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Soil and root nutrient chemistry structure root-associated fungal assemblages in temperate forests

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Summary

Root-associated fungi (RAF) link nutrient fluxes between soil and roots and thus play important roles in ecosystem functioning. To enhance our understanding of the factors that control RAF, we fitted statistical models to explain variation in RAF community structure using data from 150 temperate forest sites covering a broad range of environmental conditions and chemical root traits. We found that variation in RAF communities was related to both root traits (e.g., cations, carbohydrates, NO_3^-) and soil properties (pH, cations, moisture, C/N). The identified drivers were the combined result of distinct response patterns of fungal taxa (determined at the rank of orders) to biotic and abiotic factors. Our results support that RAF community variation is related to evolutionary adaptedness of fungal lineages and consequently, drivers of RAF communities are context-dependent.

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Introduction

Root-associated fungal (RAF) communities, important components of plant microbiomes, are defined as any fungi residing in and on plant roots (Porras-Alfaro and Bayman, 2011; Dean *et al*., 2014; Bergelson *et al*., 2019). RAF communities are composed of fungi that belong to different trophic guilds and encompass all major phyla (Basidiomycota, Ascomycota, Glomeromycota, Chytridiomycota and Zygomycota) (Kernaghan, 2013; Peršoh, 2015). The main functional guilds in RAF communities are symbiotrophic fungi providing mineral nutrients to their host and priming plant defence systems, saprotrophic fungi recycling dead, organic materials and pathotrophic fungi thriving on living cells (Kernaghan, 2013; Peršoh, 2015; Vandenkoornhuyse *et al*., 2015). Since RAF communities link matter fluxes between soil and roots, their composition is also critical for biogeochemical processes such as carbon sequestration (Clemmensen *et al*., 2013; Kyaschenko *et al*., 2019). Therefore, knowledge on the variation patterns and drivers that structure fungal assemblages is fundamental to better understand ecosystem functioning.

Habitat properties such as vegetation type, host species, forest age, successional stage, human disturbance (Edwards and Zak, 2011; Goldmann *et al*., 2015; Vannette *et al*., 2016; Zhang *et al*., 2016; Kolaříková *et al*., 2017; Maghnia *et al*., 2017; Kennedy *et al*., 2018) and abiotic environmental filters such as climate, soil type, soil fertility and soil pH (Moora *et al*., 2004; Gorzelak *et al*., 2012; Sterkenburg *et al*., 2015; Maghnia *et al*., 2017) shape fungal communities in soil and roots. For example, soil pH and the C/N ratio are major factors affecting the structure of fungal assemblages (Wubet *et al*., 2012; Kivlin *et al*., 2014; Glassman *et al*., 2017; Větrovský *et al*., 2019). The nature of the environmental filters varies across landscapes, highlighting the complexity and dynamics of fungi in their environment (Schröter *et al*., 2019). RAFs, which are recruited from the surrounding soil (Danielsen *et al*., 2012, 2013), are less affected by the abiotic environment than soil fungi (Goldmann *et al*., 2016). It is known that mycorrhizal fungi show distinct host preferences (Lang *et al*., 2011; Bahnmann *et al*., 2018; van der Linde *et al*., 2018). Since mycorrhizal community structure on roots varies with root nutrients (Lang and Polle, 2011), it is

conceivable that RAF community structure is also strongly influenced by interactions with host root properties such as root chemistry. Important fungal traits, e.g., decomposition, P and N transformation are phylogenetically conserved (Treseder and Lennon, 2015), which suggest relationships between nutrient resources and the composition of RAF assemblages, but these factors have not yet received much attention.

In addition to abiotic factors and interspecific competition (Koide *et al*., 2011), host-derived carbohydrates have been suggested to influence the coexistence of different trophic groups in plant roots (Kernaghan, 2013). Therefore, carbon is also expected to be a driver of RAF community structures. For example, the structure and diversity of ectomycorrhizal assemblages, which are a major component in RAF communities (Goldmann *et al*., 2016; Kolaříková *et al*., 2017), are strongly affected by host allocation to the roots (Druebert *et al*., 2009; Pena *et al*., 2010). In boreal forests, as much as 50% to 70% of stored soil carbon originates from roots and root-associated microorganisms, stressing the significance of RAF communities for the regulation of carbon dynamics in forests (Clemmensen *et al*., 2013). Despite the tight links of root carbon sources and associated fungal assemblages, it is unknown whether differences in root carbon pools drive changes in RAF community structure.

The goal of this study was to determine the importance of soil and root chemistry shaping RAF community structure in temperate forests. Here, we focused on root chemistry instead of more often applied biotic filters concerning plant identity and associated mycorrhizal assemblages. We investigated the effect sizes of carbon, nitrogen (C/N, NO_3^-, NH_4^+ in soil and roots, carbohydrates in roots), other nutrient elements (P, S, basic cations) and abiotic drivers (soil pH, soil moisture) on RAF community structure. We conducted our study in the Biodiversity Exploratories (http://www.biodiversity-exploratories.de/startseite/) on 150 plots in coniferous and deciduous forests in three regions [Schwäbische Alb (A), Hainich-Dün (H) and Schorfheide-Chorin (S)] located along a transect from the south-west to the north-east of Germany (Fischer *et al*., 2010). The regions are characterized by different climatic conditions with mean annual temperatures ranging from 6°C to 8.5°C and precipitation from 500 to 1000 mm (Fischer *et al*., 2010). The S region is characterized by the warmest and driest climatic conditions; A is the coolest and moistest region, and the climate in H is similar to that in the A region (Fischer *et al*., 2010). We hypothesized that (i) the composition of RAF is driven by soil chemistry and root traits explaining divergent taxonomic structures of RAF communities in different biogeographic regions and that (ii) the impact of root chemistry on RAF depends on the functional guild, being stronger for symbionts (mycorrhizal fungi) than for saprotrophic or pathotrophic fungi. Alternatively, we hypothesized that (iii) RAF assemblages are structured by distinct interactions of phylogenetic groups with root chemistry and abiotic habitat filters. To address these hypotheses, we characterized RAF taxonomic and trophic structures in relation to soil and root chemistry.

Results

Soil and root chemistry vary among biogeographic regions

Soils in S forests were more acidic than those in H (intermediate) and A (Table 1). Soil carbon and nitrogen contents were lowest in S and highest in the A region (Table 1). The highest soil C/N ratio was present in S (Table 1). Other soil nutrients also showed considerable differences among the regions, with the highest P and the lowest cation contents in S, the lowest P in H and the highest cation contents in A (Table 1). Soil moisture in the region S was lowest and that in A highest (Table 1). Soil moisture and soil temperatures were significantly correlated ($r = -0.53$, $p < 0.001$, Fig. S1).

