

## ***New Phytologist* Supporting Information**

Article title: **Deciphering the role of specialist and generalist plant-microbial interactions as drivers of plant-soil feedback**

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The following Supporting Information is available for this article:

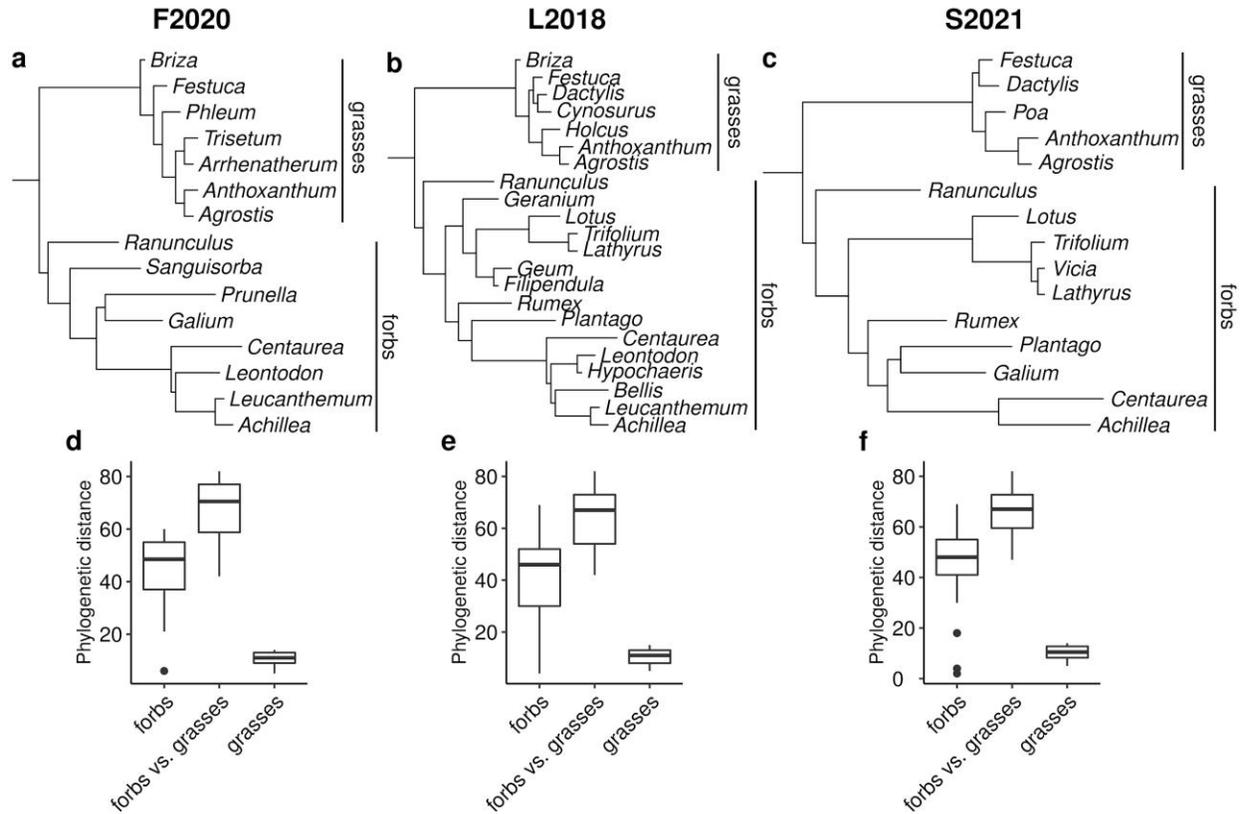
**Fig. S1** Phylogenetic distance among the host plant genera included in the case studies shown in Fig. 1b.

**Fig. S2** Host-specificity of plant-associated fungal guilds estimated by the standardised Kullback-Leibler distance ( $d'$ ).

