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

2

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40 # Abstract

41 Trait-based approaches have been increasingly used to relate plants to soil microbial
42 communities. However, the plant organs mediating this plant-microbe interaction – the roots –
43 have been largely overlooked. The recent discovery of the root economics space offers a
44 predictive framework for the structure of soil microbial communities, and specifically soil-
45 borne fungal communities. Applying this novel approach, our study in a grassland plant
46 diversity experiment reveals distinct root trait strategies at the level of the plant community. In
47 addition to significant effects of plant species richness, we show that both axes of the root
48 economics space – the collaboration and conservation gradient – are strong drivers of the
49 composition of the different guilds of soil fungi, including saprotrophic, plant pathogenic, and
50 mycorrhizal fungi. Our results illustrate that the root economics space and plant species richness
51 jointly determine the effects of plants on fungal communities and their potential role in plant
52 health and ecosystem functioning.

53

54 **Keywords:** arbuscular mycorrhizal fungi, collaboration gradient, pathogenic fungi, plant-fungi
55 interactions, root traits, root economics space, saprotrophic fungi, trait-based

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57 # Introduction

58 Soil fungi can act as mutualists or antagonists of plants and thus promote or weaken the
59 functioning of plants and ecosystems[1–3]. Understanding the drivers of the guild composition of
60 fungal communities is important for the understanding of ecological processes that shape plant
61 communities and crucial for the management of soil microbial communities[4–6]. Plant
62 communities can exert strong selective pressure on soil fungal communities[7,8]. Both plant
63 species richness[8] and composition[9,10] and, therefore, the associated plant traits are important
64 components of belowground plant-fungal relationships. In the last decades, trait-based
65 approaches have emerged to give valuable mechanistic insights into plants as drivers of the soil
66 microbial community[11,12] but these largely lack a belowground trait perspective.

67 Root traits are not just analogs of leaf traits[13]; their variation along two orthogonal axes has
68 been described in the so-called root economics space (RES)[11]. While roots, like leaves, vary in
69 root tissue density and relative root nitrogen content along the 'fast–slow' axis of the
70 conservation gradient, there is an additional trade-off in specific root length and mean root
71 diameter[11]. This trade-off has been explained by the interaction between roots and their
72 associated mutualistic arbuscular mycorrhizal fungi (AMF), with thicker roots generally
73 hosting more AMF ('outsourcing' strategy) and thinner roots maximizing root surface for
74 independent nutrient uptake ('do-it-yourself', 'DIY' strategy)[11,14]. Recent studies have largely
75 confirmed this global trait coordination of species across regions and vegetation types[15–17].
76 However, it is unclear to what extent the species-level RES is also represented at the plant
77 community-level[18,19]. Therefore, understanding how root functional strategies scale from the
78 species-level to the community-level is critical to utilizing the RES as a trait-based framework
79 for soil microbial communities and ecosystem functioning.

80 Multiple components of the fungal community can be affected by abiotic and biotic factors.
81 Based on a functional classification, soil fungal communities are composed of three main

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82 guilds: saprotrophs, pathogenic, and mycorrhizal fungi[20]. While the overall fungal abundance
83 (or biomass) can change e.g. through increased resource availability[21], changes in the functional
84 or taxonomic composition of the community likely result from interspecific processes such as
85 competition or differences in resource utilization among taxa or guilds[22]. Approaches
86 integrating quantitative and qualitative measures of fungal communities are needed for a
87 holistic view of the effects of plants on fungal communities, especially as both components are
88 relevant to ecosystem processes[23]. Generally, soil fungal communities are closely linked to the
89 plant community, as they are dependent on carbon input from the plant but also drive the
90 nutrient cycling and availability to the plant[24]. However, frameworks that explicitly link plant
91 traits to the functional composition of soil fungal communities are limited in number and
92 explanatory power[25,26], and studies that empirically tested trait-based frameworks have largely
93 omitted root traits[27]. The additional complexity of trait variation in roots compared to leaves is
94 not yet integrated into such mechanistic frameworks despite its potential opportunities to
95 reconcile trait-based approaches[28] and better understand plant communities as drivers of soil
96 fungal communities.

97 If the RES exists at the community-level, it can be used to extend the initial framework of
98 Wardle *et al.*[12] linking the 'fast–slow' plant trait gradient to the microbial community. Recently,
99 Hennecke *et al.*[17] presented a theoretical framework of how root trait gradients link with the
100 composition of fungal communities in the rhizosphere. Plant communities with dominating
101 traits on the 'outsourcing' end of the collaboration gradient should accommodate a higher
102 diversity and relative abundance of AMF and, due to their protective role[29], less plant
103 pathogenic fungal diversity and relative abundance[30,31]. Further, plant pathogenic fungi should
104 benefit more from higher nutrient availability and lower defense of plant tissue, both of which
105 align with a 'fast' strategy of the growth-defense trade-off[32] along the conservation gradient.
106 Saprotrophic fungi strongly depend on the quality and quantity of available plant litter[12,33].

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107 Roots at the slow end of the conservation gradient produce low-quality litter, yet it is unclear
108 whether this affects saprotroph diversity and abundance[17].

109 In addition to plant functional traits, plant species richness can also cause differences in soil
110 fungal composition[34,35]. Higher richness of primary producers can influence the composition
111 and diversity of soil microbes via increased heterogeneity of resources, including roots,
112 exudates, and litter[36–38]. Additionally, at similar soil fertility, plant species richness is often
113 correlated with primary productivity[39], thereby increasing the amount of plant-based resources
114 for fungi and hence fungal biomass[8,38]. Multiple studies found fungal diversity to increase with
115 plant species richness[40–42], but opposing or no effects were also reported[43,44], indicating that the
116 plant species richness-fungal diversity relationship can depend on environmental conditions[43],
117 scale[45], or differ between fungal guilds. Plant pathogenic fungi are predicted to be less abundant
118 due to decreased host density with increased plant species richness[46]. We, therefore, expect that
119 fungal guild composition differs across the plant species richness gradient, with a stronger
120 increase in the abundance and diversity of saprotrophic and arbuscular mycorrhizal fungi
121 compared to plant pathogens.

