



# Plant functional group drives the community structure of saprophytic fungi in a grassland biodiversity experiment

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## Abstract

**Aims** Saprophytic fungi are important agents of soil mineralization and carbon cycling. Their community structure is known to be affected by soil conditions such as organic matter and pH. However, the effect of plant species, whose roots provide the litter input into the soil, on the saprophytic fungal community is largely unknown.

**Methods** We examined the saprophytic fungi in a grassland biodiversity experiment with eight plant species belonging to two functional groups (grasses and forbs),

combining DNA extraction from plant roots, next-generation sequencing and literature research.

**Results** We found that saprophyte richness increased with plant species richness, but plant functional group richness was the best predictor. Plant functional group was also the main factor driving fungal saprophytic community structure. This effect was correlated with differences in root lignin content and C:N ratio between grasses and forbs. In monocultures, root traits and plant functional group type explained 16% of the variation in

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Davide Francioli and Sophie Q. van Rijssel contributed equally to this work.

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community structure. The saprophyte taxa detected in mixed plant communities were to a large extent subsets of those found in monocultures.

**Conclusions** Our work shows that the richness and community structure of the root-associated saprophytic fungi can largely be predicted by plant functional groups and their associated root traits. This means that the effects of plant diversity on ecosystem functions such as litter decomposition may also be predictable using information on plant functional groups in grasslands.

**Keywords** Decomposition · Fungal saprophytes · Grasslands · Plant functional group · Plant species richness · Root traits

## Introduction

Decomposition of plant tissue in terrestrial ecosystems regulates the transfer of carbon and nutrients to the soil (Chapin et al. 2011). The major factors involved in decomposition are soil abiotic conditions (Hättenschwiler et al. 2005; Silver and Miya 2001), plant tissue quality (Chen et al. 2017; Hobbie 1992; Jacoby et al. 2017; Veen et al. 2019) and the decomposer community (Berg 1986). The physical breakdown of plant tissue is done by metazoans, such as earthworms and mites (Eisenhauer et al. 2012), after which the saprophytic fungi perform the initial steps in the decomposition of cellulose, lignin and other complex macromolecules (Berg and McLaugherty 2014a; Gessner et al. 2010). The resulting compounds will be further processed by bacteria (Berg and Laskowski 2005; Kardol and De Long 2018). Hence, saprophytic fungi are generally considered a primary engine of the decomposition process (Maltz et al. 2017; Setälä and McLean 2004).

In grassland ecosystems, the saprophytic community is driven by the quantity of dying and decaying roots, which constitute more than half of the plant biomass (Berg and McLaugherty 2014b; Jackson et al. 1996; Poorter et al. 2012) and are a major organic matter input in soils (Lehmann and Kleber 2015; Silver and Miya 2001). It is well known from biodiversity studies in grasslands that root biomass (Cong et al. 2015; Fargione et al. 2007; Mueller et al. 2013; Oram et al. 2018; Ravenek et al. 2014) and soil organic matter content (Cong et al. 2014; Fornara and Tilman 2008; Lange et al. 2015) increase with plant species richness. This increased quantity of substrate may affect the saprophytic fungal community

(Bray et al. 2012; Cline and Zak 2015). Next to an increased quantity of substrate, the saprophytic community is driven by the *quality* of the substrate. Plant species vary in their chemical root traits, such as carbon, nitrogen and lignin content (Melillo et al. 1982; Roumet et al. 2016; Schroeder-Georgi et al. 2016; Taylor et al. 1989). For example, Chen et al. (2017) showed that the decomposition rate of standard root litter was significantly lower in plant communities with grasses than without grasses, suggesting that these different plant communities harbour different saprophytic fungal communities. Plant species richness may also affect the root litter quality (De Deyn et al. 2011; Lunghini et al. 2013; Santonja et al. 2017; Schuldt et al. 2018). Highly diverse plant communities may harbour more ecological niches to be occupied by saprotrophic fungi due to an increased diversity of organic substrates entering soils (Grayston et al. 1998; Meier et al. 2008; Waldrop et al. 2006; Zak et al. 2003).

Here we ask what are the best predictors for the saprophytic fungal community: plant species, plant functional groups or plant traits, by investigating the composition of the saprophytic fungi in the tenth year of a grassland biodiversity experiment (van Ruijven and Berendse 2005). Specifically, we studied the saprophytic fungi associated to the plant roots as they may play a pivotal role in dead root decomposition and mineralization, because they are already present on the living roots. The plant communities in the long-term field experiment investigated differed in species richness and composition, ranging from monocultures of two functional groups (grasses and forbs) to mixtures of eight plant species. We hypothesized that: (i) saprophytic fungal community richness will increase with increasing richness of plant functional groups and species, (ii) saprophytic fungal community structure in monocultures will differ between plant functional groups and species, and (iii) saprophytic fungal community structure in mixtures can be predicted from plant functional group and species composition.

## Materials and methods

### Experimental design and root collection

The Wageningen Biodiversity Experiment (Wageningen, the Netherlands) was established in March 2000. Briefly, this experiment is a randomised block design (six blocks), with plant species richness (one, two, four and eight species) as the main factor. Plant communities

comprised the following species: the grasses *Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L. and *Holcus lanatus* L. and forbs *Centaurea jacea* L., *Leucanthemum vulgare* Lamk., *Plantago lanceolata* L. and *Rumex acetosa* L.. For more information about the experiment, see van Ruijven and Berendse (2005). Here, we used 74 plots affiliated to the Wageningen Biodiversity experiment: six plots with the mixture of all eight plant species (8mix), 22 plots containing grasses and forbs in four-species mixtures: five plots with three grasses and one forb (4mix-3G-1F), 12 plots with two grasses and two forbs (4mix-2G-2F) and five plots with one grass and three forbs (4mix-3F-1G), and 16 plots with two plant species, divided into nine plots with a grass and forb species (2mix-1G-1F), four plots with only grasses (2mix-2G) and four plots with only forbs (2mix-2F). Finally, we also included 30 plots with one species (monocultures, four plots per plant per species, except for *H. lanatus* and *R. acetosa* with three plots).