The total annual N deposition was similar among the regions ranging from about 14 to 17 kg ha^{-1} a^{-1}, but the composition varied. The amounts of NO_3^- were higher in A (up to 10 kg ha^{-1} a^{-1}) than in S and H (about 7 kg ha^{-1} a^{-1}), whereas higher NH_4^+ and organic N amounts were deposited in S than in A and H (Table S1). In agreement with the atmospheric deposition, the concentration of soil NH_4^+ was highest in the S and lowest in the A region, while the concentrations of soil NO_3^- exhibited the opposite order (Table 1).

Root chemistry also showed differences among the forest regions. The root concentrations of carbon, glucose and fructose were higher in the S than the other two regions (Table 1). Root starch concentrations were higher in A than in H and S (Table 1). Root N concentrations were highest in the A and lowest in the S region. The concentrations of root NO_3^- were significantly higher in the S than the other two regions, while there were no differences in NH_4^+ concentrations among the roots from the three regions ($p = 0.173$, Table 1). The root C/N ratios did not differ between A and H but were higher in roots from S than in those from the other regions (Table 1). Root P content was lower in H and root cations lower in S than in the other regions, reflecting the variation in soil contents of these nutrients (Table 1).

Taxonomic and trophic community structure of RAF

For the comparison of fungal operational taxonomic unit (OTU) richness and diversity metrics, we used a quality-filtered, rarefied data set, which consisted of 8400 sequences

Table 1. Characterization of soil and root chemistry, soil pH and soil moisture in three forest regions (A = Schwäbische Alb, H = Hainich-Dün, S = Schorfheide-Chorin).

		Study region			*p* value			
Variables	Unit	A	H	S	A-H-S	A-H	A-S	H-S
Soil variables								
Soil C	g kg^{-1}	62.52 ± 1.92	36.85 ± 1.55	19.79 ± 0.65	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Soil N[a]	g kg^{-1}	4.68 ± 0.14	2.76 ± 0.11	1.11 ± 0.04	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Soil NH_4^{+a}	mg kg^{-1}	0.32 ± 0.04	0.44 ± 0.07	0.67 ± 0.06	**<0.001**	0.385	**<0.001**	**<0.002**
Soil NO_3^-	mg kg^{-1}	2.35 ± 0.17	1.31 ± 0.10	0.71 ± 0.08	**<0.001**	**<0.001**	**<0.001**	**<0.002**
Soil C:N[a]		13.4 ± 0.11	13.4 ± 0.16	18.3 ± 0.38	**<0.001**	<0.999	**<0.001**	**<0.001**
Soil P[a]	mg kg^{-1}	95.4 ± 10.9	49.6 ± 1.84	119.8 ± 6.2	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Soil S[a]	mg kg^{-1}	82.9 ± 3.58	75.0 ± 2.06	56.0 ± 1.50	**<0.001**	0.369	**<0.001**	**<0.001**
Soil Sum Cations[a,b]	mmol kg^{-1}	96.1 ± 3.63	77.7 ± 4.07	12.6 ± 0.60	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Soil pH[a]		5.23 ± 0.10	4.80 ± 0.12	3.55 ± 0.02	**<0.001**	**0.002**	**<0.001**	**<0.001**
Soil moisture	%	58.7 ± 1.25	43.6 ± 0.99	14.6 ± 0.61	**<0.001**	**0.001**	**<0.001**	**<0.001**
Root variables								
Root C	g kg^{-1}	436.55 ± 5.44	421.31 ± 5.57	488.36 ± 2.93	**<0.001**	0.068	**<0.001**	**<0.001**
Root glucose[a]	g kg^{-1}	2.53 ± 0.13	2.08 ± 0.09	4.36 ± 0.26	**<0.001**	0.058	**<0.001**	**<0.001**
Root fructose	g kg^{-1}	1.25 ± 0.09	1.06 ± 0.06	1.95 ± 0.17	**<0.001**	0.471	**0.004**	**<0.001**
Root starch[a]	g kg^{-1}	4.68 ± 0.85	1.83 ± 0.28	3.64 ± 0.87	**0.022**	**0.019**	0.154	0.651
Root N[a]	g kg^{-1}	13.87 ± 0.45	12.15 ± 0.26	11.51 ± 34	**<0.001**	**0.006**	**<0.001**	0.236
Root NH_4^{+a}	mg kg^{-1}	29.81 ± 3.21	22.43 ± 1.05	29.57 ± 1.98	0.173	0.443	0.797	0.156
Root NO_3^-	mg kg^{-1}	1716.2 ± 123.8	1735.1 ± 89.0	2207.5 ± 136.7	**0.006**	0.993	**0.012**	**0.017**
Root C:N[a]		32.8 ± 0.96	35.4 ± 0.85	44.4 ± 1.53	**<0.001**	0.087	**<0.001**	**<0.001**
Root P[a]	mg kg^{-1}	1100.7 ± 64.8	815.8 ± 25.6	1090.2 ± 34.5	**<0.001**	**<0.001**	0.235	**<0.001**
Root S[a]	mg kg^{-1}	1432.7 ± 76.5	1377.6 ± 49.0	1128.7 ± 22.9	**<0.001**	0.061	0.204	**<0.001**
Root Sum Cations[a,b]	mmol kg^{-1}	424.8 ± 14.7	426.5 ± 16.7	192.5 ± 5.68	**<0.001**	<0.999	**<0.001**	**<0.001**

Significant differences at $p \leq 0.05$ are indicated with bold letters. Data are means of $n = 50$ plots per region ± SE.
[a]Data were subjected to ln transformation before ANOVA analysis.
[b]Sum of K, Ca and Mg molar compositions.

per plot corresponding to a total of 3366 different OTUs (Table S2). Mean observed fungal OTU richness per plot was almost twice higher in the H than in the S region (Table 2). This result was also supported by the calculation of maximum OTU richness (via Michaelis–Menten fit, Table 2). Classification of fungal guilds revealed higher richness of symbiotrophic, saprotrophic and pathotrophic fungi on roots in H than in S, while A exhibited intermediate OTU richness (Table 2). Shannon diversity and Evenness were not different among the regions (Table 2).

The taxonomic composition of RAF varied among the forest regions, resulting in distinct clusters for RAF community structures in A, H and S (ANOSIM, $p < 0.001$, $R = 0.40$, Fig. 1A). The OTU dissimilarities of the communities were explained by all tested soil properties and root traits except root NH_4^+ concentrations (Fig. 1A, Table S3).

Classification of OTUs according to fungal orders revealed phylogenetic differences among the RAF assemblages (ANOSIM, $p < 0.001$, $R = 0.46$, Fig. 1B). Ten

Table 2. OTU richness and diversity estimates of RAF in three biogeographical regions.