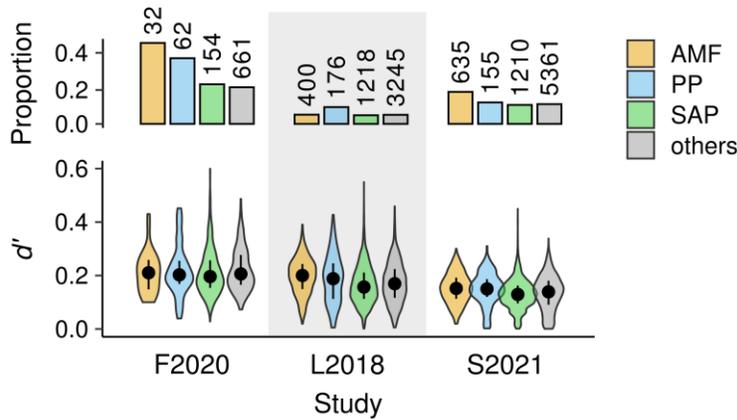
**Table S1.** Information on the case studies used in the analysis of likely host ranges of plant-associated fungi.

**Methods S1.** Analysis of likely host range breads of plant-associated fungal guilds.

**Table S2.** Overview of the studies examining the effect of plant traits on plant-soil feedback.



**Fig. S1.** Phylogenetic distance among the host plant genera included in the case studies shown in Fig. 1b. The trees in **a–c** show the phylogenetic relationships among the host plant genera included in the studies of Francioli *et al.* (2020), Leff *et al.* (2018) and Sweeney *et al.* (2021), respectively. The classification of genera into grasses and forbs is highlighted. For each study, the box plots in **d–f** summarise the distribution of pairwise phylogenetic within all grass hosts (**grasses**), within all forbs (**forbs**), and between all pairs of grasses and forbs (**forbs vs. grasses**).



**Fig. S2.** Host-specificity of plant-associated fungal guilds estimated by the standardised Kullback-Leibler distance ( $d'$ ). The analysis is analogous to that shown in Fig. 1b, including case studies from experimental mesocosms with temperate grassland plant species (S2018: Leff *et al.*, 2018; S2021: Sweeney *et al.*, 2021; F2020: Francioli *et al.*, 2020), except that taxa detected in a single host were also included in the analysis. Violin plots represent the distribution of  $d'$  values, with points and error bars indicating median values and interquartile ranges, respectively. The values at the top indicate the total number of fungal amplicon sequence variants (ASV; Callahan *et al.*, 2017) included in each dataset. Specificity index  $d'$  was calculated using data on ASV relative abundance; low values of  $d'$  indicate generalism and the value of 1 indicates strict host specificity. Bar plots at the top represent the proportion of fungal taxa with  $d'$  values significantly higher ( $P < 0.05$ ) than those obtained by a randomised null model. Abbreviations: **AMF**, arbuscular mycorrhizal fungi; **PP**, plant pathogens; **SAP**, saprotrophs.

**Table S1.** Information on the case studies used in the analysis of likely host ranges of plant-associated fungi.

	<b>F2020:</b> Francioli <i>et al.</i> (2020)	<b>L2018:</b> Leff <i>et al.</i> (2018)	<b>S2021:</b> Sweeney <i>et al.</i> (2021)
Plant species ( <i>n</i> )	16	25	21
Plant genera ( <i>n</i> )	15	22	15
Fungal rDNA region sequenced	ITS1	ITS1	ITS2
Primers used for amplification (ref.)	ITS1F/ITS2 (White <i>et al.</i> , 1990; Gardes & Bruns, 1993)	ITS1F/ITS2 (White <i>et al.</i> , 1990; Gardes & Bruns, 1993)	ITS3F/ITS4R (White <i>et al.</i> , 1990)
ASV ( <i>n</i> ) <sup>1</sup>	909	5039	7361
Substratum	Roots	Soil	Rhizosphere
Growth conditions	Plants in common garden, outdoors	Plants in common garden, outdoors	Plants in pots with soil, glasshouse
Experiment duration (from establishment to harvest)	40 months	4 years (approx.)	12 weeks
Predictors of fungal community variation tested	Plant (phylogenetic) composition, plant functional group, plant traits, water availability	Plant (phylogenetic) composition, plant functional group, plant traits, soil conditions	Plant (phylogenetic) composition, plant traits, soil conditions, light intensity, water availability

<sup>1</sup>Amplicon sequence variants (Callahan *et al.*, 2017).

**Methods S1.** Analysis of likely host range breadths of plant-associated fungal and oomycete guilds.

All analyses were performed using the program R v3.6.3 (R Core Team, 2020) with use of relevant packages (see below). We visualised the results using violin and bar plots built with package ggplot2 v3.3.5 (Wickham et al. 2019). The data and code used in these analyses are available online at Figshare (10.6084/m9.figshare.19169609).

