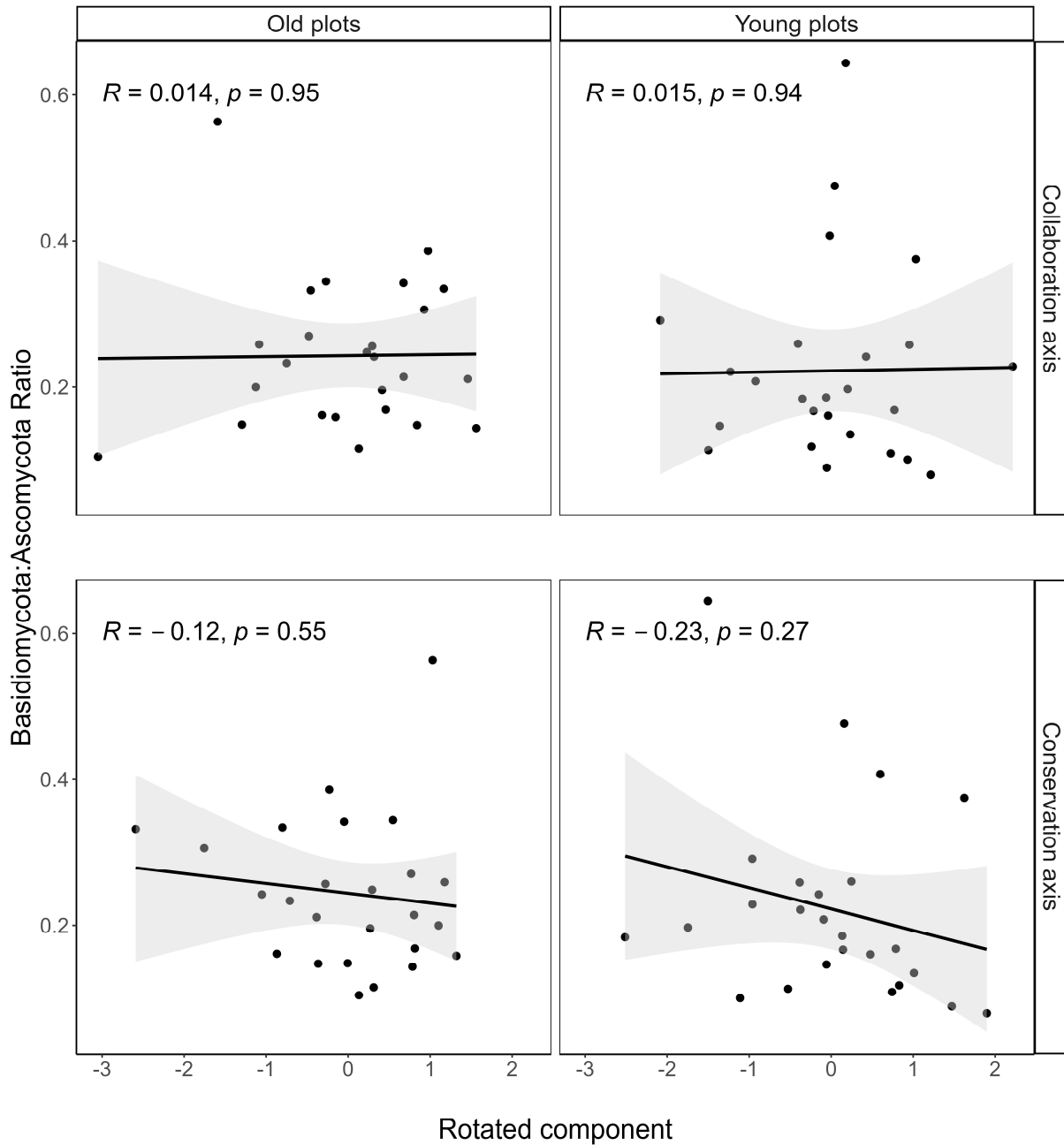


Table S1 List of plant species of which roots and soil were sampled.