	Mean ± standard error			p value			
Parameter	A	H	S	A-H-S	A-H	A-S	H-S
Observed OTU richness	157 ± 7	201 ± 8	119 ± 3	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Michaelis–Menten Fit[a]	182 ± 9	240 ± 9	135 ± 4	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SY richness	47 ± 2	62 ± 3	27 ± 1	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SA richness	30 ± 2	34 ± 2	26 ± 1	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PA richness	9 ± 1	15 ± 1	10 ± 1	**<0.001**	**<0.001**	**0.251**	**<0.001**
Shannon (H')	4.23 ± 0.1	4.37 ± 0.1	4.04 ± 0.1	0.072	0.591	0.382	0.058
Evenness (E_H)	0.85 ± 0.0	0.83 ± 0.0	0.85 ± 0.0	0.624	0.760	0.973	0.622

A = Schwäbische Alb; H = Hainich-Dün; S = Schorheide-Chorin; SY = symbiotrophic fungi; SA = saprotrophic fungi; PA = pathotrophic fungi. Differences at $p < 0.05$ are indicated with bold letters. Data are means of $n = 50$ plots per region ± SE. Generalized linear model (Poisson) was used to analyse the count data (OTU richness). ANOVA were used for analysis of the continuous variables (Shannon, Evenness and Michaelis Menten fit).
[a]Calculated OTU richness using the Michaelis–Menten algorithm.

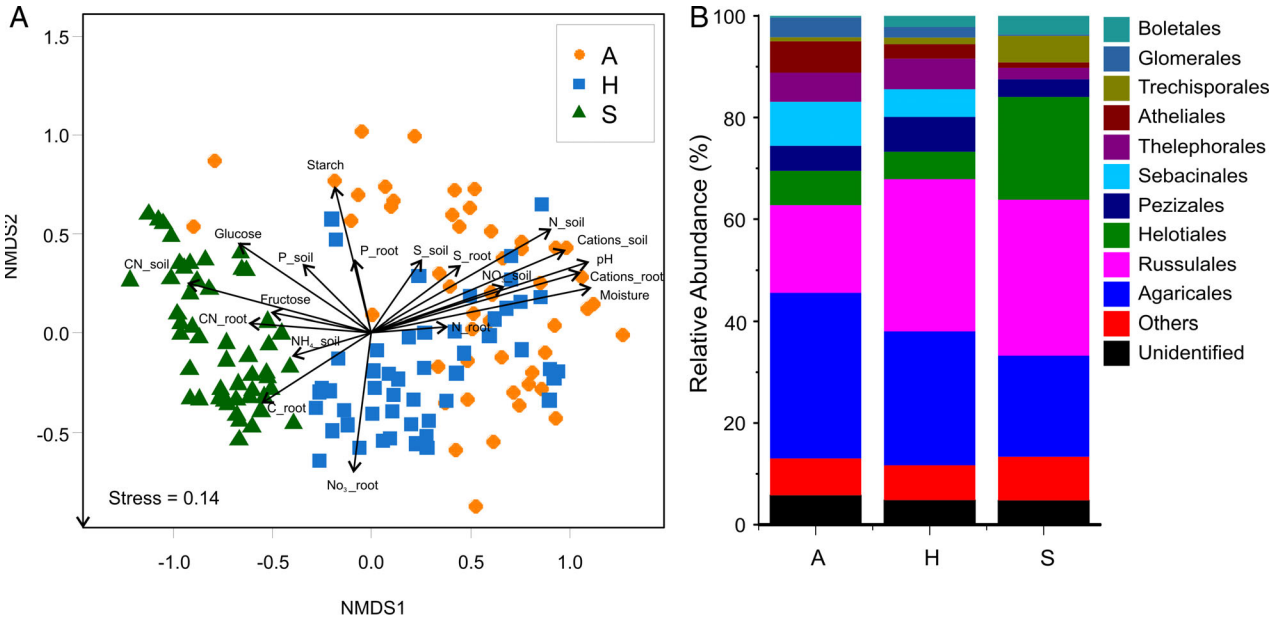

Fig 1. NMDS of fungal species (OTU-based) (A) and relative abundance of orders (B) of root-associated fungal communities in 150 forest plots in three regions.
Fungal taxonomic dissimilarities were based on Bray–Curtis dissimilarity matrices. Abundance data are means of $n = 50$ plots per region, each rarefied to 8400 reads per plot. A = Schwäbische Alb (orange circle), H = Hainich-Dün (blue square) and S = Schorfheide-Chorin (green triangle). Statistical information is found in Table S3. [Color figure can be viewed at wileyonlinelibrary.com]

fungal orders were dominant with OTU abundances greater than 2% of the total number of OTUs. These orders accounted together for about 87.3% of the total OTUs abundances (Fig. 1B). Only 5.1% of the reads could not be assigned to any fungal order (Fig. 1B). Agaricales dominated in A and Russulales in S (Fig. 1B, Table S4). These Basidiomycota are known to form ectomycorrhizal associations and accounted together for more than 50% of the RAF communities (Fig. 1B). Ascomycota, mainly of the orders Helotiales and Pezizales, which contain also ectomycorrhizal fungal species, were more abundant on roots in the S than on those in the H and A forests (Fig. 1B, Table S4).

Root traits and soil properties as drivers of RAF communities

We explored the relationships of the fungal guilds and orders with environmental factors (soil properties, root traits as predictors) by calculating their standardized coefficients for interaction (SCI) (Fig. 2, Table S5). This approach did not yield any SCI as predictor for symbiotrophs, five for saprotrophs (negative: root NO_3^-, positive soil C/N, root C/N, root glucose, root NH_4^+) and three for pathotrophs (negative: root NO_3^-, soil C/N, positive: soil P) (Fig. 2). The percentage of explained variance ranged from 21% to 27% (Fig. 2). When the fungal guilds were split into orders, each of the resulting 15 groups (9 symbiotrophic, 5 saprotrophic, and 1 pathotrophic order) exhibited more complex patterns of the SCIs than those found for the guilds alone and the percentage of explained variance ranged from 5% to 46% (Fig. 2). The most frequent SCIs, which were detect in more than half of the fungal groups, were root NO_3^- (in 10/15 group), root starch, root sulfur, soil NH_4^+, and soil basic cations (each in 8/15 fungal groups). For example, the symbiotrophic Russulales, Boletales, and Pezizales as well as the saprotrophic Pezizales were positively related to root NO_3^-, whereas negative SCIs for root NO_3^- were obtained for all categories of the Helotiales (symbiotroph, saprotroph, pathotroph), the saprotrophic Agaricales and Glomerales (symbiotrophic). The SCIs for other variables, which might have been expected such as soil pH, soil P or soil C/N, root C/N occurred in 7 out of the 15 groups. Notably, in three orders, the mycorrhizal groups (Agaricales, Sebacinales and Thelephorales) showed negative relationships with soil P, indicating that these groups were increased under low P, whereas others decreased with increasing soil P (Fig. 2). Soil pH also showed contrasting SCIs: positive values for the symbiotrophic Pezizales, Thelephorales and Sebacinales, and negative values for Russulales (symbiotrophs) and saprotrophic Atheliales and Trechisporales (Fig. 2). Some of the groups showed similar responses. For example, symbiotrophic Agaricales, Sebacinales and Thelephorales showed positive SCIs for soil moisture and negative SCIs for soil pH (Fig. 2). As the consequence of contrasting SCIs, environmental drivers can be masked when different order and

