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RESEARCH

Native and exotic grasses share generalist foliar fungi in a Canterbury high country grassland

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Abstract: Communities of foliar fungal pathogens and endophytes can influence the success and impacts of exotic plants. A key unresolved question concerns how these foliar fungal communities are structured, including whether they systematically differ between native and exotic plants, or are influenced by plant phylogeny and host abundance. To address these questions, we used culturing and Sanger sequencing to characterise the culturable foliar fungal communities of three native and seven exotic grass species that co-occurred in a high-country grassland in Canterbury, Aotearoa New Zealand. We tested the following predictions: Diversity and community composition of culturable foliar fungi differs (1) between native and exotic grass species, (2) between common and rare grass species, and (3) more closely related grass species host more similar fungal communities. We identified 39 fungal operational taxonomic units (OTUs) from 201 isolates. Overall, native and exotic grass species did not differ in their foliar fungal diversity, community composition, or the relative isolation frequency (i.e. abundance) of potential pathogens. However, native grasses hosted a higher proportion of foliar fungi that were isolated from only one host species than exotics (i.e. rare or specialist fungi), possibly due to their longer coevolutionary history with resident fungi. Common grass species (three exotics and two natives) also hosted more fungi that were isolated from only one host species than rare grasses, potentially driven by their greater connectivity and reduced dispersal limitation of foliar fungi. Closely related grass species did not host more similar fungal communities, indicating that host species phylogeny was not a useful predictor of foliar fungal community structure in this high-country grassland. Taken together, our results suggest that exotic grasses have integrated into the resident community via generalist foliar fungi and do not escape from foliar fungal pathogens, with potential for indirect impacts of exotics on co-occurring native plants.

Keywords: community composition, culturing, endophyte, pathogen, plant-fungal interactions

Introduction

Foliar fungal communities play an integral and diverse role in plant life, with impacts on plants occurring on a spectrum from antagonism to mutualism. Fungal pathogens can cause disease and death, whereas endophytes (fungi that occur asymptomatically within plant tissue; Clay & Schardl 2002; Rodriguez et al. 2009) can sometimes aid plant growth, metabolism and defence. Foliar fungi influence the success and impacts of exotic plant species through a variety of direct and indirect mechanisms, sometimes depending on the degree to which exotic plants interact with the resident fungal community (Dickie et al. 2017a). Because exotic species have long been assumed to be novel to their introduced communities (Darwin 1859), they may therefore interact with only a subset of resident species, particularly generalist fungi. Thus, compared to native plant species, exotics may be expected to host different foliar fungal communities, including fewer fungi that are rare, specialists, or pathogens (i.e. the enemy release hypothesis; Elton 1958; Keane & Crawley 2002).

Previous work largely supports these expectations. For example, fungal communities have been demonstrated to differ between roots of native (*Spinifex sericeus*) and exotic (*Ammophila arenaria*) dune grasses in Aotearoa New Zealand (Johansen et al. 2017) and even between the leaves of conspecific native and exotic genotypes of common reed (*Phragmites australis*) in the United States (Allen et al. 2020a). However, without sampling multiple co-occurring native and exotic species in the community, it is unclear whether these results are indicative of systematic differences between native species and novel exotic host plants, or simply reflect patterns of host plant specificity. Broader analyses of fungal host plant databases have begun to hint at differences being systematic. For example, Bufford et al. (2016) found contrasting patterns in the formation of novel plant-fungal associations for native and exotic plants, while another study showed that exotic plants tend to share more ectomycorrhizal fungi with one another than with native species (Dickie et al. 2017b). On the other hand, evidence for the enemy release hypothesis from plant-fungal systems has been mixed. Some studies report fewer

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fungal pathogens for plants in their introduced than native range (Mitchell & Power 2003), some find no differences in fungal diversity and pathogen damage between native and exotic plant species (Johansen et al. 2017; Allen et al. 2020a), and others indicate that pathogens could be a crucial driver of invasive species population dynamics (Flory & Clay 2013; Bever et al. 2015; Flory et al. 2017). Exotic plant species may also dominate communities through stronger mutualistic plant-fungal interactions than co-occurring native species (i.e. the enhanced mutualism hypothesis; Reinhart & Callaway 2006). However, evidence supporting this prediction has also been mixed (Lekberg et al. 2013; Bunn et al. 2015; Reinhart et al. 2017; Allen et al. 2020b).

One reason behind the mixed evidence for the enemy release and enhanced mutualism hypotheses could be that the majority of studies have focussed on pairwise comparisons of specific plant-fungal interactions between native and exotic species, rather than considering multiple co-occurring plant and fungi species in the community. Furthermore, understanding the broader community context of plant-fungal interactions is important because exotic plants can also indirectly interact with other plant species via the accumulation and subsequent spread of shared fungi, termed "spillover" if the fungi are exotic or "spillback" if the fungi are native (Mangla et al. 2008; Day et al. 2016; Dickie et al. 2014; Allen et al. 2020b). These indirect interactions may contribute to the success, impacts, and invasion dynamics of exotic species (Flory & Clay 2013; Dickie et al. 2017a), but are likely to be most important when exotic plants are integrated into the local community and share foliar fungi with native plants.

In addition to the native or exotic status of host, plant-fungal interactions and foliar fungal community composition may be determined by deeper phylogenetic relationships and more broadly conserved traits, such as those that confer constitutive resistance to pathogens or the ability to form mutualistic associations. This phylogenetic conservatism of species interactions, in which closely related species tend to associate with similar partners (Gómez et al. 2010; Peralta 2016), has previously been observed for some plant-fungal interactions (Gilbert & Webb 2007; Bufford et al. 2016; Gilbert & Parker 2016; Schroeder et al. 2019), suggesting that plant phylogenetic relatedness may predict foliar fungal community similarity. However, few studies to date have directly tested this relationship, and the available evidence both supports (Liu et al. 2019) and questions (Vincent et al. 2016) the presence of a phylogenetic signal. Furthermore, fundamental ecological processes could override or interact with these evolutionary considerations. For example, a combination of the phylogenetic rarity of a plant species plus its relative abundance was the best predictor of foliar pathogen damage in a California grassland (Parker et al. 2015). Populations of abundant plant species may have greater connectivity, which could result in less diverse and more specialised fungal communities, stronger negative density dependence via pathogens (Janzen 1970; Connell 1971), and spillover and spillback onto closely-related and co-occurring plant species (Parker et al. 2015; Dickie et al. 2017a). Such density-dependent effects have been observed for plant responses to soil biota (Mangan et al. 2010; Maron et al. 2016; Chung & Rudgers 2016) and foliar pathogen damage (Parker et al. 2015), but it is unclear whether foliar fungi diversity, community composition, and specialisation change with plant dominance.