### *Global analysis*

The global analysis relied on the dataset provided by Pölme *et al.* (2021), which includes information, among other data, about the origin and host associations for 871,138 fungal and oomycete records from the International Nucleotide Sequence Databases consortium (INSDc), classified into 132,773 Species Hypotheses (SHs; Koljalg *et al.*, 2013) based on a 1 % ITS sequence dissimilarity threshold (see Table S3 of Pölme *et al.*, 2021). We retained only SHs with a defined association with plant hosts. For each SH, we obtained a genus-level phylogeny of the host plants where it was reported, by subsetting the phylogeny provided by Slik *et al.* (2018), including most plant genera, using the *ph\_phyloomatic()* function of the package phylocomr v0.3.2 (Ooms & Chamberlain, 2018). We then obtained the mean phylogenetic distance (MPD; Webb *et al.*, 2002) between all pairs of plant genera in the host phylogenies for each SH using function *mpd()* of the R package picante v1.8.2 (Kembel *et al.*, 2010). Only SH recorded in at least two host genera could be used to calculate MPD, but we also report the number of SH with a single host genus in the results.

Although the Pölme *et al.* (2021) dataset includes information about the relative classification of SHs into different functional guilds, to simplify the analysis we performed a *de novo* functional annotation by collating the SH genera with the FungalTraits database provided by the same study (see Table S1 in Pölme *et al.*, 2021). We only show the MPD data for those guilds that have been identified as drivers of plant-soil feedbacks, namely arbuscular mycorrhizal fungi, ectomycorrhizal fungi, plant pathogens, root endophytes, and saprotrophs. For simplicity, we combined several guilds of saprotrophs from different substrata (litter, soil, wood, unspecified) into a single saprotroph category. We combined

all other fungal guilds into a joint category, 'others'. We tested for differences in MPD across fungal guilds using the Kruskal-Wallis rank sum test.

### *Analysis of case studies*

We performed a second set of analyses using a selection of three published case studies, all encompassing experimental mesocosms with a fixed set of temperate grassland plant species, in which the occurrence of fungal taxa, or lack thereof, was examined by high-throughput amplicon sequencing (Leff *et al.* 2018; Francioli *et al.* 2020; Sweeney *et al.* 2021). For each study, we obtained the data and used the original settings for clustering of sequence reads into amplicon sequence variants (ASVs; Callahan *et al.*, 2017), taxonomic classifications of fungi, and filtered read abundance data. We re-annotated the ASVs functional classification with the FungalTraits database and obtained host genus-level phylogenies as explained above. We then used the host phylogenies to calculate the host MPD per ASV, but this time using the *ses.mpd()* function of picante, which in addition to calculating MPD, compares the observed MPD values with MPD values obtained in a null model, generated by randomly shuffling 999 times the tips of the host phylogeny. This enabled us to detect ASVs with MPD values significantly lower ( $P < 0.05$ ) than those expected by chance, indicative of host specialisation.

We repeated these analyses using an alternative estimator of host specialisation, the Kullback-Leibler distance ( $d'$ ; Blüthgen *et al.*, 2006), which is independent of host phylogenetic relationships. The  $d'$  index provides a standardised measure of 'partner-diversity', taking into account both the identity and frequency of interactions between organisms, and in which values close to 1 indicate high host specialism, and those close to 0 generalism. We calculated  $d'$  using ASV abundance data and the function *dfun()* of package bipartite v2.16 (Dormann *et al.*, 2021). As for MPD, we compared the observed values of  $d'$  with those obtained from the null model, generated by randomly shuffling the host plant identities 999 times, and used these to identify significant associations at  $P < 0.05$ .

A repetition of these analyses, but using plant species-level instead of genus-level phylogenies, led to the same conclusions about the likely host range breadths of different plant-associated fungal guilds (results now shown). In this case, the host plant phylogenies were based on the reference phylogenetic tree provided by Zanne *et al.* (2014).