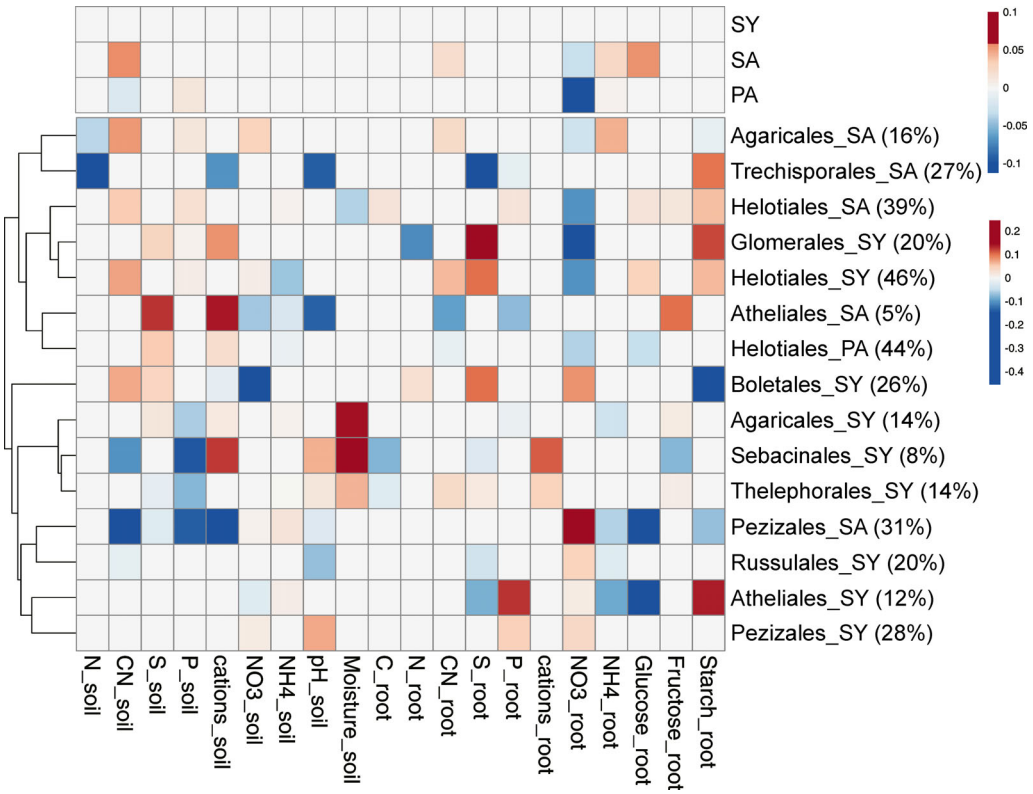

Fig 2. SCI between the abundance of root-associated fungal orders and guilds with soil and root chemistry.
Percent variance explained by soil and root variables is shown in parentheses. Orders with a relative abundance >2% were included in the analysis. All taxa per guild together (SY = symbiotroph, SA = saprotroph, PA = pathotroph). Rows for groups according to guild and order were clustered using correlation distance and average linkage. Blue colours indicate negative SCIs and red colours indicate positive SCIs. [Color figure can be viewed at wileyonlinelibrary.com]

functional guilds are analysed together or SCIs can be enhanced, when the responses of different groups are similar.

To test whether the responses of orders were more dissimilar than the guilds within an order, we used the three orders for which several symbiotrophic and saprotrophic species (OTU-based) were available (Agaricales, Heliotales, Pezizales). Two-way permutational multivariate analysis of variance (PERMANOVA) showed that the SCIs differed significantly among orders and guilds but showed no interaction (Table 3). Comparing symbiotrophic orders among each other (PERMANOVA), we found significant differences between Agaricales and Heliotales ($p = 0.010$), a marginal difference between Agaricales and Pezizales ($p = 0.067$), and no significant difference between the Ascomycota Heliotales and Pezizales ($p = 0.132$). No differences were found for SCIs of the saprotrophs in the order of Agaricales, Heliotales and Pezizales ($p > 0.05$).

Comparing the effect sizes of root- and soil-related factors showed that symbiotrophs in four orders had stronger root than soil responses (Atheliales, Glomerales, Boletales and Helotiales) but three orders (Thelephorales, Agaricales and Sebacinales) showed stronger soil than root effects (Fig. 3). Comparing the effect sizes of root and soil on the

Table 3. Two-way PERMANOVA for SCI of Agaricales, Heliotales and Pezizales according to order and guild.

Factor	Sum of squares	*df*	Mean square	*F*	*p*
Guild	0.209	1	0.209	1.026	**0.030**
Order	0.360	2	0.180	0.884	**0.031**
Interaction	−9.013	2	−4.507	−22.13	0.268
Residual	20.76	102	0.204		
Total	12.32	107			

Permutation *N* = 9999.

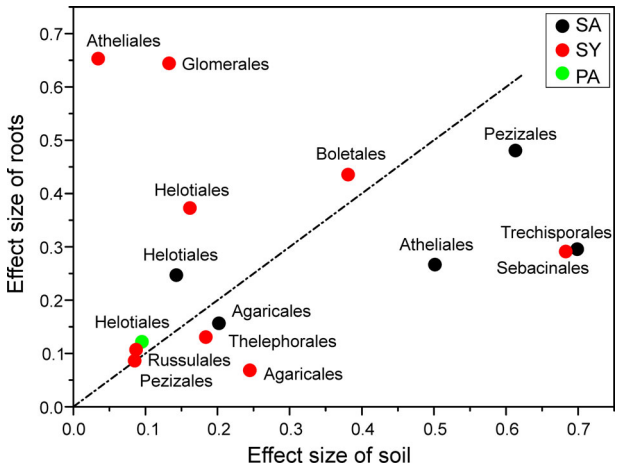

Fig 3. Effect sizes of soil and root factors, based on SCI of root-associated fungal orders and guilds.
Orders with a relative abundance >2% were included in the analysis. SY = symbiotroph, SA = saprotroph, PA = pathotroph. The hatched line separates root (upper) and soil (lower) effects. [Color figure can be viewed at wileyonlinelibrary.com]

symbiotrophs in our study, no significant differences were found (paired rank test, $p = 0.406$). Similarly, there were dominant soil effects for saprotrophs in distinct orders (Atheliales, Trechisporales, Pezizales) (Fig. 3), but the overall comparison of root and soil effect sizes did not reveal a clear dominance of either compartment in the group of the saprotrophs (paired rank test, $p = 0.208$).