Despite exotic and indigenous grasslands comprising almost 50% of Aotearoa New Zealand's terrestrial ecosystems (Ministry for the Environment & Statistics New Zealand 2015), there is still little known about the foliar fungal communities of the many native and exotic grass species that occur there. Most studies of plant-fungal interactions in Aotearoa New Zealand grasslands to date have focussed on a subset of fungal taxa considered to be highly influential to plant growth (e.g. *Epichloë* spp.; Hume et al. 2020). Moreover, most of this research has been conducted in highly modified agricultural systems, in contrast to in naturally occurring grasslands that harbour an exceptionally high diversity of plant and fungi species (Wood et al. 2017). Whether Aotearoa New Zealand's native and exotic grass species associate with disparate communities of foliar fungi, like has been observed with mycorrhiza (Dickie et al. 2017b) and general root fungi (Johansen et al. 2017), is a question that remains to be tested in the field.

Here, we conducted a field survey of ten co-occurring grass species, comprising seven exotics and three natives, to simultaneously investigate how host plant species, provenance (native or exotic), rarity (common or rare), and phylogenetic relatedness influence the diversity and community composition of their culturable foliar fungi. Based on invasion ecology theory, density dependence, and the phylogenetic conservatism of species interactions, we tested the following predictions: The diversity and community composition of culturable foliar fungi differs (1) between native and exotic grass species, (2) between common and rare grass species; and (3) more closely related grass species host more similar foliar fungal communities.

Methods

Field survey of grasses at Cass Mountain Research Area

We surveyed the foliar fungal communities of grasses at the Cass Mountain Research Area (CMRA; 43°02′05.1S, 171°45′31.1E) in the Te Waipounamu South Island of Aotearoa New Zealand, on 13 January, 2020. The survey area was a single homogenous grassland community (150 × 100 m), selected because several grass species co-occurred in close proximity, allowing the control of local environmental conditions that can influence foliar fungal communities (Wang et al. 2018).

Ten grass species (seven exotics and three natives; Table 1) were abundant enough to sample. The native grass species *Festuca novae-zelandiae* and *Poa colensoi* are widespread and common in Canterbury high country grasslands, whereas *Anthosachne solandri* is naturally uncommon. All of the exotic grass species surveyed were naturalised in Aotearoa New Zealand between 1844–1872 (Gatehouse 2008; Table 1) and several are now widespread and frequently dominate habitats in which they occur (e.g. *Agrostis capillaris*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Festuca rubra*, *Holcus lanatus*). Thus, our survey area represents a typical grassland community of the Canterbury high country and the CMRA (Young et al. 2016), where many exotic species have been present since the location was first used for research in 1916, although they were only considered a minor component of the flora at the time (Cockayne & Foweraker 1916). All species except *Anthosachne solandri* were included in the checklist of flora identified at the CMRA from over 40 years ago (Burrows 1977), indicating that these species are not recent arrivals to the area. Finally, all the grass species use the C3 metabolic pathway for photosynthesis and all species are perennial except for the annual *Briza minor*.

A combination of systematic and haphazard sampling was used to collect leaf tissue for fungal culturing. First,

Table 1. List of grass species and their provenance, frequency of occurrence in the grassland, and year of naturalisation in Aotearoa New Zealand (Gatehouse 2008). Species were considered rare if found in the study area but only occurring in one or fewer quadrats.

Grass species	Provenance	Occurrence	Year naturalised
Agrostis capillaris	Exotic (Europe)	Common	1867
Anthoxanthum odoratum	Exotic (Europe)	Common	1855
Festuca rubra	Exotic (Europe)	Common	1872
Briza minor	Exotic (Europe)	Rare	1867
Dactylis glomerata	Exotic (Europe)	Rare	1867
Phleum pratense	Exotic (Europe)	Rare	1864
Holcus lanatus	Exotic (Europe)	Rare	1844
Festuca novae-zelandiae	Native (Aotearoa New Zealand)	Common	-
Poa colensoi	Native (Aotearoa New Zealand)	Common	-
Anthosachne solandri	Native (Aotearoa New Zealand)	Rare	-

common grass species (Table 1) were sampled systematically along a 100 m transect through the middle of the grassland by tossing a 0.5 m^2 circular quadrat over the shoulder every five metres, alternating between the left and right of the transect and varying the strength of the toss. Within each quadrat, we sampled one individual of each grass species that we encountered until a total of 10 individuals had been sampled for each grass species (requiring a total of 15 quadrats). When quadrats contained more than one individual of a species, we sampled the individual closest to the quadrat centre. Second, five grass species were identified from within the study area but were rare (i.e. found in one or no quadrats) and so were sampled using a haphazard approach. To sample these rare grass species, we walked through the study area parallel to the transect, locating and randomly selecting individual grasses that were at least 5 m away from a previously sampled conspecific. We sampled 10 individuals of each grass species (with the exception of *Phleum pratense*, for which only nine individuals could be located), including three native species (*Festuca novae-zelandiae*, *Poa colensoi* and *Anthosachne solandri*) and seven exotic species (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Holcus lanatus*, *Briza minor*, *Dactylis glomerata*, *Phleum pratense and Festuca rubra*) (Table 1). Although the number of native grass species was limited, these were the only native species present in the grassland at sufficient abundance for sampling. Grass species were identified using the Manaaki Whenua – Landcare Research New Zealand Grass Key (Ford et al. 2007) and Champion et al. (2012), alongside voucher specimen matching at the University of Canterbury herbarium. To collect leaf tissue samples for culturing foliar fungi, each grass was sampled by removing a randomly selected tiller at the base using garden scissors sterilised with ethanol wipes (Janola Household Wipes, Pental, Australia) between samples. Tillers were stored in individual zip-lock bags and immediately transferred to a cooler containing icepacks, where they were stored overnight before processing the following day.