122 In a grassland biodiversity experiment, we aimed to disentangle the effects of root traits and
123 plant species richness on saprotrophic, plant pathogenic, and arbuscular mycorrhizal fungi as
124 the most relevant fungal guilds in grassland soils. We test three overarching hypotheses: (1)
125 Root trait organization at the plant community-level mirrors the RES (i.e. the collaboration and
126 conservation gradients) previously found at the species-level. (2) The diversity and relative
127 abundance of soil fungal guilds are structured by the community RES (3) Plant species richness
128 is linked to increased plant biomass and thereby increases fungal diversity and biomass, but not
129 all fungal guilds benefit equally: we expect fungal mutualists and saprotrophs to benefit more
130 from plant species richness than plant pathogens.

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131 Overall, we aim to advance the potential of trait-based frameworks by integrating root
132 functional strategies. As a first major step, we show that the root trait gradients at the
133 community-level are strong determinants of the diversity and relative abundance of soil fungal
134 guilds. While both, root traits and plant species richness, are correlated with root biomass, soil
135 fungal biomass is only driven by species richness and not root traits. Our study illustrates that
136 root traits and plant species richness affect different properties of soil fungal communities and
137 therefore jointly mediate the effects of plants on fungal communities.

138 Results and discussion

139 Root traits at the community-level

140 To determine root traits at the community-level, we sampled roots from bulk soil without
141 separation by plant species. The PCA of these root traits shows two clear axes that explain a
142 cumulative 79.4% of the variation (Fig. 1). The trait organization closely resembles the root
143 economics space (RES) found at the species-level across a large number of species and biomes
144 in Bergmann *et al.*[11]. The first axis of the varimax-rotated PCA, explaining 42.2% of the
145 variation in community root traits, represents the collaboration gradient of the RES ranging
146 from high root diameter ('outsourcing' strategies) to high specific root length ('do-it-yourself',
147 'DIY'). The second axis explained 37.2% and represents the conservation axis ranging from
148 high root tissue density ('slow') to high root nitrogen ('fast'). This is in line with Da *et al.*[19]
149 who found the community RES to be nearly identical to the species-level RES when using
150 community weighted-mean root traits of woody species in a temperate forest. In contrast,
151 Lachaise *et al.*[18] identified the RES on community weighted-mean traits in observational
152 grasslands with root nitrogen not following expected patterns. Taken together, we show for the
153 first time that directly measured plant community root traits, rather than community traits
154 calculated from species-specific traits, follow the same functional trade–offs as at the species-
155 level. This finding suggests that even under the same abiotic conditions, different economic

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156 strategies of a community can be successful. It further demonstrates the robustness of the trait
157 organization across a wide plant species richness gradient but also highlights the need to better
158 understand the conditions that lead to deviations from the RES found in other studies.
159 The community root traits varied along the gradient of sown plant species richness. While the
160 traits of the collaboration axis, represented by the scores of the first rotated component (RC1)
161 of the PCA, were not significantly related to plant species richness (Estimate = 0.092, P =
162 0.404), scores along the conservation axis (RC2) showed a stronger relationship with plant
163 species richness (Estimate = -0.246, P = 0.023, Fig. 2, Supplementary Table S1). Accordingly,
164 more diverse plant communities were characterized by a ‘slower’, more resource-conservative
165 strategy. This was primarily driven by the decrease of root nitrogen in more diverse plant
166 communities (Supplementary Table S1), which, in line with other studies, can be attributed to
167 a ‘nutrient dilution effect’ with higher plant biomass and increased nitrogen use efficiency[47,48].

168 **Links between root traits and soil fungal guilds**

169 We analyzed how the diversity and relative abundance of saprotrophic, plant pathogenic, and
170 arbuscular mycorrhizal fungi were related to the sown species richness and root traits of the
171 plant communities. We found that the Shannon diversity of fungal saprotrophs was positively
172 related to plant species richness (Table 1, Fig. 3), in line with our expectations. This suggests
173 that plant species richness effects increase root biomass[49,50] and reduce litter quality[51],
174 ultimately affecting saprotrophic diversity. In addition to plant species richness, the root
175 functional strategies of the plant community had strong effects on the saprotrophic community.
176 Easily-available carbon from high-quality litter in roots with ‘fast’ traits and exudates is also
177 used by bacteria[12,52] and therefore putatively less available to fungal saprotrophs, resulting in
178 lower fungal saprotroph diversity (Table 1, Fig. 3). However, we found no significant change
179 in saprotroph relative abundance with ‘fast’ root traits (Table 1, Fig. 3), suggesting that higher
180 resource quality favors fewer fungal taxa that still form a similar proportion of the fungal

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181 community. 'Outsourcing' root strategies along the collaboration axis did not affect fungal
182 saprotrophic diversity but significantly increased the relative abundance of saprotrophic fungi
183 (Table 1, Fig. 3). As the mechanisms behind this are not obvious and previously reported
184 relationships between the collaboration gradient of the RES and the saprotrophic fungal
185 community and decomposition rates are variable[17], we see this as an exciting avenue for further
186 studies.
187 Plant pathogenic fungi did not change in their Shannon diversity along the plant species richness
188 gradient (Table 1, Fig. 3), suggesting that higher morphological and chemical diversity of roots
189 at higher species richness does not increase pathogen diversity. Instead, the lower resource
190 quality and lower host density for specialist pathogens in diverse plant communities[53,54] might
191 limit pathogen diversity. The result, however, is in contrast to studies on aboveground
192 pathogens that found plant species richness to also increase pathogen richness[55], indicating that
193 pathogen dynamics belowground do not follow the same trends as aboveground. The root trait
194 gradients, on the other hand, were strong predictors of pathogen diversity, with 'outsourcing'
195 traits along the collaboration axis and 'slow' traits along the conservation axis being linked with
196 lower pathogen diversity (Table 1, Fig. 3). The relative abundance of fungal pathogens also
197 decreased with 'outsourcing' traits (Table 1, Fig. 3). These results are in line with our
198 predictions and with studies that found traits of the collaboration axis at the species-level to be
199 closely related to the fungal pathogen community[30,31,56]. To the best of our knowledge, our
200 results show for the first time that these effects scale from the plant species- to the community-
201 level, as well as from the root or rhizosphere to bulk soil. Similar to the species-level, we expect
202 the suppression of plant pathogens by mycorrhizal symbionts to be the most likely explanation
203 for the change along the collaboration axis[17]. Additionally, higher root diameter itself might
204 also be a beneficial strategy against plant pathogens, as it decreases the relative root surface[57]
205 and therefore the potential contact points with pathogens. The decreased plant investments into
206 defense in more resource-acquisitive plant communities along the conservation gradient[32] likely

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207 allows more plant pathogenic fungi to colonize the plant and thus be more diverse and abundant
208 in the soil as well.