Discussion

Habitat filtering and root traits explain divergent structures of root-associated fungal communities in different temperate forest regions

We found a strong taxonomic differentiation of RAF communities among the three forest regions. Soil N and pH were strong drivers of those dissimilarities, in agreement with other studies on soil and root fungi in forest ecosystems (Philippot *et al*., 2013; Zhang *et al*., 2013; Moeller *et al*., 2014; Walker *et al*., 2014; Goldmann *et al*., 2015, 2016; Sterkenburg *et al*., 2015; Glassman *et al*., 2017; Kolaříková *et al*., 2017; Maghnia *et al*., 2017). It is further known that vegetation composition influences mycorrhizal fungal communities (Ishida *et al*., 2007; Lang *et al*., 2011; Bahnmann *et al*., 2018; van der Linde *et al*., 2018). Significant relationships among tree species, root nutrient chemistry and mycorrhizal diversity were found (Lang and Polle, 2011). Here, beech (*Fagus sylvatica*) was the dominant species across all plots and previous studies indicated approximately 70% beech roots in topsoil samples (Pena *et al*., 2017). Therefore, the variation in root resources in the present study might have been caused in parts by plant species composition, but a detailed dissection of host effect was beyond the scope of this work.

In addition to soil moisture, soil NO_3^-, basic cations and P were significant factors, explaining the separation of fungal assemblages among different regions. In agreement with other studies (Zavišić *et al*., 2016) P had less effects on the RAF community structure than N. Different N forms are known to influence mycorrhizal assemblages (Kranabetter *et al*., 2015). Still, the effects of soil NO_3^- and NH_4^+ in the current study may appear surprising because the concentrations of inorganic N in soil show rapid temporal fluctuations (e.g., Dannenmann *et al*., 2009) and one time measurements of inorganic N may not reflect soil N availability (Binkley and Hart, 1989). However, available inorganic nitrogen forms are generally linked with soil pH and prevalent as NH_4^+ in acid and as NO_3^- in calcareous environments (Lapeyrie *et al*., 1987; Dames *et al*., 1999) as observed here as well. Earlier measurements in a subset of our plots also reported higher NO_3^- in less acidic soils (A region) and higher NH_4^+ in more acidic soils (S region) (Pena *et al*., 2017). Recently, we repeated the measurements of inorganic N across all 150 plots and confirmed higher concentrations NO_3^- in the A and lower in the S region; vice versa, we found higher NH_4^+ in the S and lower in the A region (Polle and Pena, unpublished results). Furthermore, the observed regional differences of NO_3^- and NH_4^+ in soils also correspond to higher deposition of NO_3^- and NH_4^+, respectively, in these areas (Schwarz *et al*., 2014). Collectively, these reports underpin long-term differences in the inorganic N forms in the soils and, thus, support the role of inorganic N forms as drivers of RAF community structure in temperate forests.

In general, high N deposition results in reduced mycorrhizal species richness (Lilleskov *et al*., 2002a; Lilleskov *et al*., 2008; Cox *et al*., 2010; Suz *et al*., 2014; de Witte *et al*., 2017), while naturally nitrogen-rich soils can also show high mycorrhizal diversity (Kranabetter *et al*., 2009). Here, we found higher fungal symbiotrophic species (OTU) richness in forests with higher inorganic N concentrations in soil than in those with lower N availabilities, which might have been caused by positive effects of moderate N increases, in agreement with other studies (Lilleskov *et al*., 2002a; Cox *et al*., 2010; van der Linde *et al*., 2018). Other explanations are also possible. For example, mycorrhizal fungi are known to be sensitive to elevated temperatures (Marx *et al*., 1970; Sánchez *et al*., 2001; Lazarević *et al*., 2016; Mucha *et al*., 2018) and drought (Leberecht *et al*., 2016a; Taniguchi *et al*., 2018). Since the region with reduced RAF species richness also exhibited lower soil moisture and higher temperatures, we suggest that these conditions might have played a critical role in reducing OTU richness in RAFs in warmer, drier regions than in cooler, moist climate.

The importance of the vegetation structuring mycorrhizal communities has often been shown (e.g., plant community composition: Wubet *et al*., 2012; stand type and management: Pena *et al*., 2017; Bahnmann *et al*., 2018; host identity: van der Linde *et al*., 2018). Our study goes a step further towards a mechanistic understanding of the influence of the vegetation community on the fungal communities by testing if variation in root chemistry (which we expect to vary with environment and host community) is an important driver of fungal community structure. Beside soil factors, root nutrient resources (carbohydrates, C, NO_3^-, N, cations) also exhibited significant relationships with the composition of RAF assemblages supporting our initial hypothesis. While the abiotic environment is generally a driver of the fungal communities, root features and mycorrhizal functions can be both response or effect traits (Koide *et al*., 2014). Girdling studies revealed that root carbohydrates affect the mycorrhizal species spectrum on roots (Druebert *et al*., 2009; Pena *et al*., 2010; Pena and Polle, 2014) and, therefore, root chemistry can be considered as a driver of fungal communities. However, root nutrient status and fungal nutrient demand can influence each other (Markkola *et al*., 2004; Valtanen *et al*., 2014; Leberecht *et al*., 2016a) and thereby may affect the direction of the response. Here, root carbohydrates as well as root N and cations were strong explanatory variables for the separation RAF assemblages among different regions. Overall, our results highlight relationships of soil habitat and root carbon and nitrogen contents with RAF community structure.