Culturing and identification of foliar fungi

To isolate foliar fungi, leaf tissue samples were surface-sterilised (30 seconds in 95% ethanol followed by 1 minute in 70% sodium hypochlorite solution and a 5 second rinse with running distilled H_2O, before being left to dry for 1 minute in a sterile petri dish) and cut into 1–2 cm lengths using sterilised forceps and scalpel (2 minutes in 70% ethanol). This resulted in an average of 6.3 ± 0.1 (mean ± SE, range of 3–9) leaf tissue fragments per individual grass that were plated onto potato dextrose agar (PDA) with chloramphenicol antibiotic (PDA: 5 g agar, 7.6 g potato dextrose agar, 1 mL chloramphenicol per 1 L distilled H_2O) for the isolation of emerging foliar fungi. Plates were sealed with parafilm, stored at room temperature (21°C), and checked every 2 days for fungal growth. Fungi that emerged from the grass leaf tissue were isolated and grown as axenic cultures using the same method as above. If multiple fungi emerged from a single leaf fragment, we attempted to isolate them all. Because the number of tissue samples differed among each grass individual, we do not compare OTU isolation frequency or raw OTU richness. Instead, our analyses focussed on rarefied richness and community diversity and composition metrics, which are not influenced by minor variation in sampling effort.

After 7 days of growth, DNA was extracted from the fungal isolates using the Sigma-Aldrich REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich, New Zealand), following manufacturer instructions. A small section of the mycelium of each fungal culture was picked using a sterile pipette tip and crushed into 50 µL REDExtract extraction solution in 1.5 mL Eppendorf tubes. The samples were vortexed, centrifuged (5 seconds at 10 000 rpm) and allowed to rest for 15 minutes, before being heated for 10 minutes at 95°C. Once cooled, 50 µL REDExtract dilution solution was added to each sample before being vortexed, centrifuged, and stored frozen overnight before Polymerase Chain Reaction (PCR).

The PCR protocol used Primers ITS1F (5' – CTTGGTCATTTAGAGGAAGTAA – 3') and ITS4 (5' – TCCTCCGCTTATTGATATGC – 3') (White et al. 1990). Each reaction contained 4 µL of extracted fungal DNA, 11 µL of REDExtract PCR mix, 8.2 µL of PCR grade water, 0.4 µL of 50 µM ITS1F Primer and 0.4 µL of 50 µM ITS4 Primer (total reaction volume of 24 µL). The extracted fungal DNA was vortexed and centrifuged briefly before being added to the PCR mix. All PCR amplifications were run on the Eppendorf Mastercycler pro PCR system (Thermo Fisher Scientific) at the University of Canterbury. The parameters for this protocol used an initial ramp up period of 1 hour 25 minutes at 94°C, 13 cycles of 35 seconds at 95°C, 55 s at 55°C and 45 s at 72°C, 14 cycles of 35 s at 95°C, 55 s at 55°C and 2 minutes at 72°C, and 9 cycles of 35 s at 95°C, 55 s at 55°C and 3 minutes at 72°C. This was followed by a 10 minute annealing period at 72°C, before a final cooling to 4°C.

PCR amplification was confirmed via electrophoresis of 3 µL of PCR product through a 1% high-grade agarose gel in TAE buffer (40 mM Tris [pH = 7.6], 20 mM acetic acid, 1 mM EDTA) with Red Safe™ nucleic acid staining solution (iNtRON Biotechnology, South Korea) for 30 minutes at 100 V and 500

mA. Successful PCR amplification products were sequenced using Sanger sequencing at Te Whare Wānaka o Aoraki Lincoln University, Aotearoa New Zealand. The resulting sequences were grouped into operational taxonomic units (OTUs, 97% similarity) and identified via the NCBI Nucleotide BLAST software using one sequence of each OTU, optimising for similar sequences (blastn). Multiple sequence alignment of our ITS sequences plus reference sequences from GenBank was conducted using MAFFT software (https://mafft.cbrc.jp/alignment/server/) (Katoh & Toh 2010) and phylogenetic analysis of the fungal ITS sequences was performed using MrBayes (Huelsenbeck & Ronquist 2001) and based on the Jukes-Cantor nucleotide substitution model. Posterior probabilities of phylogenetic relationships were inferred using a Bayesian Markov chain Monte Carlo (MCMC) simulation run for 200 000 generations, with trees sampled every 100 generations.

Finally, to investigate differences in the prevalence of potential pathogens between sampled native and exotic grasses, we identified putative functions (i.e. plant pathogen, saprotroph, endophyte) for each OTU based on the FUNGuild database (Nguyen et al. 2016). We used these data to calculate relative pathogen abundance for each grass species (i.e. the proportion of total isolates that were identified as potential pathogens). We recognise that many fungi can fulfil multiple functions depending on life stage or environmental conditions (Suryanarayanan 2013), and this is reflected by OTUs with multiple putative functions in Figs 1, 2; Appendix S1 in Supplementary Materials.

Data analysis

To test our hypotheses of the drivers of culturable foliar fungal community composition, we first used *t*-tests to compare several metrics of fungal diversity between native and exotic grass species, and between common and rare grass species. The diversity metrics investigated were rarefied OTU species richness, effective diversity (i.e. Hill number 1, or the exponent of the Shannon diversity index), and the Chao1 diversity estimator. Rarefied OTU richness (rarefied to 10 isolates, the lowest number obtained from any one grass species) was used to correct for any sampling bias that could arise from the unequal number of leaf tissue samples obtained from each individual grass. We also compared the proportion of fungi that were isolated from only one host grass species and the relative abundance of potential pathogens between native and exotic grasses and between common and rare grasses using *t*-tests.