209 AMF diversity was significantly linked with the collaboration axis, with higher AMF diversity
210 found in plant communities with 'outsourcing' root strategies (Table 1, Fig. 3). Based on the
211 reliance of AMF on high cortex volume and root diameter[58], higher intra-radical mycorrhizal
212 colonization rate and higher extra-radical hyphal length[59] and therefore potentially also higher
213 abundance and diversity is expected with 'outsourcing' roots. Generally, AMF communities in
214 bulk soil are more diverse than in the root, as the plant only recruits a fraction of species from
215 the available species pool in the soil[60,61]. While we did not measure mycorrhizal colonization
216 in this study, the positive correlation with root diameter has been previously shown for a subset
217 of species in our field site[62] and our data now highlight that these trait-fungal relationships at
218 the plant species-level also transfer to AMF diversity in the soil at the plant community-level.
219 Plant species richness and the conservation axis were not related to AMF diversity (Table 1,
220 Fig. 3). The general direction of effects on the relative abundance of AMF was similar to effects
221 on AMF diversity, but there was no significant relationship with the collaboration axis. While
222 we calculated AMF diversity from sequence data of the AMF-specific primers, relative
223 abundance compared to other fungal guilds was calculated from the ITS2 sequence data, in
224 which AMF only account for a very small portion due to the primer bias[63]. We therefore
225 attribute these weaker effects on the relative abundance of AMF to the sequencing methods
226 rather than ecological effects.

227 Overall, we found strong effects of the plant community root trait gradients on the diversity and
228 relative abundance of fungal guilds, with each being significantly correlated with at least one
229 trait axis. Plant species richness, however, was considerably less important than the trait axes.
230 Specifically, we found no change in the relative abundance of any of the three fungal guilds in
231 response to the plant species richness gradient (Table 1, Fig. 3), suggesting that the fungal guild
232 composition of the fungal community is less sensitive to the diversity of the root system and

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233 quantity and quality of plant litter input determined by plant species richness compared to root
234 trait axes.

235 **Drivers of fungal and microbial biomass**

236 Sequencing studies have substantially advanced our knowledge of the community composition
237 of soil microbial communities and are an indispensable part of soil ecology. Yet, the increased
238 use of compositional sequence data has partly shifted focus away from more quantitative
239 measures of soil microbial communities. To gain a more holistic view of the effects of plant
240 species richness and root traits on soil fungal communities, we also quantified lipid biomarkers
241 from soil samples.

242 The biomass of fine roots, a critical carbon source for the majority of soil fungi[38], increased
243 with plant species richness but was also significantly higher in plant communities with
244 'outsourcing' and 'slow' root trait strategies (Table 2, Fig. 4). Traits associated with these
245 strategies (i.e. high root diameter and high root tissue density) generally show a positive
246 relationship with root life span[64,65] and can therefore enhance root standing biomass. The fungal
247 phospholipid fatty acid (PLFA) marker 18:2ω6,9, indicative of overall fungal biomass,
248 increased strongly with plant species richness but was not associated with the collaboration and
249 conservation axis of root traits (Table 2, Fig. 4). The soil microbial biomass carbon, calculated
250 from substrate-induced soil respiration, showed a similar positive effect of plant species
251 richness but no effect of the root trait gradients (Table 2, Fig. 4). The AMF-specific neutral
252 lipid fatty acids (NLFA) marker 16:1ω5, however, was not significantly related to either plant
253 species richness or trait axes (Table 2, Fig. 4). Given that plant species richness was previously
254 shown to increase carbon transport to AMF[66] and that the root length colonized by AMF is
255 correlated with AMF biomass in the soil[67], it is surprising that the AMF biomarker did not show
256 a positive relationship with 'outsourcing' root strategies and increasing plant species richness.
257 NLFA, unlike PLFA, are mainly storage lipids or found in spores[68] and are therefore not as

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258 directly relatable to fungal biomass, potentially explaining the weak effect. While our study
259 was not designed to specifically test this, our results do not support the hypothesis that the
260 diversity, relative abundance, or biomass of AMF mediate positive biodiversity-ecosystem
261 functioning (BEF) relationships[69,70].

262 The ratio between fungal and bacterial biomass (F/B) is considered a proxy for nutrient cycling
263 rates in soils as a higher fungal proportion decreases nutrient cycling rates and increases nutrient
264 retention compared to bacterial-dominated communities[71,72]. We found no change in the F/B
265 ratio along the plant species richness gradient and the collaboration axis but a marginally
266 significant decrease with 'fast' root traits along the conservation axis (Table 2, Fig. 4). This
267 aligns with previous concepts and results suggesting that bacteria benefit from the higher litter
268 quality of 'fast' above- and belowground traits[72].

269 Generally, the quantification of absolute abundances or biomass of individual fungal guilds is
270 not possible in the same way as for relative abundances. Approaches using qPCR methods can
271 quantify gene copies under certain circumstances but are sensitive to biases during the DNA
272 extraction or require standardization[73,74]. Since the PLFA biomarker 18:2ω6,9 is largely
273 determined by the biomass of Ascomycota and Basidiomycota[75], we used it as an indicator of
274 fungal biomass of non-arbuscular mycorrhizal fungi, which include saprotrophic as well as
275 pathogenic fungi. Because PLFA biomarkers and sequencing rely on very different components
276 of the fungal community, the results are not directly comparable. However, as the relative
277 abundance of saprotrophs and plant pathogens changes along the root trait axes but the fungal
278 biomass is not affected, we conclude that the trait axes affect the ratio of guilds in the fungal
279 community, but not the biomass of individual guilds. Studies using a combined qualitative (e.g.
280 sequencing) and quantitative (e.g. PLFA and respiration) approach provide valuable
281 opportunities to overcome the limitations of the compositional nature of sequencing data in the
282 absence of appropriate qPCR methods.