Complex interactions of fungal taxa with root traits and soil properties structure the fungal assemblages

The RAF communities in this study were composed of symbiotrophic, saprotrophic and pathotrophic guilds, in which ectomycorrhizal fungi, i.e., symbionts, formed the dominant group. This composition was expected in agreement with other investigations of root-residing fungi in temperate forests (Goldmann *et al*., 2016; Kolaříková *et al*., 2017; Schröter *et al*., 2019). Here, we tested the hypothesis that different trophic guilds of fungi harbour signatures related to features of their ecological niche, i.e., that the saprotrophic guild would be less affected by root than by soil properties, whereas the symbiotrophic guild would show strong root and less soil impact. However, in contrast to this expectation, the saprotrophic guild showed a higher number of significant interactions with root (C/N, NO_3^-, NH_4^+, glucose) than with soil properties, whereas significant soil and root effects on the whole assemblage of symbiotrophs were absent. Fungal biomass of saprotrophs was also stronger affected by plant than by soil factors, while these factors had less impact on symbiotrophs (Awad *et al*., 2019). The reason that we found no significant effects on the whole group of symbiotrophs could be related to the fact that the fungal guilds were composed of different orders, each being characterized by unique response patterns to abiotic drivers and roots traits. For example, for symbiotrophs in distinct orders, soil pH was an important driver, but in opposing directions, negative for Russulales and positive for Pezizales and Sebacinales. As a result, in composed assemblages, contrasting effects may neutralize each other or disappear when the abundance of pH-dependent orders is low compared with dominant orders such as the Agaricales and, thus, explain the apparent pH independence of guilds. Our findings underpin that evolutionary differences among distinct fungal groups determine their responses to abiotic filters and root traits. Along similar lines, Treseder and Lennon (2015) reported that maximum variance of fungal traits related to N and P transformation could be explained at the levels of order to phylum. These results are also compatible with the general principle of niche-selection filtering (Chase and Myers, 2011), which results, for instance, in a stronger phylogenetic clustering of mycorrhizal fungi in the drier and more acid habitat than under more favourable, moist soil conditions (Pena *et al*., 2017). These considerations imply that the relationship of a guild with habitat filters such as that of the symbiotrophic fungi with root NO_3^- and NH_4^+ is the outcome of the combination of the characteristics of the taxa that make up that assemblage and thus, will vary, if the community composition was changed. Thus, the drivers of any multi-taxa trophic guild in RAFs must be context-dependent.

In contrast to guilds as a whole, which showed mainly moderate or no responses to different soil- or root-related variables, our analyses of fungal orders uncovered larger differences for the standardized effects of root traits and environmental filters. Glomerales were most strongly responsive to roots traits, in particular to root NO_3^-. Negative effects of elevated soil nitrogen levels on arbuscular mycorrhizal fungi have often been reported (Bradley *et al*., 2006; Rodríguez-Echeverrá *et al*., 2017; Xu *et al*., 2017; Ceulemans *et al*., 2019), but this effect can be counteracted by higher richness and land cover of arbuscular mycorrhizal plants in more productive soils (Kranabetter and MacKenzie, 2010). The present results suggest that at the plot level, root nitrogen may be more important than soil nitrogen levels for the abundance of arbuscular mycorrhizal fungi. In contrast to the Glomerales, the groups of ectomycorrhizal symbionts and saprotrophs in different orders did not show a clear dominance of root-respective soil-related factors but were distinguished by distinct patterns of interaction coefficients. The most frequent discriminating factor was root NO_3^- (10/15 groups), followed by root starch and sulfur besides soil basic cations and NH_4^+ (8/15 groups). While root starch might have been

expected, the importance of root NO_3^- was surprising. We can currently only speculate that this result might have been caused by different fungal abilities to use and translocate NO_3^- (Leberecht *et al*., 2016b; Nguyen *et al*., 2017). In general, fungal species grow well on NH_4^+ (Finlay *et al*., 1992; Keller, 1996; Yamanaka, 1999), whereas the fungal efficiencies to utilize NO_3^- vary strongly among taxa (Nygren *et al*., 2008). The main agaricoid fungi in our study, *Laccaria* and *Hebeloma* can use both inorganic N sources (Yamanaka, 1999, 2003), but for important russoloid taxa (*Russula* sp., *Lactarius* sp.) divergent results have been reported. *Russula* spp. are nitrophilic species (Lilleskov *et al*., 2002b), but their growth was inhibited by NO_3^-; *Lactarius* spp. transform NO_3^- into NH_4^+ and exude this compound into the medium (Nygren *et al*., 2008). In agreement with lower NO_3^- usage, Russulales were dominant in regions with low soil NO_3^- and high soil NH_4^+ concentrations, explaining differences in RAF community composition. Roots showed inverse NO_3^- and NH_4^+ patterns, which might have been caused by complementary resource use.

Taken together, our results support that adaptation to distinct ecological niches drive RAF community structure. Symbiotrophic fungi in different orders showed divergent patterns in response to root and soil chemistry. Symbiotrophic taxa in several orders, especially the Glomerales, which reside inside roots, were more strongly affected by root than by soil factors. Differences at the level of fungal orders suggest that widely conserved properties dominated the responses. This conclusion is supported by conserved genes for functional traits at higher phylogenetic levels (Amend *et al*., 2016; Treseder and Lennon, 2015) that could explain trait differences among the orders found here. However, in contrast to our initial hypothesis, the symbiotrophic and saprotrophic guilds were not distinguished by the effect sizes of root- or soil-related factors. The mechanisms that result in the segregation of RAFs across temperate forests remain speculative because our knowledge on fungal traits and their nutritional preferences is still very limited. To better understand the outcome of ecological interactions, comprehensive analyses of fungal species and their traits are required. In combination with the current genome projects on ectomycorrhizal fungi, which provide information on the molecular tool boxes of distinct taxa (Grigoriev *et al*., 2014), these efforts will greatly be expedited.

Experimental procedures

Study site description

This study was conducted in the Biodiversity Exploratories (http://www.biodiversity-exploratories.de/startseite/) encompassing three biogeographic regions located in the south-west (Schwäbische Alb), centre (Hainich-Dün) and north-east (Schorfheide-Chorin) of Germany (Fischer *et al*., 2010). Key bioclimatic and geographic data have been summarized in Table S1. In addition to climatic differences, the soil types differ among the regions with loamy soils in A and silty soils in S (Table S1). In each region, 50 forest plots (100 × 100 m) were established. The locations of the plots can be found at http://www.biodiversity-exploratories.de/exploratories/. The plots are dominated by Fagaceae (*Fagus sylvatica* or *Quercus* sp.) or Pinaceae (*Picea abies* or *Pinus sylvestris*), with a mean age of 114 years.

Root and soil sampling

In early May 2014, 150 mineral topsoil samples (0–10 cm) and 150 root samples were collected in 150 forest plots as follows: in each forest plot, two transects of 40 m length were established. Fourteen soil cores were taken with a split tube (56 mm in diameter) along the transects from north to south and from west to east at the sampling points 2.5, 8.5, 14.5, 20.5, 26.5, 32.5 and 38.5 m from the starting points. When present, the forest floor was removed before sampling. The collected soil cores were mixed yielding one a composite sample per plot. An aliquot of soil from the composite sample was used for element analysis. We used 200 g of the composite sample to separate soil and roots by sieving. A sieved soil sample was kept for further analyses. The roots were gently washed with cool tap water, the fine roots cut off, immediately frozen in liquid nitrogen in the field and stored at −80 °C. Sorting of roots according to vitality was not possible, but previous analyses showed no differences in root tip vitality among the different regions (Pena *et al*., 2017).