Second, we compared culturable foliar fungal community composition between native and exotic grasses and common and rare grasses using ordination analyses. We summed the number (i.e. frequency) of isolates of each OTU for each grass species and calculated Bray-Curtis dissimilarity of foliar fungal communities between grass species. We then subjected this dissimilarity index to principal co-ordinate analysis (PCoA) to visualise whether foliar fungal community composition differed between native and exotic grasses or between common and rare grasses. Permutational Analysis of Variance (PERMANOVA) with 999 permutations (the 'adonis' function in the vegan R package; Oksanen et al. 2019) was then used to test whether foliar fungal community composition differed between these groups more than would be expected based on chance. To ensure we did not give too much weight to more frequently isolated OTUs, we repeated these analyses using the Jaccard dissimilarity index (i.e. based on presence-absence of OTUs). We observed no qualitative change in the results, so present only the results using Bray-Curtis dissimilarity here.

Third, to test whether closely related grasses had more similar culturable foliar fungal communities, we quantified phylogenetic distance between each grass species using Phylomatic (Webb & Donoghue 2005) and based on the dated phylogenetic Supertree R2G2_20140601 (Parker et al. 2015; Li et al. 2019). We then used a linear mixed model to test whether pairwise Bray-Curtis dissimilarities of foliar fungal communities between grass species (response variable) were related to their pairwise phylogenetic distance (explanatory variable). To control for multiple occurrences of each grass species in the dataset, we included random intercepts for the identity of each grass species. Because native and exotic grass species were not phylogenetically clustered, we were able to test the influence of phylogenetic distance independent of plant provenance. To further assist in visualising the comparison between foliar fungal community similarity and plant phylogeny, we used hierarchical clustering with Bray-Curtis dissimilarities to construct a cluster dendrogram of the grass species based on the similarity of their foliar fungal communities.

Finally, to test whether culturable foliar fungal communities differed among the ten grass species, we repeated the same ordination analyses as described above, but using an OTU abundance (i.e. frequency of isolation) matrix at the individual grass level (as opposed to summed within grass species). Post-hoc contrasts were conducted using pairwise PERMANOVA analyses (Bonferroni-corrected) to identify significant pairwise differences in foliar fungal communities between the grass species. All data analyses were performed using R version 3.6.1 (R Core Team 2019), and the vegan (Oksanen et al. 2019), lme4 (Bates et al. 2019), phangorn (Schliep 2011), ggtree (Yu et al. 2017), and bipartite (Dormann et al. 2008) packages.

Results

From 206 foliar fungal isolates, 201 were successfully sequenced (deposited into GenBank under accession numbers MW054250-MW054450) and 39 distinct operational taxonomic units (OTUs) were obtained based on 97% sequence similarity (Table S1). Using the FUNGuild database, nine OTUs were identified as potential pathogens (23%), one as an endophyte (3%), ten as saprotrophs (26%), and eleven (28%) as having multiple potential functions (all including plant pathogen), while no information on function was available from FUNGuild for the remaining six identified OTUs (15%) (Figs 1, 2). *Ascomycota* was the dominant phylum (36 OTUs, 92%), followed by *Basidiomycota* (2 OTUs, 5%) and *Mucoromycota* (1 OTU, 3%). Among the *Ascomycota*, the dominant orders included *Pleosporales* (36%), *Heliotales* (13%), and *Xylariales* (8%). One isolate (OTU34, Unknown fungus aff. *Anthostomelloides* sp.) is thought to be previously undetected in Aotearoa New Zealand. We identified a diverse group of fungal taxa that encompassed widespread pathogens like *Botrytis cinerea* (OTU35), a generalist across many plant species, including those of agricultural and horticultural value (Williamson et al. 2007). Conversely, several potentially beneficial fungi were also identified, such as *Epicoccum* sp. (OTU8), a genus of fungi that can stimulate plant growth and produce secondary metabolites that inhibit pathogenic fungi (Fávaro et al. 2012).