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284 **Conclusions**

285 In summary, our study demonstrates that in experimental grassland communities, fine root
286 functional strategies of the root economics space scale from the species-level to the community-
287 level. We further demonstrate that these functional strategies of plant communities structure the
288 guild composition of soil fungal communities, with saprotrophic, plant pathogenic and
289 arbuscular mycorrhizal varying in diversity or relative abundance depending on the root traits
290 of the plant community. Plant species richness, however, is only a weak driver of the fungal
291 and microbial community composition but drives microbial and fungal biomass in the soil.
292 Ultimately, root trait gradients drive the soil fungal guild composition, but plant species
293 richness controls the fungal biomass. These contrasting results on the role of plant species
294 richness and root trait gradients highlight that a diversity of mechanisms need to be considered
295 in future predictions of how changes in plant communities will affect soil biodiversity and
296 functioning.

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297 # Methods

298 ## Sampling design and soil collection

299 We conducted our study at the Jena Experiment, a large-scale long-term biodiversity
300 experiment. The area is located on a former arable field near the river Saale 51° N, 11° E, 135
301 m above sea level[76]. In 2002, experimental plots varying in sown plant species richness from 1
302 to 2, 4, 8, 16, and 60 species were set up. The plots are grouped in four blocks to account for
303 the differences in initial soil conditions[76]. The initial size of the plots was 10 × 10 m before
304 being reduced to 6 × 5.5 m in 2010. The plots are mown twice a year with the plant material
305 being removed and not fertilized. They are further weeded manually two to three times per year
306 to maintain the designed plant communities. There was no resowing of any plant species,
307 leading to local extinction of some species. However, sown and realized plant species richness
308 are strongly positively correlated[77].
309 For root trait measurements and DNA extraction, we took 4 soil cores (3.5 cm diameter, 5 cm
310 depth) between May 31st and June 11th 2021 in four locations across each plot. The soil cores
311 were stored at 4 °C until final preparation (no longer than 24 h after sampling). The four soil
312 samples were pooled, and a subsample was collected for the DNA extraction and frozen at –20
313 °C immediately. For respiration measurement and fatty acid analysis, we took four soil cores
314 (2 cm diameter, 10 cm depth) in each plot between June 14th and 24th 2021, and stored them at
315 4 °C until measurements were done.

316 ## Root trait measurements

317 Of the 80 plots in the Jena Experiment, 7 plots did not contain any of the sown plant species or
318 did not yield enough root material to measure root traits and were therefore not further
319 considered in our sampling. The pooled soil cores for trait measurements were soaked in water
320 for around 15 minutes and then washed with tap water over a sieve and manually cleaned.
321 Coarse roots with a diameter larger than 2 mm were manually removed from the sample. A

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322 random subset of fine roots was scanned and measured using an Epson Expression 11000XL
323 (Epson, Tokyo, Japan) flatbed scanner at 600 dpi and the software RhizoVision Explorer[78] to
324 quantify root length, root diameter, and root volume. The scanned roots were weighed, dried
325 (48 h at 70 °C), and then weighed again for dry mass. Specific root length (SRL) was calculated
326 as root length : dry mass and root tissue density (RTD) as root dry mass : root volume. Fine
327 root biomass in g/m^2 was calculated based on root dry mass per area of the soil cores. The roots
328 were freeze-dried and ground using a zirconium kit in a ball mill (MM400, Retsch, Haan,
329 Germany). For 46 random samples, relative nitrogen content (RN, % of dry weight) was
330 quantified using an elemental analyzer (Elementar vario ELII, Hanau, Germany) at the Max-
331 Planck-Institute for Biogeochemistry in Jena, Germany. All samples were freeze-dried again to
332 measure near-infrared spectra (NIR) in the range of 9090–4000cm^{-1} at 8cm^{-1} resolution in
333 transmission mode (Multi-Purpose FT-NIR-Analyzer, Bruker Corporation, Billerica, USA).
334 For each sample, five independent measurements were averaged. We converted transmission
335 to absorbance as $\log_{10}$(1/Transmission) and used it in combination with a bootstrapped CARS-
336 PLSR procedure[79] to predict nitrogen content for the remaining 26 samples.

337 The sampling of root traits at the community-level, as used in our study, differs from the more
338 common species-level sampling. In grasslands, our method allows for faster measurement of
339 community traits, while still including plasticity in species traits that would not be captured if
340 a species was only sampled in some communities. Additionally, belowground community
341 weighted-mean traits are usually calculated based on aboveground plant community
342 composition, assuming similar belowground composition. Disproportional above- or
343 belowground allocation at the species-level can therefore cause a misrepresentation of
344 community weighted-mean traits, whereas they do not affect our directly measured community
345 traits. At the same time, every plant community is only described by one value per trait, rather
346 than a weighted mean calculated from individual species' traits, and it is therefore not possible
347 to quantify functional diversity.

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348 **Fungal amplicon sequencing**

349 We extracted genomic DNA from 0.25 - 0.3 g of thawed and homogenized soil using the Quick-
350 DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research Europe, Freiburg, Germany) following
351 the manufacturer's instructions. We measured the DNA content using a NanoDrop 2000c
352 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) and stored the DNA at −20
353 °C until amplification. We amplified the internal transcribed spacer (ITS) region 2 using ITS4
354 and ITS7:ITS7o primers[80,81]. Library preparation and sequencing followed the protocol
355 described in Hennecke *et al.*[17]. In short, three positive PCR products were pooled, purified with
356 AMPure XP beads (Beckman Coulter), and indexed with the Nextera XT Illumina Index Kit
357 (Illumina Inc., San Diego, USA). The samples were then pooled to equal molarity and the
358 paired-end sequencing of 2 × 300 bp was performed using a MiSeq Reagent Kit v3 on an
359 Illumina MiSeq System at the Department of Soil Ecology of the Helmholtz-Centre for
360 Environmental Research (UFZ, Halle/Saale, Germany). To overcome the amplification bias
361 against AMF with ITS primers[63], we additionally sequenced AMF. For this, the SSU of AMF
362 was amplified using a nested PCR with primer pair Glomer 1536 / WT0 for the first PCR and
363 NS31 / AML2 for the second PCR according to Wahdan *et al.*[82] and the sequencing was
364 performed as for the ITS sequencing. Detailed description of the sequencing of AMF
365 communities is described in Albracht *et al.*[83].