In June 2010, two soil cores (30 mm diameter, 50 cm deep) were taken (30 cm apart) in each plot. For fungal community analyses in roots, we used two soil layers: 0–5 cm (shallow layer) and 20–35 cm (deep layer). The root material collected inevitably was a mixture of dead and living roots. However, due to root washing, albeit careful, the most decayed, slimy parts have been removed. Soil samples were sieved through a 2 mm mesh. Root and soil samples were kept at 4 °C for chemical analyses and at –80 °C for molecular analyses. The soil samples of the shallow layer collected in all the plots were used to

measure total organic C (TOC) and total N (TN), soil pH, and soil organic matter (SOM). The root samples from the shallow layer in the monoculture plots were used to measure root C and N and lignin content (Table 1). Soil and root chemical analyses were conducted as reported in Cong et al. (2014) and Elle et al. (2019), respectively.

#### Molecular analyses of the root associated saprophytic fungi

DNA from 80 to 100 mg of plant roots per sample (and thus also DNA of the root-associated fungi) was extracted from the 0–5 and 20–35 cm core fractions using the DNeasy Plant mini kit (Qiagen). Fungal DNA amplification was performed using the forward primer ITS1F and reverse primer ITS2 (White et al. 1990) and characterized using 454 GS FLX pyrosequencing by Plant Research International, Wageningen UR, Wageningen, the Netherlands. Reads were analysed using the QIIME pipeline and its associated modules (Caporaso et al. 2010). Subsequent procedures regarding read filtering, chimera removal, OTUs clustering were performed using the bioinformatics pipelines described in Mommer et al. (2018) and available via Dumbrell et al. (2017). Representative sequences of each OTU were assigned taxonomy by comparing each OTU's representative sequence against sequences from the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST, Altschul et al. 1990). Fungal OTU accumulation curves were computed using

**Table 1** Soil and root properties measured in the shallow layer in the monoculture plots

Plant community	Soil parameters					Root parameters	
	pH	TOC (g/kg)	TN (g/kg)	C/N	SOM (%)	Root lignin	Root C/N
<i>A. capillaris</i>	6.2(0.1)	70.0(6.5)	8.2(0.9)	8.6(0.6)	1.8(0.3)	6.2(0.1) <sup>a</sup>	57.4(2.7) <sup>a</sup>
<i>A. odoratum</i>	6.4(0.1)	76.4(3.4)	8.9(0.5)	8.5(0.3)	1.8(0.5)	5.7(0.3) <sup>ab</sup>	43.5(0.8) <sup>b</sup>
<i>F. rubra</i>	6.2(0.1)	71.1(3.2)	7.1(0.2)	10.0(0.5)	1.7(0.1)	6.5(0.3) <sup>a</sup>	42.8(0.7) <sup>b</sup>
<i>H. lanatus</i>	6.1(0.2)	72.4(5.5)	9.2(1.1)	7.9(0.8)	1.6(0.1)	6.8(0.4) <sup>a</sup>	37.4(1.6) <sup>c</sup>
<i>C. jacea</i>	6.3(0.2)	79.7(3.4)	9.6(1.3)	8.7(1.0)	1.8(0.1)	4.1(0.1) <sup>cd</sup>	33.2(1.1) <sup>cde</sup>
<i>L. vulgare</i>	6.3(0.1)	71.5(6.4)	8.5(1.4)	8.8(0.8)	1.6(0.03)	4.0(0.3) <sup>cd</sup>	34.2(1.6) <sup>cd</sup>
<i>P. lanceolata</i>	6.3(0.1)	73.2(2.7)	7.9(0.4)	9.2(0.7)	1.7(0.1)	4.7(0.7) <sup>bc</sup>	31.1(1.5) <sup>de</sup>
<i>R. acetosa</i>	6.4(0.1)	69.2(3.2)	7.7(0.7)	9.2(0.8)	1.6(0.1)	3.1(0.6) <sup>d</sup>	29.8(0.4) <sup>c</sup>

Values are means and standard error of the means are indicated in parentheses

Abbreviations: TOC total organic carbon, TN total nitrogen, SOM soil organic matter

Different letters denote significant ( $P < 0.05$ ) differences between plant species

rarefaction and visually inspected. In general, most samples had OTU accumulation curves that were beginning to reach an asymptote and for those that did not, the rates of OTU accumulation were not significantly different from those in other samples, indicating statistical comparisons at lower sequencing intensity were unlikely to be qualitatively different to those conducted with greater sequencing depth. To account for differences in sequencing depths, samples were rarefied to 1000 sequences per sample. All sequences have been submitted to the European Nucleotide Archive (study accession number PRJEB18545). Because we were mainly interested in the fungi involved in the decomposition of root litter, we focused on the ecological guild of saprophytic fungi that were associated to our root samples of mature plants, likely providing a mix of young and older roots. We refer to these saprophytic fungi as Root Associated Saprophytic Fungi (RASf). We have excluded the arbuscular mycorrhizal fungi (AMF) from the analysis since they lack substantial saprophytic capability (Azcón-Aguilar et al. 1999; Hodge 2014) and the primer set employed for the fungal identification in this study has a low resolution for this fungal group. Functional characterisation of the obtained fungal OTUs into saprophytes was performed using a combination of FUNGuild (Nguyen et al. 2016) and complementary literature research (Arnolds and van den Berg 2013; Dighton 2016; Dighton and White 2017; Domsch et al. 2007; Farr and Rossman 2014; Griffith and Roderick 2008). In this study we opted for a pragmatic approach, only considering those fungal taxa with their saprophytic capability as known from literature in August 2019. This means that some fungal endophytes that have only been suggested to have saprophytic potential have not been included in our analyses. Please note that the pathogenic fungal community of this experiment has already been described in another study (Mommer et al. 2018).