Species accumulation and rarefaction curves indicated that

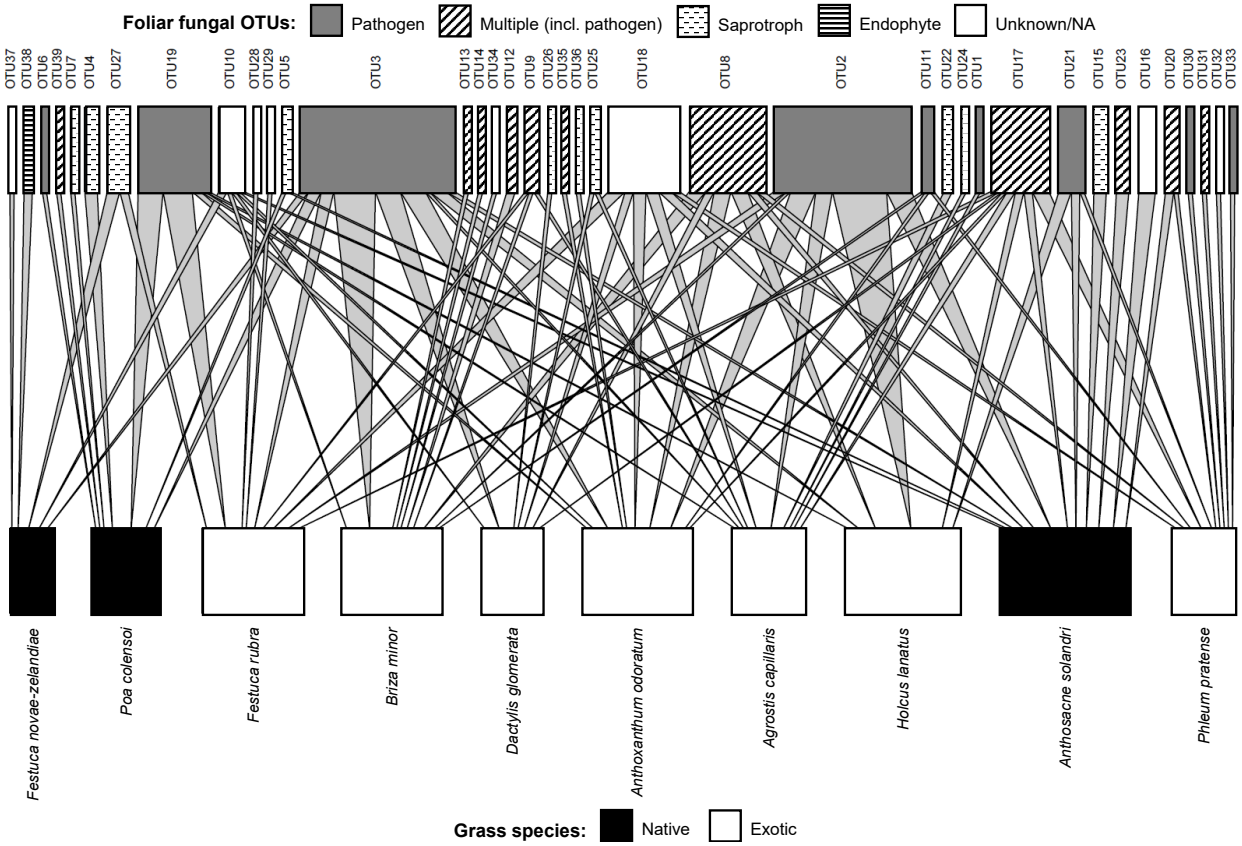

Figure 1. Plant-fungal interaction network illustrating the frequency (link width) of interactions between grass species and culturable foliar fungi OTUs at the Cass Mountain Research Area, Canterbury, Aotearoa New Zealand. Node width represents the isolation frequency of each OTU (top) and the number of isolates obtained from each grass species (bottom). Node colour represents the putative function of each fungal OTU based on the FUNGuild database (top) and provenance of each grass species (bottom).

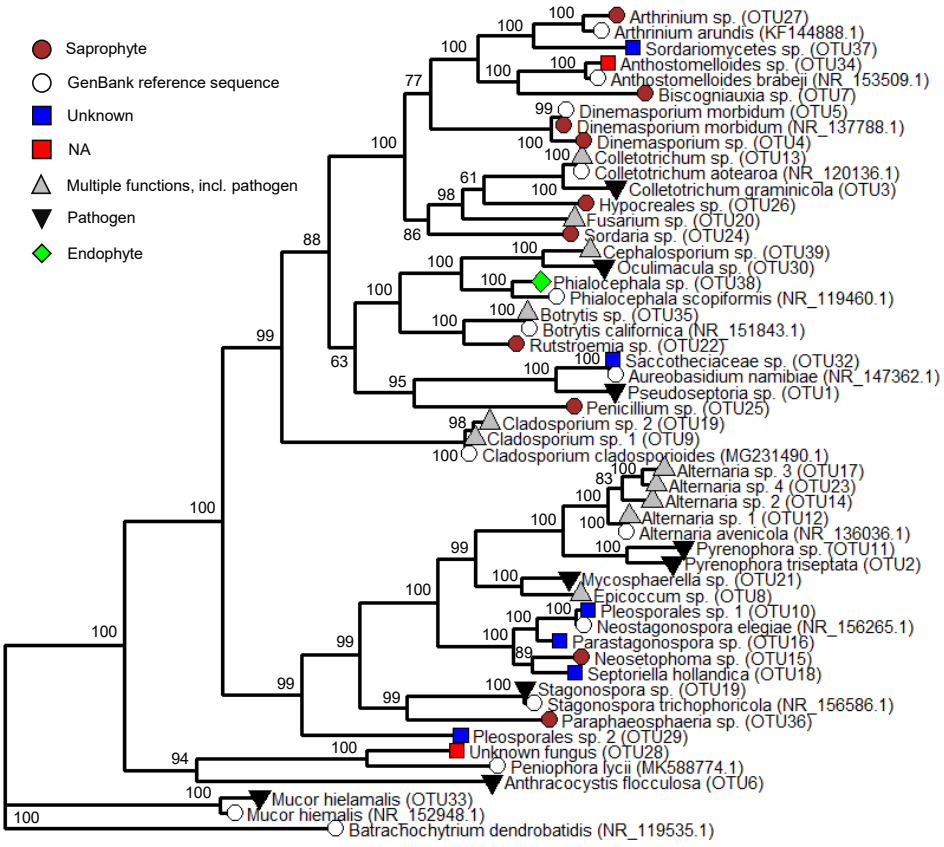

Figure 2. Phylogenetic tree showing the relationships among the foliar fungal OTUs isolated from ten grass species at the Cass Mountain Research Area, Canterbury, Aotearoa New Zealand. The tree was inferred from sequences of the ITS1-5.8S-ITS2 rDNA region using a Bayesian Markov chain Monte Carlo (MCMC) simulation run for 200 000 generations, with trees sampled every 100 generations. Numbers adjacent to nodes indicate the posterior probability of each branch. Symbol shapes represent the putative function of OTUs based on the FUNGuild database: blue or red square = Unknown or NA, respectively; brown circle = saprophyte; grey triangle = multiple functions; including pathogen; black inverted triangle = pathogen; green diamond = endophyte; unfilled circle = reference sequence from GenBank.

we captured less than half of the total estimated foliar fungal diversity present in grasses in the study area (estimated at 193 ± 24 OTUs using the Chao1 diversity estimator) (Appendix S2). Community composition of culturable foliar fungi varied among the grass species (PERMANOVA: $F_{9,75} = 2.41$, $p = 0.001$; Appendix S3), and post-hoc pairwise PERMANOVAs revealed pairwise differences between the fungal communities of *Holcus lanatus* and *Festuca novae-zelandiae*, *Holcus lanatus* and *Poa colensoi*, and *Poa colensoi* and *Anthosachne solandri* (all $p = 0.045$).

Native grass species hosted more single-host fungi, but had similar diversity to exotic grass species

Foliar fungal diversity did not differ between native and exotic grass species (all $p > 0.378$ for rarefied OTU richness, effective diversity, and the Chao1 diversity estimator; Table 2; Appendix S4) and neither did foliar fungal community composition (PERMANOVA: $F_{1,8} = 1.41$, $p = 0.131$; Fig. 3). Although the relative abundance of potential pathogens did not differ between native and exotic grasses ($t_8 = 2.15$, $p = 0.064$), native grasses hosted 4.5 times greater relative abundance of fungi that were isolated from only one host species ($t_8 = 3.35$, $p = 0.010$).