366 The processing of sequences was executed using the snakemake implementation *dadasnake*[84]
367 of the *DADA2* pipeline[85]. To summarize, *cutadapt*[86] was used to trim the primer sequences from
368 raw reads. Quality trimming was performed with a minimum length of 70 bp for ITS and 260
369 bp (fwd) / 210 bp (rvs) for AMF, truncation of reads at positions with a PHRED score below
370 15 for ITS, and exclusion of reads with an expected error higher than 2. The identification of
371 exact sequence variants (Amplicon Sequence Variants, ASVs) included merging read pairs with
372 a minimum overlap of 15 bp (ITS) and 12 bp (AMF) and a maximum of three (ITS) and zero
373 (AMF) mismatches. Chimeras were filtered using DADA2's 'consensus' algorithm. Taxonomic

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374 classification was conducted using the *mothur*[87] implementation of the Bayesian classifier
375 against the UNITE v8.2 database[88] (ITS) and SILVA v138 SSUref database[89] for AMF. Non-
376 fungal ASVs (for ITS sequences) and non-Glomeromycotinian ASVs (for AMF sequences)
377 were discarded. For ITS data, ASVs were then assigned to putative fungal guilds based on their
378 taxonomic annotation and the FungalTraits database[20]. Further, all Glomeromycotinian ASVs
379 in the ITS data were assigned to be arbuscular mycorrhizal. For the AMF data, ASVs were
380 blasted against the MaarjAM database[90] and assigned to virtual taxa (VTX). ASVs without
381 VTX assignment were extracted, singletons removed, and used for the construction of a
382 maximum likelihood phylogenetic tree based on a general time-reversible, discrete gamma
383 (GTR+G) model using raxML[91] and FasttreeMP[92]. Consequently, these ASVs were associated
384 with custom virtual taxa (VTC) characterized by cophenetic distances below 0.03. For the
385 analysis of fungal diversity, the dataset was rarefied to address any potential impact of
386 sequencing depth on ASV richness (Supplementary Fig. S1).

387 **Lipid fatty acid and respiration measurement**

388 Soil fungal biomass was determined using phospholipid fatty acids (PLFA) analysis. We
389 extracted PLFAs from 5 g of soil following Frostegård *et al.*[93] and fractioned them into PLFAs,
390 neutral lipid fatty acids (NLFA), and glycolipids. PLFAs and NLFA were then measured using
391 a gas-chromatograph (GC-FID Clarus 500; PerkinElmer Corporation, Norwalk, USA) with an
392 Elite-5 column (PerkinElmer Corporation, Norwalk, USA). PLFA and NLFA concentrations
393 were calculated from the internal standard C19:0 (Methylnonadecanoat). Based on the
394 classification of Ruess and Chamberlain[94], we used 18:2ω6,9 PLFA marker as a measure of
395 fungal biomass and the 16:1ω5 NLFA marker as a measure of AMF biomass. For the fungal :
396 bacteria (F/B) ratio, the sum of both fungal markers was divided by the sum of the bacterial
397 PLFA markers a15:0, i15:0, i16:0, i17:0, 16:1ω7, cy17:0, and cy19:0. For three samples,
398 chromatograms showed very high peaks suggesting erratic measurements but could not be
399 repeated due to small sample amount and were therefore excluded from the analysis. As recent

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400 studies raised the issue that plant biomass can also contribute to 18:2ω6,9 PLFA[95], we further
401 quantified soil microbial biomass carbon (C_{mic}) using an O2-micro-compensation apparatus[96].
402 The soil was sieved at 2 mm to remove stones, large organic materials, and larger organisms,
403 and added watery glucose solution to determine the maximal initial respiratory response
404 (MIRR). C_{mic} was calculated from MIRR following Beck *et al.*[97]. Soil water content was
405 estimated via drying after the end of measurements.

406 **Statistical analysis**

407 Data analyses were conducted in R v.4.3.2[98]. We used a principal component analysis (PCA)
408 of the root traits RD, SRL, RN, and RTD, followed by varimax rotation for better
409 interpretability of the components and inversing the scores and loadings to match the direction
410 of trait gradients in Bergmann *et al.*[11]. Rotated components (RC) 1 and 2 of the PCA captured
411 the root economics space's main axes (Fig. 1), representing the collaboration and conservation
412 axes. Subsequent analyses used the scores from these axes to examine the effects on fungal
413 communities. The unrotated PCA is shown in Supplementary Fig. S2.
414 Fungal abundance and taxonomy were organized in a phyloseq object[99]. Read numbers of ASVs
415 with a primary lifestyle as litter saprotrophs, soil saprotrophs, wood saprotrophs, and
416 unspecified saprotrophs were summed for the total number of reads of saprotrophs. Shannon
417 diversity was calculated within the three fungal guilds (saprotrophs, plant pathogens, and
418 AMF). Relative guild abundance was calculated as the number of reads per guild relative to the
419 total number of reads. A broad description of the sequenced fungal ITS2 community is
420 presented in Supplementary Methods S1.
421 To test how plant species richness affected the root trait axes, we used two separate linear mixed
422 models with RC1 and RC2 as the response and plant species richness (log) as a fixed effect and
423 the experimental block as a random term. We tested how plant species richness and root traits
424 axes are linked with fungal guild diversity and relative abundance, as well as root biomass,
425 PLFA and NLFA biomarker concentration, F/B ratio, and soil microbial carbon, using a linear

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426 mixed-effect models of the lme4 package[100] and tested for significance using lmerTest[101] with
427 type III sum of squares. Log-scaled sown plant species richness and RC1 and RC2 were
428 included as the fixed effects and the experimental block as a random term to account for any
429 spatial effects of the field site. Standardized effect sizes were extracted using z-transformed
430 model variables. In case the random terms did not explain any variance, linear regression was
431 used instead. Collinearity was checked for all models but was considered unproblematic with
432 variance inflation factors well below 2 for all models. An additional analysis with sequential
433 fitting of variables is presented in Supplementary Table S2.
434

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449

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450 ## Author contributions

451 J.H., J.B., N.E., A.H.-B., T.W.K., M.L., L.M. and A.W. conceived the idea of the study. J.H.,
452 L.B., C.A., A.A., L.H., Y.P., A.R. and M.D.S. collected the data; J.H. and A.H.-B. processed
453 the sequence data and J.H. analyzed the data. J.H. led the writing of the manuscript and all
454 authors contributed to reviewing and editing.

455 ## Data availability

456 The data associated with this study will be published with a unique accession number. The raw
457 Illumina sequences generated in this study are available in the NCBI Sequence Read Archive
458 (BioProject: PRJNA1074103 and PRJNA988299).