### Statistical analyses

In our analyses of the root-associated saprophytic fungal richness and community structure, we follow a two-step approach, in which we first determine the effects of plant species and functional group in the monocultures. In the next step, we use the full dataset to determine the effects of plant species richness, plant functional group richness and plant functional group composition on the saprophytic fungal community structure and richness.

### Saprophytic fungal richness

In the monoculture dataset, saprophytic fungal richness was analysed using linear mixed effects models (LME) with either plant species or functional group identity, and depth (nested within plot to account for the fact that the two depths are not independent) as fixed factors, and block as a random factor. In the full dataset, we investigated the effects of plant functional group richness (PFR; the number of different plant functional group within a plant community) and plant species richness (PSR) on saprophytic fungal richness using linear mixed effects models (LME) with block as a random factor, soil depth nested within plot as fixed factor and plant species or functional group richness as a covariate. To identify the most parsimonious model, the Akaike information criterion corrected for small sample sizes (AICc; Burnham and Anderson (2002)) was used. The variance explained (conditional  $R^2$ ) by the mixed effect models was calculated by the *r.squaredLR* function in MuMIn Package (Bartón 2016). All the models were analysed using the lme4 R package (Bates et al. 2015). Lastly, the relationships between the fungal OTU richness and root traits and soil properties were calculated using non-parametric Spearman's rank correlation.

### Saprophytic fungal community structure

The differences in overall community composition between samples were calculated using the Bray-Curtis dissimilarity index after Hellinger transformation (Legendre and Gallagher 2001). Non-metric multidimensional scaling (NMDS) was used to visualize the effects of plant community structure on the saprophytic fungal community. Permutational analysis of variance (PERMANOVA), as implemented in the *adonis* function from the vegan package (Oksanen et al. 2018), was used to test for differences in saprophytic fungal community composition across factors.

In the monoculture dataset, PERMANOVA was first applied to explore the effects of plant species and soil depth (model 0) on the saprophytic fungal community structure. Furthermore, to assess the extent to which plant functional group captured the differences in the saprophytic fungal community between plant species, we performed an additional PERMANOVA analysis where plant functional group was fitted before species plant species and soil depth in the model (model 1). To assess which OTUs were responsible for the observed

differences in community structure among the different monocultures, SIMPER (similarity percentage) analysis was applied. To assess the relationships between the environmental variables (soil properties and root chemical traits) and saprophytic fungal community structure in the shallow layer in monoculture plots we used the *envfit* R function within the *vegan* R package (Oksanen et al. 2018). Furthermore, we performed variance partitioning based on redundancy analysis (RDA) to quantify the contribution of soil and root properties, and plant functional groups to the structure of the saprophytic fungal community in the shallow layer within the monoculture plots. Following Blanchet et al. (2008) we first tested the significance of the global model using all predictors. Variable selection was then done using forward selection implemented with function *forward.sel* in the R package *packfor* (Dray et al. 2011) following the recommended stopping rules in Blanchet et al. (2008). Variance partitioning was conducted using the *varpart* function in the *vegan* R package (Oksanen et al. 2018).

In the full dataset, PERMANOVA was employed to explore the effects of plant functional group richness (PFR), plant species richness (PSR) and soil depth on saprophytic fungal community structure. First, we analyzed the effects of PFR and PSR on saprophytic fungal community in separate models together with soil depth. Second, PFR was fitted before PSR and soil depth to assess whether PSR could explain variation that was not captured by PFR. Furthermore, we investigated whether plant fungal group composition affected the saprophytic fungal community structure across the plant species richness gradient. We first assessed whether there was difference in saprophytic fungal community structure between the grass only, forb only and grass-forb communities (mixed communities). We then performed pairwise PERMANOVA tests between all the different functional group combinations examined in this study. Statistical significances were based on 9999 permutations and Bonferroni-corrected *P* values were used when more than two groups were compared. SIMPER (similarity percentage) analysis was employed to assess which OTUs were primarily responsible for the observed differences in community structure among the different plant communities across the plant richness gradient. Voronoi diagrams were made using the *treemap* package in R. All statistical analyses were carried out using R version 3.50 (R Core Team 2014).

## Results

Our analyses on 148 root samples from two soil depths resulted in 605,766 high quality sequences representing 988 fungal OTUs of which 355 OTUs were characterized as ‘saprophytic’ (Table S1). Of the 355 root-associated saprophytic fungal (RASf) OTUs, 245, 218, 236, and 112 were found in monocultures, 2-, 4-, and 8- species plots, respectively. Ascomycota was the dominant phylum (216 OTUs, 61%), followed by Basidiomycota (119 OTUs, 33%) and Mucoromycota (20 OTUs, 6%) (Fig. S1). The 355 saprophytic fungal OTUs clustered in 157 fungal genera, most of which were affiliated to the Ascomycota (105). The remaining OTUs were associated with the genera Basidiomycota (49) and Mucoromycota (3). Among the Ascomycota, common saprophytic fungal genera like *Pezizella*, *Lachnum*, *Tetracladium* and *Preussia* were the most abundant, accounting for 14, 12, 10 and 4% of the total saprophyte fungal reads, respectively. *Coprinellus* (15%) and *Cotylidia* (3%) were the dominant Basidiomycota genera, while *Mortierella* (1%) was the most abundant genus within Mucoromycota.