Rare grass species hosted more single-host fungi, but had similar diversity to common grass species

Foliar fungal diversity metrics (all $p > 0.599$; Table 2), community composition (PERMANOVA: $F_{1,8} = 1.63$, $p = 0.107$; Fig. 3), and the relative abundance of potential pathogens ($t_8 = 1.59$, $p = 0.150$) also did not differ between common and rare grass species. However, rare grasses (i.e. those surveyed using haphazard sampling) hosted almost five times greater relative abundance of fungi that were isolated from only one host species than their common counterparts ($t_8 = 2.35$, $p = 0.047$).

Phylogenetic relatedness of grasses does not predict the similarity of their fungal communities

We did not detect a correlation between pairwise phylogenetic distance of grass species and the dissimilarity of their foliar fungal communities ($F_{1,13} = 0.68$, $p = 0.424$). This result was further demonstrated through visual comparison of the grass phylogeny and cluster dendrogram, where patterns of phylogenetic relatedness did not align with foliar fungal community similarity for our sampled grass species (Fig. 4).

Table 2. Mean (± SE) values of various diversity metrics describing foliar fungal communities cultured from native or exotic grass species and common or rare grass species at the Cass Mountain Research Area, Canterbury, Aotearoa New Zealand. Bold text represents comparisons where means differed significantly between grass provenance or rarity based on t-tests ($p \leq 0.05$). Potential pathogen relative abundance was determined using the FUNGuild database, from which we assigned taxa as potential pathogens, saprotrophs, endophytes, unknown (for unidentified fungi), and NA (if no information on function was available on FUNGuild).

Diversity metric	Grass species provenance		Grass species rarity	
	Native (n = 3)	Exotic (n = 7)	Common (n = 5)	Rare (n = 5)
Rarefied OTU richness	5.89 ± 0.69	6.11 ± 0.38	5.97 ± 0.37	6.11 ± 0.55
Effective diversity	6.94 ± 1.87	6.88 ± 0.61	6.75 ± 0.72	7.05 ± 1.14
Chao1 estimated diversity	10.06 ± 2.54	13.21 ± 1.91	11.60 ± 1.89	12.93 ± 2.63
Relative abundance of potential pathogens	0.50 ± 0.15	0.77 ± 0.06	0.59 ± 0.10	0.79 ± 0.07
Proportion of OTUs isolated from only one host species	0.29 ± 0.06	0.06 ± 0.04	0.22 ± 0.06	0.05 ± 0.04

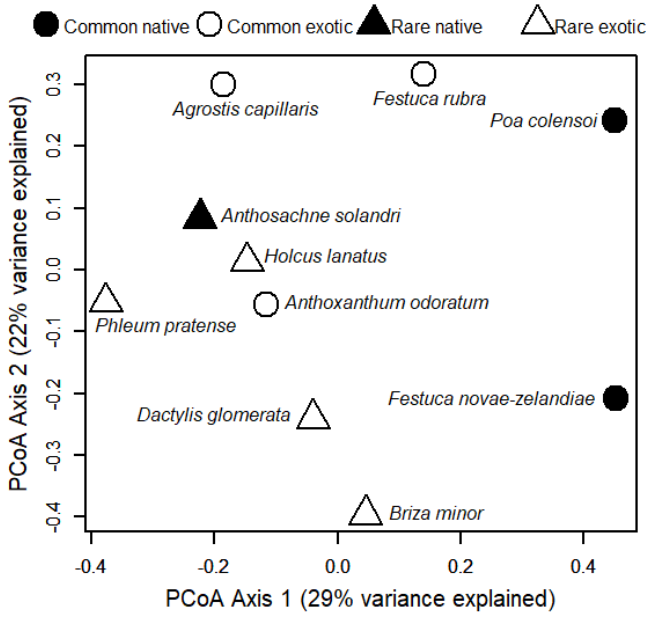

Figure 3. Ordination showing principal coordinates analysis of Bray-Curtis dissimilarities among the culturable foliar fungal communities of grass species sampled at Cass Mountain Research Area, Canterbury, Aotearoa New Zealand. Each point represents the foliar fungal community of a native (filled) or exotic (open) grass species that was either common (circles) or rare (triangles) in the plant community. Species that are closer together in ordination space hosted more similar foliar fungal communities.