459 ## Code availability

460 The code used for the analyses and to produce the figures will be deposited together with the
461 data.

462 ## Conflict of interest

463 None declared.

464 ## References

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700 # Figures and Tables

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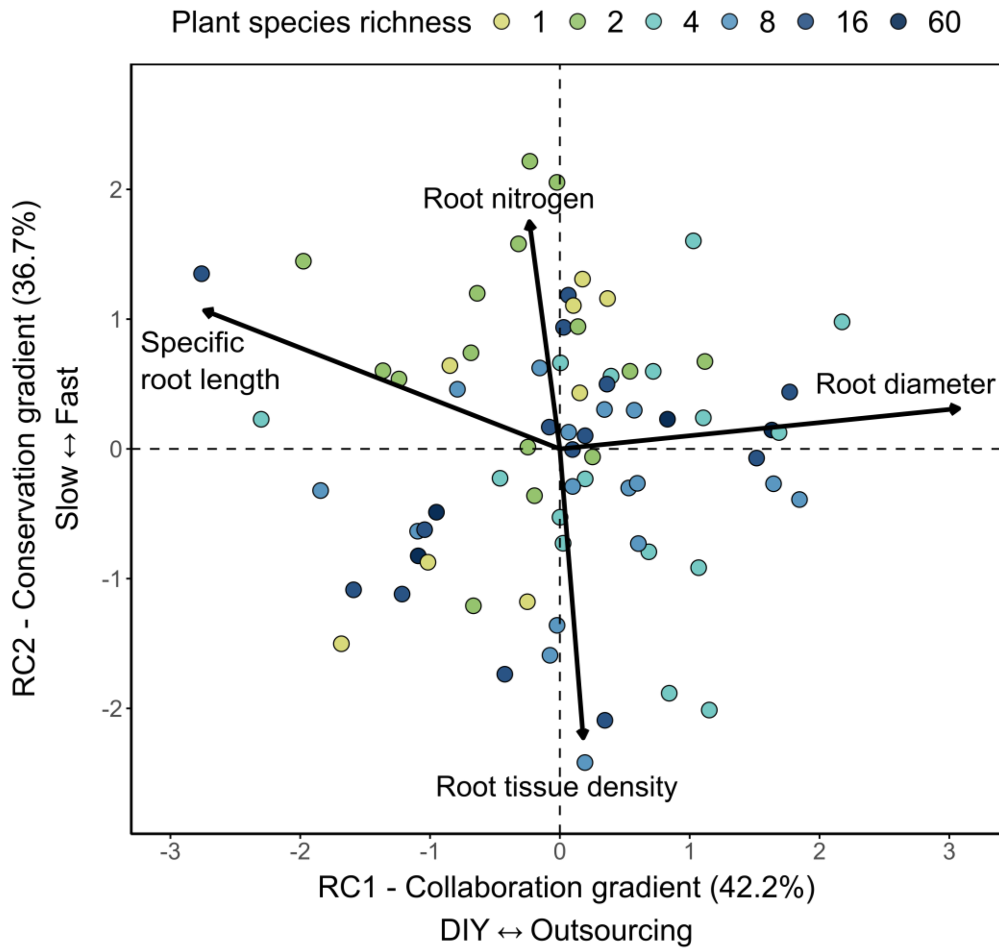

703

704 **Fig. 1: PCA of the community root traits.** Each point represents a plant community (n = 73).
705 The two axes closely resemble the species-level root economics space[11]. The traits of the
706 collaboration gradient load on the first axis while traits of the conservation gradient load on the
707 second axis. Varimax rotation was used to increase interpretability of the two axes. Points are
708 color-coded for plant species richness of the plot. RC, rotated component.

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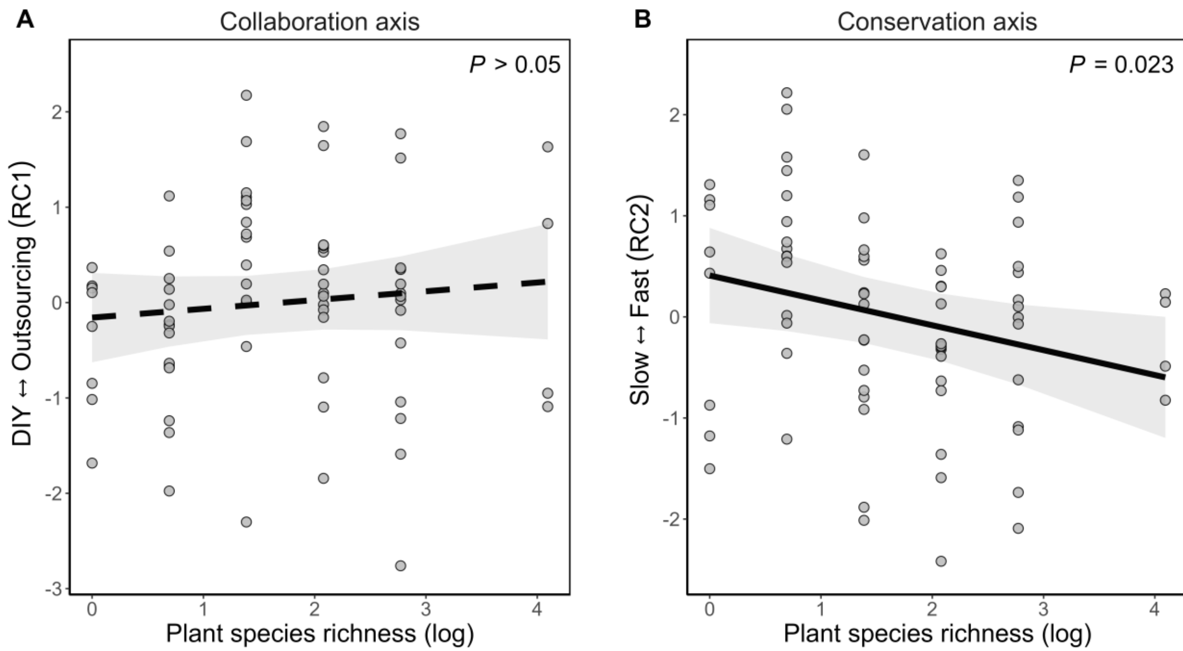

711

712 **Fig. 2: Change of root functional strategies along the plant species richness gradient.**
713 Scores of the first and second rotated component (RC) of the root trait PCA, representing the
714 (A) collaboration axis and (B) conservation axis, in relation to the plant species richness
715 gradient. Each point represents the plant community of one experimental plot (*n* = 73).
716 Regression lines are based on mixed-effects model predictions, solid lines indicate significant
717 relationships (P<0.05), dashed lines indicate non-significant relationships (P>0.05). The grey
718 bands around the regression lines depict the 95% confidence interval.