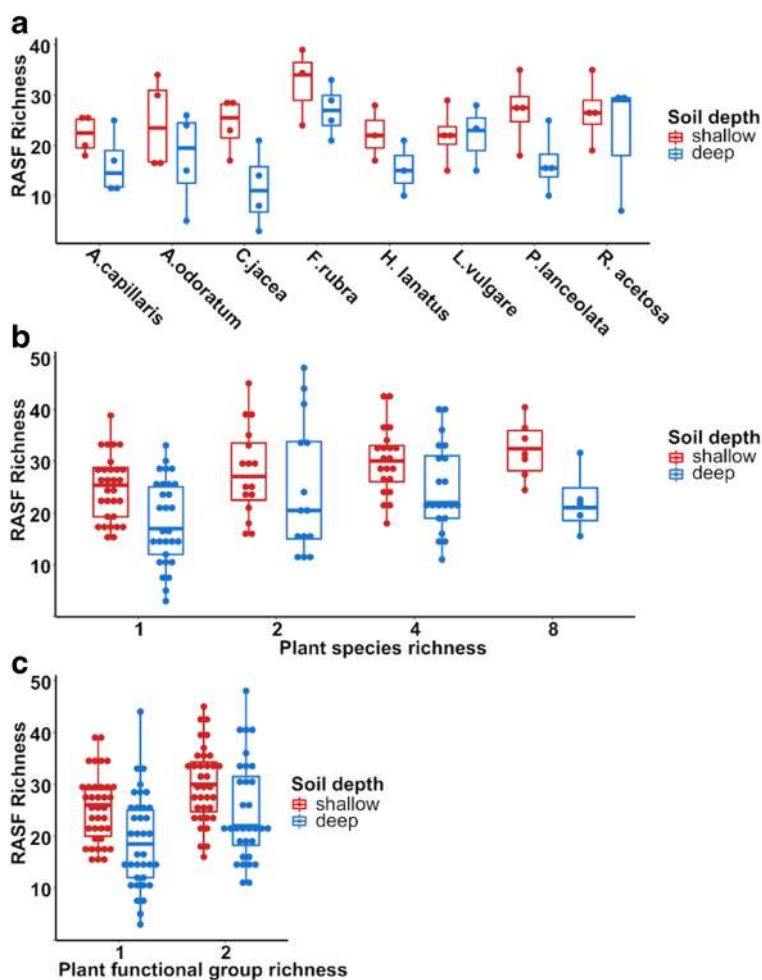
### Fungal saprophytic richness

In the monoculture plots, RASf richness was significantly higher in the shallow than in the deep layer ( $F_{1,29} = 16.6$ ,  $P < 0.001$ ) (Fig. 1a). Plant species identity ( $F_{7,29} = 1.91$ ;  $P = 0.12$ ) and plant functional group ( $F_{1,29} = 0.21$ ;  $P = 0.65$ ) did not have an effect on RASf richness. No interaction was found between soil depth and plant species identity ( $F_{7,28} = 0.69$ ,  $P = 0.67$ ) nor between soil depth and plant functional group ( $F_{1,28} = 0.19$ ,  $P = 0.66$ ). In monocultures, RASf richness was not significantly related to any of the soil properties or root traits measured (Table S2).

When analysing all plots (i.e. monocultures and mixtures), RASf richness increased significantly with both plant species richness (Fig. 1b;  $F_{1,68} = 5.57$ ,  $P < 0.05$ ) and plant functional group richness (PFR) (Fig. 1c;  $F_{1,68} = 10.87$ ,  $P < 0.01$ ). However, the model with PFR (AICc = 986.64,  $R^2 = 0.188$ ) performed better than the one with PSR (AICc = 994.79,  $R^2 = 0.153$ ), suggesting that PFR is a better predictor for RASf richness.

RASf richness was significantly higher in the shallow than in the deep layer (Fig. 1b;  $F_{1,64} = 7.12$ ,  $P < 0.001$ ). No significant interactions were observed

**Fig. 1** Root-associated saprophytic fungal (RASf) richness across the different plant communities investigated in this study. Boxplot representing RASf richness in the shallow and deep soil layers across the **a**) eight monocultures, **b**) plant species richness levels and **c**) plant functional group richness



between soil depth and PSR ( $F_{1,64} = 0.16$ ,  $P = 0.68$ ) and PFR ( $F_{1,64} = 0.12$ ,  $P = 0.73$ ).

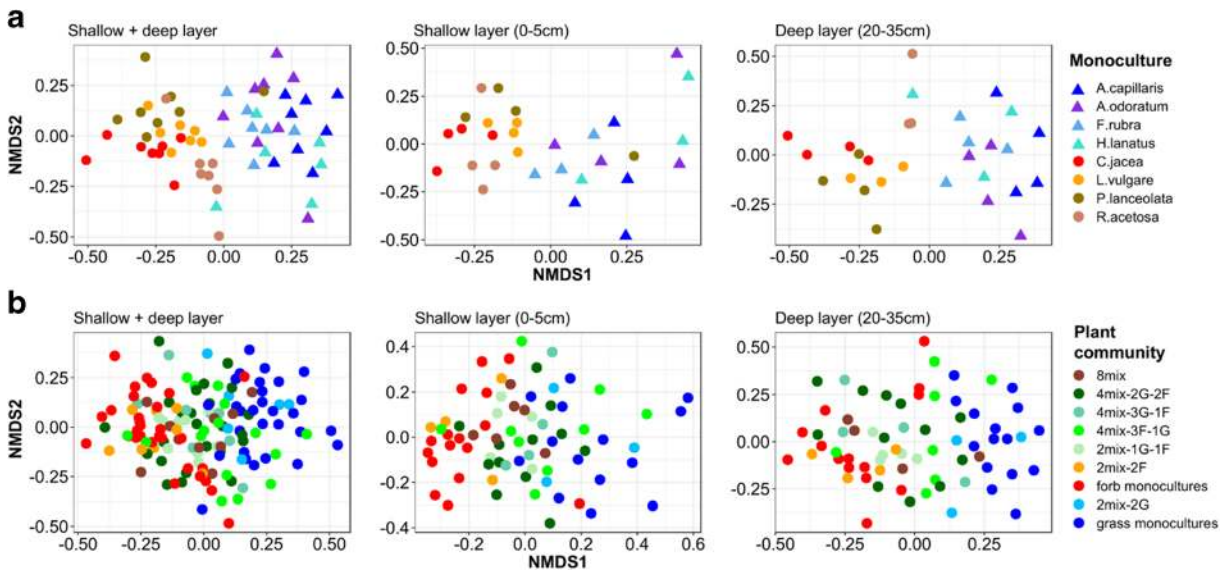
Total organic carbon, C:N ratio and soil organic matter in the shallow soil layer increased significantly with plant species richness (Table S3) and they were also significantly ( $P < 0.05$ ) positively correlated with RASf richness (Table S4). However, among the models tested, which included plant species richness or PFR or the soil parameters as fixed factors, the model that included only PFR was the most parsimonious in predicting RASf richness in the shallow layer across the species richness gradient ( $AICc = 491.52$ ,  $R^2 = 0.143$ ).