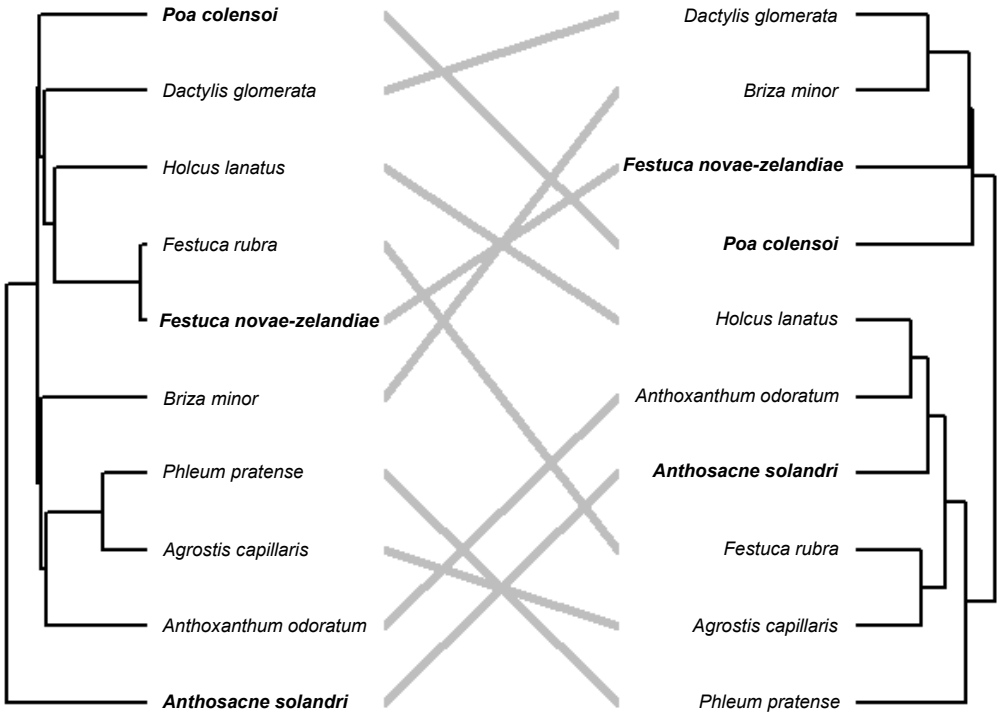

Figure 4. Tanglegram showing the lack of congruence between the phylogenetic tree (left) of native (bold text) and exotic (normal text) grass species and a cluster dendrogram (right) of their corresponding fungal communities at Cass Mountain Research Area, Canterbury, Aotearoa New Zealand. Phylogenetic distance between each grass species was quantified using Phylomatic (Webb & Donoghue 2005) and based on the dated phylogenetic Supertree R2G2_20140601 (Parker et al. 2015), while the cluster dendrogram was based on the Bray-Curtis dissimilarity of plant foliar fungal communities.

Discussion

In contrast to our expectations based on coevolutionary and invasion biology theory, we found that the diversity and community composition of culturable foliar fungi largely did not differ between native and exotic grass species or between common and rare grasses. However, both exotic and rare grass species hosted fewer fungi that were isolated from only one host species than native and common grasses, respectively. We also found that phylogenetic relatedness of grass species did not predict the similarity of their culturable foliar fungal communities. Taken together, these findings indicate that exotic grasses, especially those that were common in the grassland, have integrated into the resident foliar fungal community at the CMRA. Moreover, the significant sharing of foliar fungal OTUs between native and exotic grass hosts suggests the potential for indirect interactions via fungal spillover and spillback.

In contrast to the enemy release hypothesis (Elton 1958; Keane & Crawley 2002), the enhanced mutualism hypothesis (Reinhart & Callaway 2006), and our first prediction, we found no differences in the diversity of culturable foliar fungi between native and exotic grass species at CMRA. The lack of support for the enemy release hypothesis is consistent with previous field surveys of native and exotic plants (Johansen et al. 2017; Allen et al. 2020a) and analyses of database records (Clay 1995; Vacher et al. 2010). Moreover, we found no systematic differences in the foliar fungal community composition of native and exotic plant species, further adding to the mixed results of previous studies (Koyama et al. 2019; Allen et al. 2020a). These mixed results could be due to differences among study systems and species, such as their time since introduction (Diez et al. 2010; Mitchell et al. 2010). For example, the seven exotic species included in our survey have all been present in Aotearoa New Zealand for at least 148 years, which could be sufficient time to accumulate interactions with both native and co-introduced fungi (Sikes et al. 2018). Regardless of when these interactions were realised, the exotic grasses found at CMRA appear to have integrated into the resident fungal community, enhancing potential for indirect interactions with other co-occurring native and exotic grasses, mediated by pathogens and mutualists (i.e. spillover or spillback, depending on fungi provenance) (Mangla et al. 2008; Day et al. 2016; Dickie et al. 2014; Allen et al. 2020b). Although we were unable to directly test for the occurrence of spillback or spillover, these indirect interactions have the potential to contribute to the dominance of exotic grasses in the community (Flory & Clay 2013).

Both native and common grass species hosted a higher proportion of foliar fungi that were isolated from only one host species than their exotic and rare counterparts, respectively. Fungal OTUs isolated from a single host species likely represent fungi that were rare (e.g. the preferred host was not present or the fungi was not abundant at this time of the year) or specialists, although our results point towards the latter explanation. For example, the higher proportion of these fungal OTUs on native grasses could be driven by a shared coevolutionary history (i.e. specialisation). In contrast, exotic plants may primarily interact with generalist species that are shared with other hosts, potentially explaining the integration of the exotic grasses into the resident plant-fungal community at the CMRA. This finding contrasts with those of Bufford et al. (2016; 2019), who analysed database records to show that native plants disproportionately form novel associations with exotic generalist fungi. Moreover, the greater proportion of single-host fungi isolated from common grass species could be a result of greater connectivity among individuals, which may reduce dispersal limitation and allow competitively superior specialists to dominate their preferred plant host.

In contrast to our third prediction, phylogenetic relatedness of grass species was not related to the similarity of their culturable foliar fungal communities. This finding is consistent with a study of the foliar fungal communities of 11 tree species

in Papua New Guinea (Vincent et al. 2016) but conflicts with the frequently-observed phylogenetic conservatism of plant-fungal interactions (Gilbert & Webb 2007; Gilbert & Parker 2016), including for foliar fungal communities among 47 *Ficus* species in a botanical garden in China (Liu et al. 2019). One reason why we did not detect a relationship between phylogenetic distance and community dissimilarity could be because we surveyed only a small portion of the plant phylogenetic tree (i.e. within the grass family - Poaceae). Phylogenetic conservatism is commonly tested at broader taxonomic scales (i.e. across multiple families or orders; Gómez et al. 2010; Anacker et al. 2014) and may be less important at finer taxonomic scales or within certain taxonomic groups. In other words, it is possible that the phylogenetic similarity of all our grass species was relatively high and thus it was likely that most fungi could interact with most grasses. Alternatively, it is possible that the lack of co-evolutionary history between exotic plants and the resident community may confound phylogenetic predictions, although the lack of a plant provenance effect on foliar fungal community composition does not support this interpretation.