Species	Functional	
	Group	Family
<i>Alopecurus pratensis</i> L.	Grass	Poaceae
<i>Arrhenatherum elatius</i> L.	Grass	Poaceae
<i>Bellis perennis</i> L.	Herb	Asteraceae
<i>Centaurea jacea</i> L.	Herb	Asteraceae
<i>Crepis biennis</i> L.	Herb	Asteraceae
<i>Daucus carota</i> L.	Herb	Apiaceae
<i>Festuca rubra</i> L.	Grass	Poaceae
<i>Galium mollugo</i> L.	Herb	Rubiaceae
<i>Glechoma hederacea</i> L.	Herb	Lamiaceae
<i>Helictotrichon pubescens</i> Huds.	Grass	Poaceae
<i>Holcus lanatus</i> L.	Grass	Poaceae
<i>Leontodon hispidus</i> L.	Herb	Asteraceae
<i>Leucanthemum vulgare</i> Lam.	Herb	Asteraceae
<i>Medicago lupulina</i> L.	Legume	Fabaceae
<i>Medicago x varia</i> Martyn	Legume	Fabaceae
<i>Onobrychis viciifolia</i> Scop.	Legume	Fabaceae
<i>Plantago lanceolata</i> L.	Herb	Plantaginaceae
<i>Plantago media</i> L.	Herb	Plantaginaceae
<i>Ranunculus repens</i> L.	Herb	Ranunculaceae
<i>Rumex acetosa</i> L.	Herb	Polygonaceae
<i>Sanguisorba officinalis</i> L.	Herb	Rosaceae
<i>Taraxacum officinale</i> L.	Herb	Asteraceae
<i>Trifolium fragiferum</i> L.	Legume	Fabaceae
<i>Trifolium pratense</i> L.	Legume	Fabaceae
<i>Veronica chamaedrys</i> L.	Herb	Plantaginaceae

Table S2 Mean root traits per plant functional group. SRL, specific root length; RDMC, root dry matter content.

Plant functional group	Mean diameter (mm)	Mean SRL (cm/g)	Mean RDMC (g/g)	Mean relative nitrogen (%)
Grasses	0.25	113.12	0.28	0.89
Herbs	0.34	66.07	0.20	1.17
Legumes	0.39	43.57	0.21	2.51

Tab. 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.

	DF	Old monocultures			Young monocultures		
		Estimate	SE _{Estimate}	P-value	Estimate	SE _{Estimate}	P-value
Fungal richness (Shannon Diversity)							
Overall community							
Collaboration gradient (Outsourcing)	1	0.027	0.043	0.533	0.013	0.05	0.794
Conservation gradient (Fast)	1	-0.085	0.053	0.13	0.026	0.045	0.574
Saprotroph							
Collaboration gradient (Outsourcing)	1	0.046	0.044	0.301	-0.097	0.07	0.184
Conservation gradient (Fast)	1	-0.04	0.049	0.423	0.144	0.065	0.037
AMF							
Collaboration gradient (Outsourcing)	1	0.028	0.064	0.664	-0.014	0.084	0.873
Conservation gradient (Fast)	1	-0.104	0.084	0.229	-0.027	0.08	0.735
Pathogen							
Collaboration gradient (Outsourcing)	1	0.042	0.083	0.619	0.009	0.08	0.907
Conservation gradient (Fast)	1	0.064	0.093	0.494	0.109	0.077	0.173
Relative guild abundance (log)							
Saprotroph							
Collaboration gradient (Outsourcing)	1	0.006	0.022	0.795	0.027	0.027	0.324
Conservation gradient (Fast)	1	0.024	0.028	0.412	-0.001	0.025	0.966

AMF

Collaboration gradient (Outsourcing)	1	0.079	0.104	0.458	-0.113	0.128	0.389
Conservation gradient (Fast)	1	-0.260	0.135	0.070	-0.118	0.124	0.353

Pathogen

Collaboration gradient (Outsourcing)	1	0.276	0.212	0.206	0.161	0.160	0.326
Conservation gradient (Fast)	1	0.079	0.237	0.743	0.141	0.145	0.342

ASVs without guild annotation or with multiple annotations were not included. Relative abundances of the guilds were log transformed to meet the criteria of normal distribution of residuals. Experimental blocks were included as random factors. DF, degrees of freedom. SE, Standard Error.