#### Fungal saprophytic communities in monocultures

In the monocultures, plant species had a significant effect on RASf community structure, explaining 32% of variation (Fig. 2a and Fig. S2;  $F_{7,58} = 3.43$ ,  $P < 0.001$ )

(Table S5), while soil depth did not have a significant effect ( $F_{1,58} = 1.34$ ,  $P = 0.13$ ) and there was no significant interaction between depth and plant species ( $F_{7,58} = 0.94$ ,  $P = 0.64$ ). Our subsequent PERMANOVA analysis revealed a significant effect of plant functional group on RASf community structure (i.e. grasses vs forbs; Fig. 2a and Fig. S2;  $F_{1,58} = 9.08$ ,  $P < 0.001$ ), capturing 12.1% of the total variance (Table S5). Hence, functional group captured 38% of the variance explained by plant species. We did not find a significant interaction between soil depth and plant functional group ( $F_{1,58} = 1.13$ ,  $P = 0.31$ ).

Of the 245 OTUs encountered in the monocultures, 90 were found in at least one grass and one forb (only 9 OTUs were detected in all eight plant species, see Fig. S3), indicating that approximately 40% of the OTUs is shared among the grasses and forbs. This may suggest that approximately 60% is either grass or forb specific.



**Fig. 2** Root-associated fungal saprophyte (RASF) communities across the plant communities investigated in this study. Nonmetric multidimensional scaling (NMDS) ordinations, based on Bray-Curtis distance of RASF communities associated (a) with the eight

monocultures and (b) with all the different plant species richness levels. Results are displayed in combination (left) and separately for the shallow (0–5 cm, middle) and deep (20–35 cm, right) soil layers

However, within each plant functional group, we found that the proportion of unique OTUs detected in each plant species ranged from the 17 to the 35% in the grasses and from 28 to 40% in the forb monocultures (Fig. S3).

In each monoculture, the seven most abundant OTUs accounted for approximately 60–85% of the sequence reads, indicating that each monoculture was dominated by a few saprophytic taxa (Fig. 3a). Within a plant functional group, each plant species was dominated by the same OTUs (Fig. 3a), whereas between plant functional group these OTUs were different (Fig. 3a; Fig. S4). The dominant functional group-specific OTUs were further identified as the major contributors to the dissimilarity (66.87%) in fungal saprophytic community composition between grass and forb monocultures (Table 2). Indeed, the forb monocultures showed high abundances of OTU 4 (*Tetracladium furcatum*), OTU 6 (*Pezizella discreta*), OTU 2 (*Coprinellus micaceus*) and OTU 10 (*Coprinellus disseminatus*), which were not well represented in the grasses (Table 2). On the contrary, OTU 5 (*Lachnum spartinae*), OTU 7 (*Lachnum virgineum*) and OTU 8 (*Pezizella chrysostigma*) were remarkably more abundant in grass than in forb monocultures (Table 2).

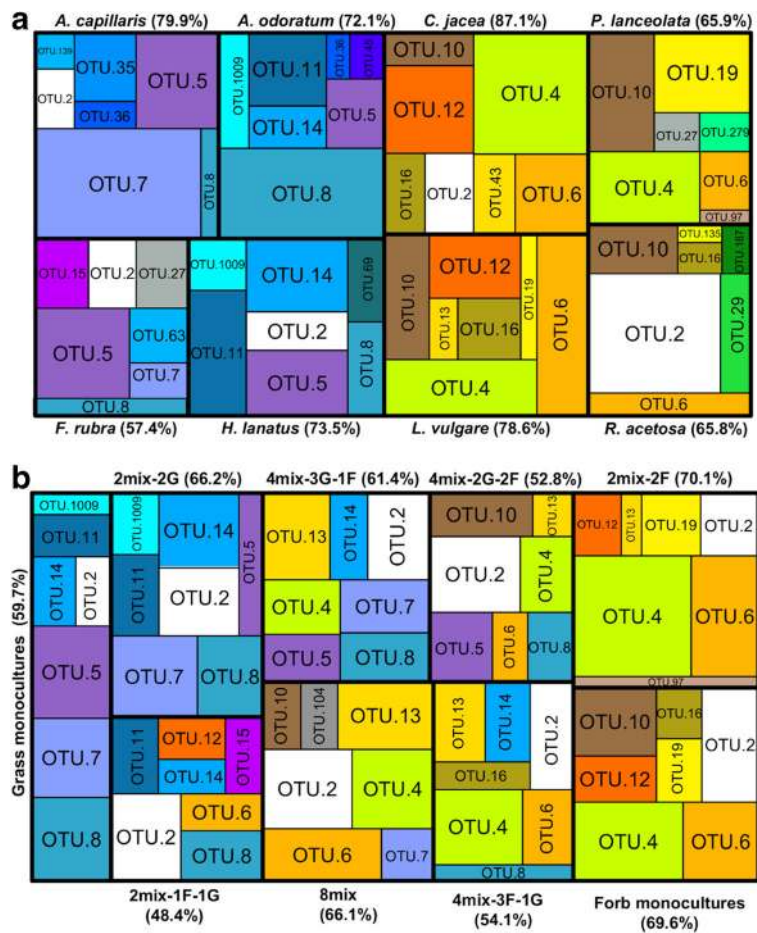
Soil properties did not differ significantly between the eight plant species, but root C:N ratio ( $F_{1,28} = 14.47$ ,