**Table S2.** Overview of the studies examining the effect of plant traits on plant-soil feedback. In August 2021, we used Web of Science to perform a literature search with the search terms: plant\* AND soil feedback\* AND functional trait\*. This search is deliberately inclusive to ensure that we included all literature that examined the effect of plant traits on plant-soil feedback. This search returned 207 articles. We briefly screened these to see if they were likely to include both a plant-soil feedback experiment and the measurement of plant functional traits across a range of species. Papers that did not meet both of these criteria were eliminated. PSF type refers to the way PSF was estimated: Specific – comparison of plant performance in conspecific *versus* heterospecific soil; Total – comparison of plant performance in live *versus* sterile soil (see Box 1). The studies are colour-coded by the trait gradient (see Fig. 3) that they find significant support for: green – conservation gradient; blue – fungal collaboration gradient; orange – a combination of traits correlated with the conservation, collaboration and dispersal gradients.

Citation	PSF type	Ecosystem	Traits	Main finding
Baxendale <i>et al.</i> , 2014	Specific*		Specific leaf area (SLA), leaf dry matter content (LDMC)	More shoot mass in soil conditioned by fast growing species in the absence of interspecific competition; Positive PSF for slow- and fast-growing species when grown in species mixtures
Fitzpatrick <i>et al.</i> , 2017	Specific		Longest root length, root angle, LDMC, SLA, root hair density, specific root length (SRL)	Higher SLA and lower root biomass of the conditioning species reduce plant growth in the feedback phase. Species with higher SLA experience more negative PSF.
Heinen <i>et al.</i> , 2020	Specific*		Functional group, growth rate	Lower biomass when soil was conditioned by fast- than slow-growing forbs, higher biomass when soil was conditioned by fast- than slow-growing grasses
Münzbergová & Šurinová 2015	Specific		Height, leaf nitrogen content (LNC), leaf phosphorus content (LPC)	More negative PSF when the heterospecific soil was conditioned by shorter species or species with higher LNC and LPC, but responses are dependent on focal species

Lekberg <i>et al.</i> , 2018	Specific/ Total***		Competitive ability	Stronger competitors experienced more negative total PSF
Lemmermeyer <i>et al.</i> , 2015	Specific/ Total		Relative growth rate, SRL, root C:N ratio	Slower-growing species had more positive total PSF
Bergmann <i>et al.</i> , 2016	Specific/ Total		SRL	On disintegrated soil, specific PSF was positively correlated with SRL
Cortois <i>et al.</i> , 2016	Specific/ Total		RGR, SRL, SLA, arbuscular mycorrhizal (AM) colonization	Species with lower SRL and higher mycorrhizal colonization had more positive total PSF
Teste <i>et al.</i> , 2019	Specific		Nitrogen acquisition strategy	Ectomycorrhizal species had positive specific PSF, N fixing and non-mycorrhizal species had negative specific PSF
Liu <i>et al.</i> , 2015	Total**		SLA, wood density, seed mass, photosynthetic rate, LNC	More negative PSF with more dissimilar trait values between the focal and other populations
Bennett & Klironomos 2018	Specific		LNC, SLA, height, seed weight, drought tolerance, mycorrhizal type	Ectomycorrhizal species, species with larger seeds and lower drought tolerance had more positive PSF
Semchenko <i>et al.</i> , 2018	Specific/ Total		SLA, LDMC, LNC, RDMC, RNC, root diameter, shoot and root dry mass	Root diameter positively related to total PSF; LNC negatively related to specific and total PSF.
Xi <i>et al.</i> , 2021	Specific***		SLA, LNC, SRL, fine root density, plant height, seed dry mass	More negative PSF with increasing SLA, LNC and SRL, and more positive PSF with increasing root diameter, height and seed mass
Bukowski <i>et al.</i> , 2017	Specific		Height, seed production, rosette formation, life form, AM colonization, life span, N fixation	Strength of PSF was not significantly related to traits
Heinen <i>et al.</i> , 2018	Specific*		Root system size	PSF not significantly affected by root system size of the conditioning or feedback species
Heinze <i>et al.</i> , 2015	Total		Plant biomass, local abundance	More negative PSF for large species with low abundance
Kut'áková <i>et al.</i> , 2018	Specific		Height, SLA, life span	More negative PSF when the height of heterospecifics is greater than conspecifics

Png <i>et al.</i> , 2019	Specific/ Total		N fixation strategy	Non-N-fixing plant species grew better in soil conditioned by N fixing plants
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\* PSF assessed as comparison of plant growth on soils conditioned by species with different trait values; does not include soil sterilisation or conspecific soil conditioning treatments

\*\* Intraspecific PSF comparing populations of the same species

\*\*\* Meta-analysis

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