Analysis of root and soil carbon, nitrogen and mineral nutrient elements

Aliquots of fine roots were dried at 60°C for 1 week and ground to a fine powder with a ball mill (Type MM400, Retsch GmbH, Hann, Germany). Aliquots of 0.7–1 mg were weighed into 4 mm × 6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) using a microbalance (Model: Cubis MSA 2.7S-000-DM, Sartorius, Göttingen, Germany). Samples were analysed in an Elemental Analyzer (Model SHNC-O EA1108, Carlo Erba Instruments, Milan, Italy). Acetanilide (71.09% C, 10.36% N) was used as the standard for quantification of N and C. Total carbon and nitrogen contents in soils were analysed on ground subsamples by dry combustion in a CN analyser 'Vario Max' (Elementar Analysensysteme GmbH, Hanau, Germany). Mineral element concentrations of Ca, K, Mg, P and S were determined using iCAP

6300 Duo VIEW ICP Spectrometer (Thermo Fischer Scientific GmbH, Waltham, MA, USA) after pressure digestion of samples in 65% HNO_3 for 12 h (Heinrichs *et al*., 1986).

Determination of non-structural carbohydrates

Non-structural carbohydrates in fine roots were determined enzymatically by NADPH production at the wavelength of 340 nm in a spectrophotometer (Type UV-DU640, Beckmann, CA, USA) (Schopfer, 1989). Briefly, frozen root samples were milled and extracted in dimethyl sulfoxide and 25% HCl (80:20, vol:vol). For the assay, the following enzymes were used: 10 µl hexokinase/glucose-6-phosphate dehydrogenase (3 mg ml^{-1}, 340 U hexokinase ml^{-1} and 170 U glucose-6-phosphate dehydrogenase ml^{-1}; Roche Diagnostics GmbH, Mannheim, Germany) for glucose determination, 5 µl phosphoglucose isomerase (10 mg ml^{-1}, 350 U mg^{-1}; Roche Diagnostics GmbH) for fructose and 10 µl of invertase (30 mg ml^{-1}, 200–300 U mg^{-1}; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) for sucrose. For starch analysis, amyloglucosidase [(10 mg ml^{-1}, 14 U mg^{-1}; Roche Diagnostics GmbH) in 50 µM citrate buffer pH 4.6 (1:5, vol:vol)] was used to convert starch to glucose and then the glucose assay was used.

Determination of soil and root ammonium and nitrate

Ammonium (NH_4^+) and nitrate (NO_3^-) of soil samples were extracted from 20 g of fresh soil in 40 ml of 1 mM $CaCl_2$ solution. The samples were thoroughly shaken for 10 min and filtered for 1 h using folded filter papers MN 280 (Macherey-Nagel, Düren, Germany). To remove all organisms, the filtrates were further filtered through a 0.2-µm sterile Corning® syringe filters (CLS431218 Sigma, Sarstedt, Nümbrecht, Germany) and then kept at −80°C until further analysis. The ammonium and nitrate concentrations in the filtrate were spectrophotometrically analysed using ammonium (#100683) and nitrate (#109713) test kits (Merck, Darmstadt, Germany) following the manufacturer's instructions.

Fifty micrograms of frozen root powder were extracted in 0.4 ml of methanol (VVA, Cologne, Germany) and chloroform (Th. Geyer GmbH, Renningen, Germany) (3.5:1.5, vol:vol) as described by Winter *et al*. (1992). After incubation at 40°C for 60 min, 0.2 ml demineralized water was added to the sample solution, shaken and centrifuged for 5 min at 5000 rpm (Model 5417R, Eppendorf AG, Hamburg, Germany). The lower hydrophilic phase was collected and extraction with water repeated twice. The hydrophilic phases were merged and dried in a vacuum concentrator (Model 5301, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) at 35°C. The residue was resuspended in 400 µl demineralized water and centrifuged shortly at 5000 rpm (Model 5417R, Eppendorf AG). The supernatant was used for ammonium and nitrate determinations as above.

Determination of soil pH and soil moisture

Twenty-five millilitres of 0.01 M $CaCl_2$ was added to 10 g soil. The soil pH was measured with a WTW pH meter 538 (Wissenschaftlich-Technische-Werkstätten GmbH, Germany). Two measurements per sample were taken, and final value was calculated as the average value of the two measurements. To determine soil moisture, soil samples were dried at 105°C to a constant weight. Weights were taken before and after soil drying. The percentage of water in soil was calculated as follows:

$$\text{Soil water content}\,(\%) = \frac{(\text{moist soil weight} - \text{dry soil weight})}{\text{dry soil weight}} \times 100$$

DNA extraction and amplification of internal transcribed spacer region 2

Frozen fine roots were ground in a ball mill (Type MM400, Retsch). Total DNA from roots was extracted using the innuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany) according to the manufacturer's instructions. Fungal ribosomal internal transcribed spacer (ITS) region 2 was amplified with a Mastercycler Gradient (Eppendorf AG, Hamburg, Germany) using the forward primer ITS3_KYO2 (Toju *et al*., 2012) and the reverse primer ITS4 (White *et al*., 1990). Primers were purchased from Microsynth AG (Balgach, Switzerland). Details of the PCR conditions and purification have been reported as an addendum to Table S2. Purified PCR products were quantified using a Qubit dsDNA HS assay Kit in a Qubit 3.0 Fluorometer (Thermo Fischer Scientific, Dreieich, Germany) and pooled at equimolar concentrations for sequencing. Amplicons were sequenced using the dual index paired-end approach (v3, 2 × 300 bp) for the MiSeq platform (Illumina).

Sequence processing and analyses

Demultiplexing of raw sequences was performed by CASAVA data analysis software (Illumina). Paired-end sequences were merged using PEAR v0.9.10 (Zhang *et al*., 2014) with default parameters. Subsequently, we used Quantitative Insights into Microbial Ecology (QIIME) v1.9.1 (Caporaso *et al*., 2010) with the *split_library_fastq.py* script to remove the sequences with an average quality (Phred) score lower than 20 or containing unresolved nucleotides. For the removal of reverse and forward