$P < 0.001$ ) and root lignin content ( $F_{1,28} = 48.63$ ,  $P < 0.001$ ) were significantly higher in the grass monocultures compared to the forbs (Table 1; Fig. S5). These two root traits were positively correlated to each other ( $r = 0.49$ ,  $P < 0.01$ ) and were also significantly ( $P < 0.05$ ) associated to RASF community structure in the shallow soil layer (Table S6). Variance partitioning demonstrated that 16% of the variation in the RASF community structure in the monocultures was explained by plant functional group and root traits together, with corresponding pure effect of 4% and 3%, respectively, whereas soil properties had a significant but marginal effect (Fig. 4).

#### Fungal saprophytic communities across the plant richness gradient

Across the plant species gradient, RASF community structure was significantly different between the two soil depths (Fig. 2b;  $F_{1,140} = 3.54$ ,  $P < 0.001$ ) with soil depth explaining 2.5% of RASF community variance (Table S7). Surprisingly, RASF community structure was only marginally affected by plant species richness (PSR:  $F_{3,140} = 1.37$ ,  $P = 0.057$ ; PSR  $\times$  depth:  $F_{3,140} = 0.91$ ,  $P = 0.77$ ), but we did find a significant difference between plant communities consisting of one and of two plant functional groups (PFR:  $F_{1,140} = 2.12$ ,  $P < 0.005$ ;

**Fig. 3** Most abundant OTUs in the different plant communities investigated in this study. Voronoi diagrams showing the seven most abundant root-associated saprophytic fungal (RASf) OTUs in **a**) each of the monocultures, and **b**) in the plant communities across the plant species richness levels. Cell size is proportional to the OTUs relative abundance. Numbers in parenthesis represent the cumulative relative abundance of the seven most abundant RASf OTUs for each plant community



1.5% of variance; Table S7). In addition, plant communities consisting of only grasses, only forbs or both differed significantly from each other ( $F_{2,140} = 7.83$ ,  $P < 0.001$ ). Pairwise comparisons revealed that the biggest differences were found between forb only and grass only communities (Table S8). This effect of plant functional group on the RASf structure is also evident in the NMDS plots (Fig. 2b), which show a clear separation of the grass only and forb only plant communities, with the grass-forb communities (mixed communities) clustered in between. RASf community structure did not differ between two, four and eight species mixtures consisting of grasses and forbs. Also, these mixed communities did not differ significantly from forb only or grass only communities (Table S8). We also found no evidence that the proportion of the two plant functional groups within mixtures affected the RASf community structure (Table S8).

In general, we found that the OTUs detected in plots containing mixtures of either two, four or eight

plant species were to a large extent a subset of those found in the monoculture plots (Fig. S3). For example, 93 out of the 112 OTUs (83%) detected in the 8-mix were found in the monoculture samples, while only one OTU was detected only in the 8-mix plots (Fig. S3). Interestingly, RASf communities in the mixed plots were mainly composed and dominated by the most abundant OTUs characterizing the two groups of monocultures (Fig. 3b). SIMPER analysis identified 14 taxa that together accounted for approximately 66% of saprophytic community dissimilarity between the plant communities across the plant species richness gradient (Table 3). Most of these OTUs (12 out of 14) were also found as drivers of the community dissimilarity between the grass and forb monocultures (Table 2). However, the other two taxa identified did not contribute to dissimilarity between grasses and forbs in monoculture. Of these, OTU 13 (*Cotylidia undulata*) was more abundant in

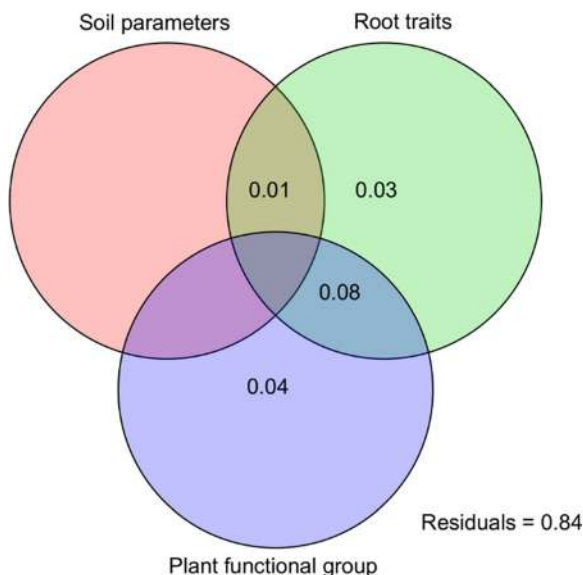


**Table 2** SIMPER analysis results showing the 14 fungal taxa that contributed most to the dissimilarity between grass and forb monocultures

OTU number	Order	Taxon	Variation explained (%)	Monocultures	
				Grasses	Forbs
OTU.4	Helotiales	<i>Tetracladium furcatum</i>	8.72	0.21	16.40
OTU.2	Agaricales	<i>Coprinellus micaceus</i>	8.02	4.63	12.50
OTU.5	Helotiales	<i>Lachnum spartinae</i>	7.12	13.30	0.31
OTU.8	Thelebolales	<i>Pezizella chrysostigma</i>	6.54	10.70	2.69
OTU.7	Helotiales	<i>Lachnum virgineum</i>	5.91	11.00	0.15
OTU.10	Agaricales	<i>Coprinellus disseminatus</i>	5.75	0.39	10.60
OTU.6	Thelebolales	<i>Pezizella discreta</i>	5.71	1.46	11.30
OTU.12	Hypocreales	<i>Flagellospora leucorhynchos</i>	3.92	0.20	7.33
OTU.11	Helotiales	<i>Cyathicula sp.</i>	3.48	6.50	0.03
OTU.14	Pleosporales	<i>Preussia africana</i>	2.89	5.42	0.09
OTU.19	Xylariales	<i>Hypoxyton rutilum</i>	2.79	0.05	5.23
OTU.16	Ascomycetes <sup>a</sup>	<i>Ypsilina graminea</i>	2.59	0.85	4.48
OTU.1009	Helotiales	<i>Hymenoscyphus scutula</i>	1.76	3.30	0.01
OTU.35	Agaricales	<i>Coprinopsis atramentaria</i>	1.67	3.13	–