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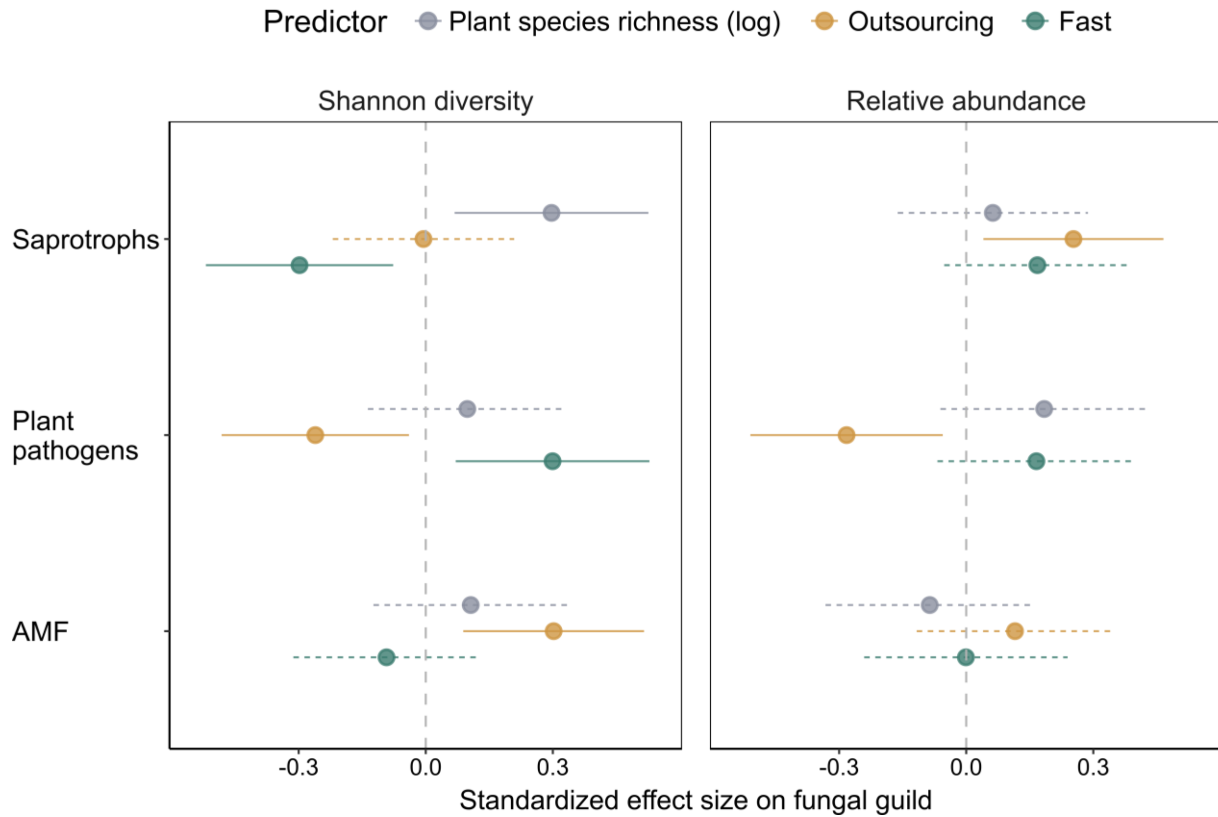

720

721 **Fig. 3: Effects of plant species richness and root trait gradients on fungal guilds.**
722 Standardized effect sizes of plant species richness (log) and root trait strategies ('outsourcing'
723 along the collaboration gradient and 'fast' along the conservation gradient) on the Shannon
724 diversity (A) and relative abundance (B) of saprotrophic, plant pathogenic and arbuscular
725 mycorrhizal fungi (AMF) in bulk soil (*n* = 73). Each point represents the predicted marginal
726 effect with the horizontal line showing 95% confidence interval from a linear mixed effect
727 model.

728

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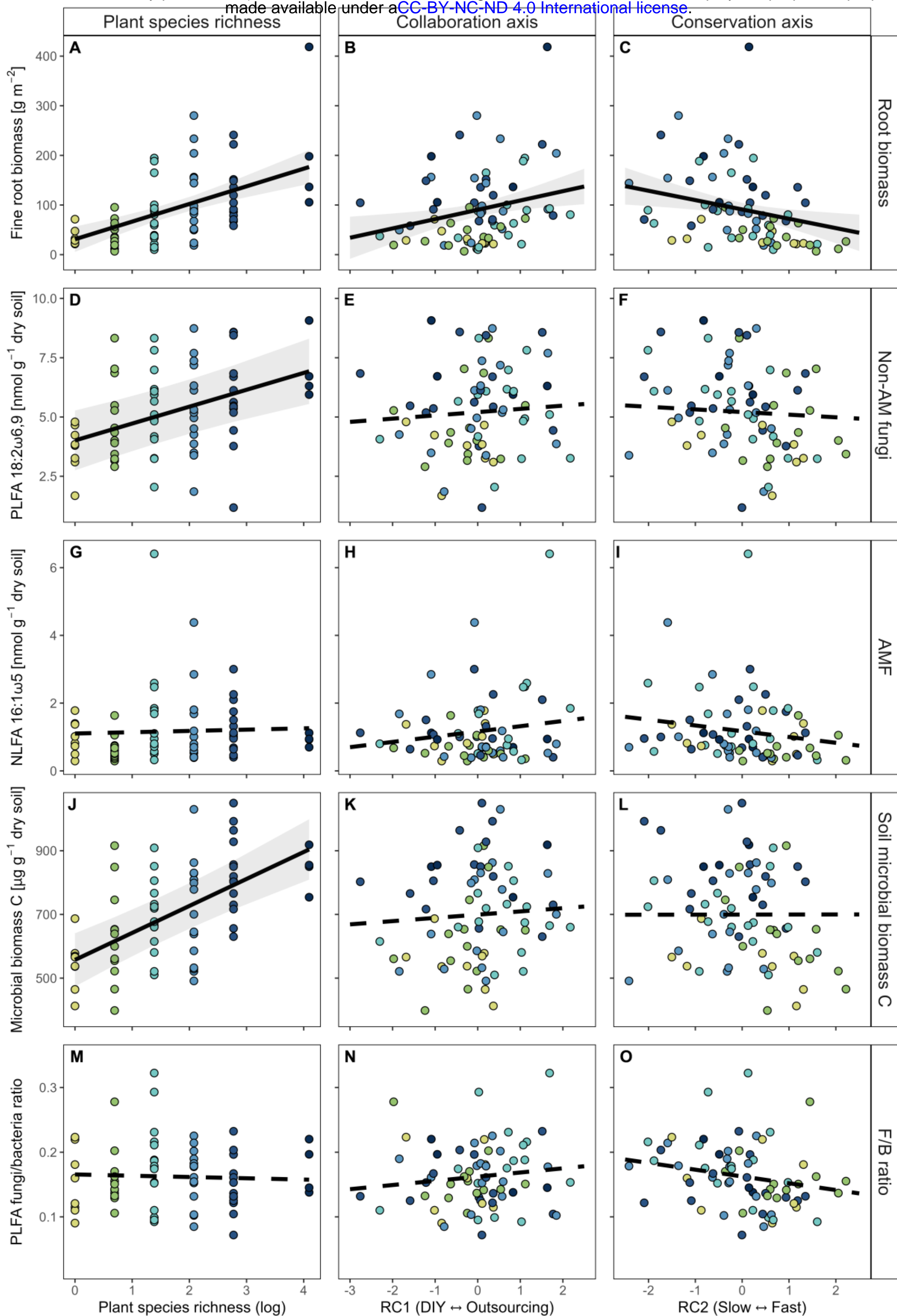