primer sequences, cutadapt v1.12 (Martin, 2011) with default settings was applied. Before OTU clustering, we employed USEARCH v9.2.64 (Edgar, 2010) with the UPARSE (Edgar, 2013) algorithm to remove sequences shorter 140 bp, discard singleton reads, merge identical sequences (dereplication) and sort sequences by cluster size. OTUs were clustered at 97% sequence identity using USEARCH. Chimeric DNA sequences were detected and removed using UCHIME2 algorithm (Edgar *et al*., 2011) with a reference data set from the Unite database Version 7.1 (Abarenkov *et al*., 2010), available at https://unite.ut.ee/repository.php. The merged paired-end sequences were mapped to chimera-free OTUs. The OTU table was generated using USEARCH. Taxonomic assignment of OTUs was performed with *parallel_assign_taxonomy_blast.py* against the UNITE database, version 7.1. Extrinsic domain OTUs and unclassified OTUs were removed from the data set by employing *filter_otu_table_py*. This procedure resulted in a total of 5,030,679 quality-filtered reads, which were clustered into 4765 OTUs. All unidentified fungal OTUs were searched by blastn against the nt database of NCBI GenBank (May 2017) to remove non-fungal OTUs (especially plant sequences), and only fungal classified reads were kept. Thereby, we acquired 4,758,344 fungal reads that were grouped into 3815 OTUs. The highest number of reads in a sample was 101,929, while the lowest number was 8471 reads. On average, there were 31,722 fungal reads per sample. For comparison, all samples were rarefied by random selection to the same number of reads utilizing the lowest number of sequences present in one of the samples (total 8400 reads). The sequences were deposited in sequence read archive under the accession number SRP140604. The OTUs were assigned to functional guilds using the software FUNGuild (Nguyen *et al*., 2016) (Table S2). An overview on reads per order and life style is shown in Table S6.

Statistical analysis and calculations

Statistical analyses were conducted using R statistical software version 3.4.1 (R Core Team, 2017). Data distribution and homogeneity of the variance were checked by visual inspection using histograms and residual plots. When the data did not show a normal distribution, the data were log-transformed for statistical analyses. We used ANOVA to compare the means of soil and root variables among the three biogeographical regions. Generalized linear models (Poisson regression, chi-square test) were used to compare the means of count data-related variables such as fungal OTU richness and read abundance among the study regions. Pairwise differences between study regions were compared using Tukey's honestly significant difference test. Differences between saprotrophs and symbiotrophs in the same fungal order were analysed by the paired-rank Kruskal–Wallis test. Two-way and one-way PERMANOVA were conducted on the matrix of interaction coefficients (see below) to test similarities between different fungal orders and guilds (using correlation as the distance measure). Non-metric multidimensional scaling (NMDS) ordination of RAF fungal communities was conducted using function metaMDS () in 'vegan' package (Oksanen *et al*., 2018) with three dimensions and 100 iterations. The root and soil-related explanatory variables were fitted to NMDS plots using the envfit() function of the 'vegan package'. Only the vectors of significant correlations ($p < 0.05$) with the ordination (random 999 permutation steps of the data) were plotted (Oksanen *et al*., 2018).

To obtain a meaningful statistical assessment of root and soil effects, the a priori selection of key variables in a balanced design is required (Borcard *et al*., 2011). With the exception of soil pH for which no corresponding parameter was available, all other parameter were mirrored in roots and soil. The contribution of root and soil factors and their combination in explaining the total variation in the fungal community response was assessed using the function varpart() in the 'vegan' package. The analysis was conducted using the two explanatory matrices, root and soil factors and the OTU abundance response matrix. The variation partitioning between the root and soil variables was based on redundancy analysis ordination models using permutational ANOVA ($p < 0.05$; 999 permutations). Abundance data were Hellinger-transformed. The contribution of each fraction was evaluated by adjusted R^2 (Peres-Neto *et al*., 2006). The relationship between the specific root and soil variables and taxa abundance was further investigated by an integrated multivariate species distribution model that analyses the taxa distribution based on their abundance, assuming different response for different taxa (Brown, 2014). This analysis was computed in the 'mvabund' packages in R (Wang *et al*., 2012) using the trait generalized linear model, function traitGLM(), where the trait argument was the taxa ID factor. The Least Absolute Shrinkage and Selection Operator (LASSO) penalty was included in the function by 'glm1path' method. The LASSO penalty shrinks the regression coefficients with a minor contribution to the model towards zero by penalizing the regression model with an L1-penalty term (Tibshirani, 1996; Warton *et al*., 2015). The LASSO approach provides both the model selection and parameter estimation to determine the contribution of each explanatory variable to taxa distribution (Warton *et al*., 2015). All variables were standardized by default; therefore, the size of interaction coefficients (standardized coefficients of interaction, SCIs) between the OTU abundance and explanatory variables can be interpreted

as a measure of importance. SCI matrices were clustered and visualized in ClustVIS (https://biit.cs.ut.ee/clustvis/) without any transformation. Both rows and columns were clustered using correlation distance and average linkage (Metsalu and Vilo, 2015).

Where applicable, data in tables and figures are shown as the mean ± standard error. p-value ≤0.05 was used to indicate a significant difference in statistical analyses.

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Author contributions

D.Q.N. sampled fine roots, conducted the root measurements, extracted DNA and prepared DNA amplicons, analysed data and wrote the first draft of the manuscript. N.B. supervised and supported DNA extraction and PCR. DS and RD performed amplicon sequencing and bioinformatic analysis. B.S. and R.P. measured soil NH_4^+ and NO_3^- concentrations. R.P. analysed data. I.S. measured soil C, N, pH and moisture. D.J. conducted statistical data analyses. A.P. designed the study and supervised data analyses. A.P. and R.P. revised the manuscript. All authors commented on and approved the final version of the manuscript.

Data Availability Statement

All data have been deposited in the BExIS database (https://www.bexis.uni-jena.de) under the following accession numbers (data owner): root-related parameters 22967, 22968, soil nutrient ions 26066 (Polle); soil NO_3^- and NH_4^+ 19966 (Pena); soil-related parameters 18386, 18787, 19067 (Schöning). The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Correlation between soil moisture and annual mean temperature in forest plots across Germany in 2014. Pearson correlation coefficient (r) with the associated *P* value is shown

Table S1. Key biogeographic characteristics of three study regions. (*Source*: Fischer et al. 2010 and http://www.

biodiversity-exploratories.de/exploratories/) with additional information on atmospheric N input determined on nine plots per region (Schwarz *et al*., 20142014).

Table S2. Master table with fungal OTU counts and taxonomic and guild assignment is presented in an additional excel file.

Table S3. Statistical information for Fig. 1A (NMDS) and Fig. 1B (Fungal abundances).

Table S4. Fungal orders found in roots collected in three biogeographical regions. A = Schwäbische Alb. H = Hainich-Dün and S = Schorfheide-Chorin. Significant differences in the abundance of fungal orders at $p < 0.05$ are indicated with bold letters. Data are means of n = 50 plots per region ± SE. Statistical analyses of fungal orders with an abundance of less than 0.1% were shown as NA (not available).

Table S5. Standardized Coefficients for Interaction (SCI) between the abundance of root-associated fungal orders and guilds, symbiotroph (SY), saprotroph (SA) and pathotroph (PA), with soil and root chemistry. Orders with a relative abundance > 2% were included in the analysis.

Table S6. Taxa and number of reads used for the analyses presented in this study.