Values in the plant community columns (grasses vs forbs) indicate the mean relative abundance of the saprophytic OTUs

<sup>a</sup>Taxonomy rank = Class



**Fig. 4** Variation partitioning analysis, illustrating the effects soil parameters, root traits and plant functional group on the community structure of the root associated saprophytic fungal (RASF) communities found in the shallow layer in the monoculture plots. Each ellipse represents the portion of variation accounted by each factor. Shared variance is represented by the intersecting portions of the ellipses. Values <0 are not shown. Unexplained variation (residuals) is reported in the plot

mixtures containing grasses and forbs than in plots with just one functional group. The other, OTU 15 (*Marasmius tricolor*), showed a less clear pattern, being absent in communities containing only forbs or two grasses, and present in the other communities (Table 3).

## Discussion

Root-associated saprophytic fungal (RASF) richness increased with plant species richness in the 10-year old grassland biodiversity experiment, but this effect was largely due to an increase in plant functional group richness with plant species richness. The RASF community structure differed between plant species in monocultures, and primarily so between the two different plant functional groups, as grasses and forbs showed distinct saprophytic fungal communities. This plant functional group effect cascaded into the RASF community composition in plant species mixtures. The clear effect of plant functional group on RASF community composition may be driven by the differences in C:N ratio and lignin content of the roots, as these traits are significantly different between the two plant functional groups.

**Table 3** SIMPER analysis results showing the 14 fungal taxa that contributed most to the dissimilarity across the different plant communities investigated in this study

OTU number	Order	Taxon	Variation explained (%)	grass mono	2mix-2G	4mix-3 G - 1F	2mix-1 G - 1F	4mix-2 G - 2F	8mix	4mix-3 F - 1G	2mix-2F	forb mono
OTU.2	Agaricales	<i>Coprinellus micaceus</i>	9.84	4.63	10.60	10.50	11.70	13.40	13.80	9.06	7.06	12.50
OTU.4	Helotiales	<i>Tetracladium furcatum</i>	8.14	0.21	0.15	8.21	3.89	7.85	12.50	13.00	26.60	16.40
OTU.8	Thelebolales	<i>Pezizella discreta</i>	6.31	10.70	10.10	8.52	7.87	6.15	3.59	4.39	2.44	2.69
OTU.10	Agaricales	<i>Coprinellus disseminatus</i>	5.91	0.39	–	5.21	–	8.73	4.97	–	–	10.60
OTU.6	Thelebolales	<i>Pezizella chrysostigma</i>	5.74	1.46	4.05	3.57	5.86	4.77	11.90	7.40	15.80	11.30
OTU.5	Helotiales	<i>Lachnum spartinae</i>	5.58	13.30	6.61	7.13	3.80	8.48	3.97	0.35	0.09	0.31
OTU.7	Helotiales	<i>Lachnum virgineum</i>	4.11	11.00	13.50	9.24	2.16	1.45	5.25	0.2	0.35	0.15
OTU.14	Pleosporales	<i>Preussia africana</i>	3.83	5.42	11.60	6.41	4.50	2.51	1.62	7.01	0.02	0.09
OTU.13	Hymenochaetales	<i>Cotyldia undulata</i>	3.77	1.27	0.41	11.4	3.39	3.44	12.60	8.07	2.48	1.42
OTU.12	Helotiales	<i>Cyathicula sp.</i>	3.12	0.20	–	0.81	5.40	0.82	0.62	2.66	5.56	7.33
OTU.11	Hypocreales	<i>Flagellospora leucorhynchus</i>	2.88	6.50	7.58	0.53	7.07	0.04	0.12	0.06	0.06	0.03
OTU.19	Xylariales	<i>Hypoxyylon rutilum</i>	2.81	0.05	–	0.19	1.57	2.93	3.27	1.65	7.04	5.23
OTU.15	Agaricales	<i>Marasmius tricolor</i>	1.85	2.02	–	0.88	5.57	3.08	0.271	0.02	–	–
OTU.1009	Helotiales	<i>Hymenoscyphus scutula</i>	1.71	3.35	5.77	0.50	4.16	0.243	0.01	0.03	0.22	0.01

Values in the plant community column indicate the mean relative abundance of the saprophytic OTUs

Abbreviations: *mono* monocultures

### Drivers of RASF richness

RASF richness was higher in the shallow soil layer than in the deeper one. This is in line with earlier findings, as the majority of fungal richness in grassland (Jumpponen et al. 2010; Mommer et al. 2018; Zajicek et al. 1986) and agricultural (Oehl et al. 2005) ecosystems is found in the top soil, where root biomass (Oram et al. 2018; Ravenek et al. 2014), soil organic matter content (Fang and Moncrieff 2005; Lange et al. 2015) and microbial activity (Debnath et al. 2015; Taylor et al. 2002) are often highest.

We found positive relationships between aspects of plant biodiversity (i.e. species and functional group richness) and saprophytic fungal richness, and between plant species and soil organic matter in the shallow soil layer. These findings together may suggest that the higher soil organic matter content as a result of plant biodiversity may lead to higher RASF richness (Lunghini et al. 2013; Santonja et al. 2017). Future work has to reveal the relative importance of plant litter inputs and soil organic matter as drivers of fungal richness and

community composition in soil (de Vries et al. 2012; Louis et al. 2016; Pellissier et al. 2014; Siles and Margesin 2016), and their links to the diversity in metabolic capacities (i.e., extracellular enzyme synthesis) of saprotrophic fungi (Baldrian 2006; Cline et al. 2018; Grayston et al. 1998; McGuire et al. 2010).