Tab. S4 Results of linear mixed effects model 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 with altered random effect structure compared to the main analyses.

	DF	Old monocultures			Young monocultures		
		Estimate	SE _{Estimate}	P-value	Estimate	SE _{Estimate}	P-value
Fungal richness (Shannon Diversity)							
Overall community							
Collaboration gradient (Outsourcing)	1	0.027	0.043	0.533	0.002	0.051	0.967
Conservation gradient (Fast)	1	-0.085	0.053	0.130	0.035	0.044	0.434
Saprotroph							
Collaboration gradient (Outsourcing)	1	0.046	0.044	0.301	-0.072	0.071	0.322
Conservation gradient (Fast)	1	-0.04	0.049	0.423	0.161	0.063	0.019
AMF							
Collaboration gradient (Outsourcing)	1	0.028	0.064	0.664	-0.014	0.084	0.873
Conservation gradient (Fast)	1	-0.104	0.084	0.229	-0.027	0.080	0.735
Pathogen							
Collaboration gradient (Outsourcing)	1	0.042	0.083	0.619	0.009	0.080	0.907
Conservation gradient (Fast)	1	0.064	0.093	0.494	0.109	0.077	0.173
Relative guild abundance (log)							
Saprotroph							
Collaboration gradient (Outsourcing)	1	0.006	0.022	0.795	0.027	0.024	0.269
Conservation gradient (Fast)	1	0.024	0.028	0.412	-0.004	0.022	0.874

AMF

Collaboration gradient (Outsourcing)	1	0.079	0.104	0.458	-0.113	0.128	0.389
Conservation gradient (Fast)	1	-0.260	0.135	0.070	-0.118	0.124	0.353

Pathogen

Collaboration gradient (Outsourcing)	1	0.328	0.210	0.132	0.196	0.159	0.232
Conservation gradient (Fast)	1	0.114	0.232	0.627	0.143	0.143	0.327

ASVs without guild annotation or with multiple annotations were not included. Relative abundances of the guilds were log transformed to meet the criteria of normal distribution of residuals. In addition to experimental blocks, the annotation rate was included as random factor. For the annotation rate, the number of sequence reads with single guild annotation (as described in the methods of the main text) per sample was divided by the total number of sequence reads per sample and this proportion was split into five quartiles that were used as random effects of the models. In case of singular fit, linear regressions were used instead of linear mixed models. DF, degrees of freedom. SE, Standard Error.

Tab. S5 Results of the partial distance-based redundancy analysis (db-RDA) of the overall fungal community and individual subsets of saprotrophs, AMF and plant pathogens constrained by the scores of the collaboration axis and the conservation axis of the varimax-rotated PCA of root traits with altered covariate structure compared to the main analyses.

Predictor	DF	Old monocultures			Young monocultures		
		R^2	F -value	P -value	R^2	F -value	P -value
Overall community							
Collaboration gradient	1	0.045	0.805	0.861	0.061	1.107	0.297
Conservation gradient	1	0.073	1.306	0.062	0.051	0.921	0.607
Saprotrophic community							
Collaboration gradient	1	0.046	0.825	0.774	0.055	0.986	0.462
Conservation gradient	1	0.079	1.425	0.049	0.05	0.891	0.596
AMF community							
Collaboration gradient	1	0.045	0.812	0.823	0.067	1.209	0.166
Conservation gradient	1	0.069	1.243	0.117	0.052	0.942	0.591
Pathogen community							
Collaboration gradient	1	0.123	2.211	0.025	0.05	0.898	0.523
Conservation gradient	1	0.055	0.983	0.446	0.051	0.918	0.503

Analyses within a guild only include ASVs that are assigned to a single guild. In addition to the experimental block, we controlled for the annotation rate. For this, the number of sequence reads with single guild annotation (as described in the methods of the main text) per sample was divided by the total number of sequence reads per sample and this proportion was split into 5 quantiles that were used as conditioning effects of the db-RDA. DF, degrees of freedom. Statistically significant values are indicated in bold text.