Fig. 4: The relationship of plant species richness and root trait gradients with root biomass and soil microbial properties. Changes in root biomass, non-arbuscular mycorrhizal (non-AM) and arbuscular mycorrhizal fungi (AMF) biomarker concentration, soil microbial biomass carbon and fungal to bacterial (F/B) ratio along the plant species richness gradient and the collaboration and conservation axis of the root economics space. Each point represents one experimental plot ($n = 70$). Regression lines are based on linear mixed-effect model predictions, solid lines indicate significant relationships (P<0.05), dashed lines indicate non-significant relationships (P>0.05). The grey bands around significant regression lines depict the 95% confidence interval.

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739 **Table 1: Summary of linear mixed-effect models testing how plant species richness and**
740 **the community root trait gradients affect Shannon diversity and relative abundance of**
741 **saprotrophic, plant pathogenic and arbuscular mycorrhizal fungi (see Fig. 3).**

Response	Guild	Predictor	Standardized Estimate	SE	P
Shannon diversity	Saprotrophs	Plant species richness (log)	0.273	0.102	**0.009**
		Collaboration gradient (‘outsourcing’)	-0.010	0.095	0.920
		Conservation gradient (‘fast’)	-0.274	0.098	**0.007**
	Plant pathogens	Plant species richness (log)	0.100	0.120	0.409
		Collaboration gradient (‘outsourcing’)	-0.273	0.113	**0.019**
		Conservation gradient (‘fast’)	0.297	0.117	**0.013**
	AMF	Plant species richness (log)	0.106	0.115	0.359
		Collaboration gradient (‘outsourcing’)	0.302	0.107	**0.006**
		Conservation gradient (‘fast’)	-0.093	0.110	0.403
Relative abundance	Saprotrophs	Plant species richness (log)	0.062	0.111	0.579
		Collaboration gradient (‘outsourcing’)	0.250	0.105	**0.020**
		Conservation gradient (‘fast’)	0.166	0.109	0.132
	Plant pathogens	Plant species richness (log)	0.190	0.127	0.138
		Collaboration gradient (‘outsourcing’)	-0.291	0.117	**0.015**
		Conservation gradient (‘fast’)	0.171	0.121	0.162
	AMF	Plant species richness (log)	-0.089	0.127	0.487
		Collaboration gradient (‘outsourcing’)	0.118	0.120	0.326
		Conservation gradient (‘fast’)	-0.001	0.124	0.995

742 The scores of each plant community (n = 73) along the first and second rotated axis in the root
743 trait PCA were extracted and used as fixed effect in the model. Standardized estimates were
744 obtained by z-transformation of variables prior to fitting the model. Experimental block was
745 included as a random effect to account for spatial effects in the field site. AMF, arbuscular
746 mycorrhizal fungi; SE, standard error.

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749 **Table 2: Summary of results of linear mixed-effect models testing how plant species**
750 **richness and the community root trait gradients affect root biomass, PLFA and NLFA**
751 **biomarkers, soil microbial biomass carbon and the fungal : bacterial ratio (see Fig. 4).**

Response	Predictor	Estimate	SE	P
Fine root biomass	Plant species richness (log)	35.552	6.594	**<0.001**
	Collaboration gradient ('outsourcing')	18.765	6.794	**0.007**
	Conservation gradient ('fast')	-18.804	7.011	**0.009**
Non-AM fungi PLFA (18:2ω6,9)	Plant species richness (log)	0.712	0.164	**<0.001**
	Collaboration gradient ('outsourcing')	0.137	0.174	0.435
	Conservation gradient ('fast')	-0.111	0.181	0.541
AMF NLFA (16:1ω5)	Plant species richness (log)	0.038	0.116	0.744
	Collaboration gradient ('outsourcing')	0.155	0.120	0.201
	Conservation gradient ('fast')	-0.171	0.124	0.173
Soil microbial biomass carbon	Plant species richness (log)	84.701	13.316	**<0.001**
	Collaboration gradient ('outsourcing')	10.163	14.115	0.474
	Conservation gradient ('fast')	0.187	14.618	0.990
Fungal : bacterial ratio	Plant species richness (log)	-0.002	0.005	0.710
	Collaboration gradient ('outsourcing')	0.006	0.006	0.253
	Conservation gradient ('fast')	-0.010	0.006	0.075

752 The scores of each plant community (n = 70) along the first and second rotated axis in the root
753 trait PCA were extracted and used as fixed effect in the model. Experimental block was included
754 as a random effect to account for spatial effects in the field site. SE, standard error; non-AM,
755 non-arbuscular mycorrhizal; AMF, arbuscular mycorrhizal fungi; PLFA, Phospholipid fatty
756 acids; NLFA, neutral lipid fatty acid.

757