### Plant functional group shapes the saprophytic community in the monocultures

Within the monocultures, RASF community was mainly structured by plant species followed by plant functional type. The monoculture communities were dominated by a few plant functional group-specific OTUs. In contrast to RASF richness, RASF community structure did not differ significantly between the two soil layers. This is surprising because other studies exploring total soil or root-associated fungal community found different results (Jumpponen et al. 2010; Mommer et al. 2018; Mujic et al. 2016; Unterseher et al. 2011). Our explanation for a lack of significant differences in RASF community structure between the two soil layers in this

study is that the dominant saprophytic OTUs characterizing the RASF communities were largely the same in the two soil layers, while nearly all the RASF taxa that were lost with increasing soil depth were rare or low abundant OTUs in the shallow layer.

The grass and forb species were characterized by significantly different root chemical properties, with higher lignin content and C:N ratio in grasses than in forbs confirming earlier findings (Chen et al. 2017; Roumet et al. 2016; Schroeder-Georgi et al. 2016). Root C:N and lignin content have been identified as plant traits shaping the soil-borne fungal community in grasslands (Cline et al. 2018; Schöps et al. 2018), which corresponds to differences in their decomposition potential (Cline et al. 2018; Melillo et al. 1982; Taylor et al. 1989). Generally, plant litter from grasses decomposes slower than the forbs (Chen et al. 2017; Cornelissen et al. 2017; Cornwell et al. 2008; Griffith and Roderick 2008). Our study confirms these differences between grasses and forbs and their differential impact on RASF. Indeed, we found that the root traits we measured together with plant functional group type explained approximately 16% of the variation in RASF community structure. However, other root traits that can affect RASF communities, such as root exudate profiles (van Dam and Bouwmeester 2016) or other chemical components as magnesium, potassium and calcium concentration (Schöps et al. 2018) have to be included to capture a greater part of the variation in RASF community structure. Alternatively, plant species may have induced effects on soil macro- and meso- fauna, which can change the structure of plant litter (Carrillo et al. 2011; Eisenhauer et al. 2009) and thus the RASF community, which may have obscured the relationship between RASF community structure and root traits.

#### Plant functional group composition affects RASF community structure in plant mixtures

The plant mixtures harboured RASF communities that were characterized by the dominant OTUs found in the grass and forb monocultures. Indeed, the most abundant OTUs detected in the monocultures were highly representative for the plant mixtures, and accounted for a large proportion of the total saprophyte fungal reads (ranging from 48.4 to 70.1%) in each mixed plant community. The abundance and occurrence of individual saprophytic fungal taxa thus appeared sensitive to particular plant functional groups, probably through an

influence of root litter biochemistry and plant community composition (Fornara et al. 2009; Spehn et al. 2000). For example, the relative dominance of grass species increased the abundance of saprotrophic taxa affiliated to the genus *Lachnum* (OTU 5, *Lachnum spartinae* and OTU 7, *Lachnum virgineum*) which have the ability to produce laccase enzymes that are involved in lignin degradation (Lyons et al. 2003), reflecting the more recalcitrant litter chemistry of the grass roots compared to forb roots (Dean et al. 2014). In the grass-dominated plots, we also detected a high abundance of the fungal saprophyte *Preussia africana* (OTU 14). This particular taxon has recently gained considerable attention due to its production of bioactive secondary metabolites, which are typically involved in defence mechanisms against other competing microbes (Bills et al. 2013; Gonzalez-Menendez et al. 2017; Zhang et al. 2012). Contrary to the grasses, forb plant communities showed an increase in saprophytic fungi affiliated to Xylariales and Agaricales orders, as already reported by Cline et al. (2018). Among these forb-enriched saprophytic taxa, *Hypoxylon rutilum* (OTU 19; Xylariales order, *Hypoxylaceae* family) represents an ecologically relevant fungus as it is able to produce secondary metabolites that may negatively affect a large spectrum of organisms, including bacteria, fungi, insects and nematodes (Helaly et al. 2018; Hellwig et al. 2005). Overall, our findings clearly indicate that RASF community composition and structure in plant mixtures can largely be predicted by the plant functional groups composing the plant community.

Interestingly, the root-associated *pathogenic* fungal communities in the same plots as in this study showed a different pattern when comparing mixtures and monocultures (Mommer et al. 2018). More than half of the pathogenic OTUs detected in the monocultures was not found in the mixed plots (Mommer et al. 2018), and pathogen communities in mixtures could not be predicted on the basis of their monocultures. This difference may be due to the fact that fungal pathogens interact with living plants, which can lead to co-evolution of plants and pathogens (Gilbert and Webb 2007; Möller and Stukenbrock 2017). This may have resulted in higher host specificity in pathogens than saprophytes. If host specificity is large, dilution of the plant host with plant species diversity will have more impact on fungal behaviour than when any plant species can be a host (Ampt et al. 2018). The relationship between plant and fungal communities clearly depends on fungal

ecological guild under study (Alzarhani et al. 2019; Kolarikova et al. 2017).

## Conclusions

We showed that plant species richness can stimulate RASF richness. However, the positive effect of plant species richness on RASF richness was mainly due to combining two plant functional groups (grasses and forbs). Also with regard to the RASF community structure plant functional group was the main driver as grasses and forbs showed clear differences in the fungal taxa dominating their RASF communities. These differences between grasses and forbs were not correlated to their effects on soil properties, but to their differences in root traits, particularly root lignin and C:N ratio. In contrast to pathogenic fungi, the plant functional type is a good predictor for saprophytic fungal communities in grasslands.

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