Instead of the variables we investigated other important factors that we did not account for could influence foliar fungal diversity and community composition, such as priority effects and microhabitat differences (Adame-Álvarez et al. 2014; van Bael et al. 2017). For example, fungi can be long lived and often demonstrate strong priority effects, meaning that some species may persist for a long time, resulting in divergent fungal communities between hosts that depend on the identity of early-colonising species (Kennedy & Bruns 2005; Hiscox et al. 2015). Another possibility is that the strength of species interactions may vary depending upon the provenance of both interaction partners (Parker et al. 2006; Dickie et al. 2017a). Due to our relatively small number of isolates and difficulty in identifying them to species level, we did not attempt to identify the provenance of our identified foliar fungi. However, Bufford et al. (2016; 2019) previously showed that exotic pathogens tend to be highly generalist in their interactions with both native and exotic plants in Aotearoa New Zealand, which may explain the lack of differentiation of fungal communities beyond the host plant species level.

Alternatively, our species accumulation and rarefaction curves indicated that we captured less than half of the total estimated foliar fungal diversity culturable from grasses in the study area (Appendix S2). Because the ordination plots comparing foliar communities of native and exotic grasses and common and rare grasses are suggestive of trends along axis 1 (Fig. 3), the drivers of foliar fungal community structure in this community may only become apparent through further sampling. The sampling of culturable fungal diversity also only identifies a subset of fungi associated with the host plant, particularly fast-growing, generalist species, whereas fungi that are poor competitors or have specialist growth requirements will be difficult to culture (e.g. biotrophic pathogens that require live host tissue in order to survive). We also used only a single type of growth media for our study, which may further select for fungi that grow well on potato dextrose agar. In comparison, next-generation sequencing approaches tend to capture more diversity. However, they are also expensive, require significant technical expertise, have their own taxonomic biases depending upon the primers used, can amplify DNA from non-viable tissue, and often lead to conclusions consistent with culturing approaches (Johnston et al. 2017; Dissanayake et al. 2018). If the goal is to obtain a full picture of the fungal diversity present in a sample, future studies should consider using a combination of culturing and next-generation sequencing. However, the evidence above suggests that culturing remains a reliable and cost-effective approach for testing hypotheses on fungal community composition, and is particularly useful because fungal cultures can also be used in future experiments to investigate their function and interactions with the host plant.

This is one of the first studies to characterise and compare the culturable foliar fungal communities that reside in co-occurring native and exotic grasses in Aotearoa New Zealand. Although we found no significant differences in foliar fungal diversity or community composition between native and exotic grasses, native grass species tended to associate with a higher proportion of fungi that were isolated from only one host species than exotic grasses, as did rare grass species. Moreover, no phylogenetic signal was detected in the structuring of culturable foliar fungal communities among plants, suggesting that other unmeasured factors may be more important to determining plant-fungal interactions, rather than fine-scale trait evolution. We conclude that exotic grasses have successfully integrated into the resident plant-fungal community at CMRA via their interactions with generalist fungi, with potential implications for exotic grass invasions and the state of Aotearoa New Zealand grasslands.

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Author contributions

WJA and IAD conceived the project and obtained funding. MLV collected the data, conducted analyses and wrote the first draft of the manuscript, with all authors contributing to subsequent revisions.

References

Adame-Álvarez RM, Mendiola-Soto J, Heil M 2014. Order of arrival shifts endophyte-pathogen interactions in bean from resistance induction to disease facilitation. FEMS Microbiology Letters 355: 100–107.

Allen WJ, Devries AE, Bologna NJ, Bickford WA, Kowalski KP, Meyerson LA, Cronin JT 2020a. Intraspecific and biogeographical variation in foliar fungal communities and pathogen damage of native and invasive *Phragmites australis*. Global Ecology and Biogeography 29: 1199–1211.

Allen WJ, Wainer R, Shadbolt M-W, Tylianakis JM, Barratt BIP, Waller LP, Dickie IA 2020b. Community-level direct and / impacts of an invasive plant favour exotic over native species. Journal of Ecology 108: 2499–2510.

Anacker BL, Klironomos JN, Maherali H, Reinhart KO, Strauss SY 2014. Phylogenetic conservatism in plant-soil feedback

and its implications for plant abundance. Ecology Letters 17: 1613–1621.
Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann H, Dai B, Scheipl F, Grothendieck G, Green P, Fox J 2019. lme4: linear mixed-effects models using 'Eigen' and S4. R package version 1.1-21. http://CRAN.R-project.org/package=lme4.
Bever JD, Mangan SA, Alexander HM 2015. Maintenance of plant species diversity by pathogens. Annual Review of Ecology, Evolution, and Systematics 46: 305–325.
Bufford JL, Hulme PE, Sikes BA, Cooper JA, Johnston PR, Duncan RP 2016. Taxonomic similarity, more than contact opportunity, explains novel plant-pathogen associations between native and alien taxa. New Phytologist 212: 657–667.
Bufford JL, Hulme PE, Sikes BA, Cooper JA, Johnston PR, Duncan RP 2019. Novel interactions between alien pathogens and native plants increase plant-pathogen network connectance and decrease specialization. Journal of Ecology 108: 750–760.
Bunn RA, Ramsey PW, Lekberg Y 2015. Do native and invasive plants differ in their interactions with arbuscular mycorrhizal fungi? A meta-analysis. Journal of Ecology 103: 1547–1556.
Burrows CJ 1977. History and science of the Cass District, Canterbury, New Zealand. Christchurch, University of Canterbury. 418 p.
Champion PD, James T, Popay I, Ford K 2012. An illustrated guide to common grasses, sedges and rushes of New Zealand. Havelock North, New Zealand Plant Protection Society. 208 p.
Chung YA, Rudgers JA 2016. Plant-soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses. Proceedings of the Royal Society B: Biological Sciences 283: 20160608.
Clay K 1995. Correlates of pathogen species richness in the grass family. Canadian Journal of Botany 73: S42–S49.
Clay K, Schardl C 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. The American Naturalist 160: 99–127.
Cockayne L, Foweraker CE 1916. Notes from the Canterbury College Mountain Biological Station 4. The principal plant associations in the immediate vicinity of the station. Transactions of the New Zealand Institute 48: 166–186.
Connell JH 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Dynamics of Population 298: 298–312.
Darwin C 1859. On the origin of species. London, John Murray. 502 p.
Day NJ, Dunfield KE, Antunes PM 2016. Fungi from a non-native invasive plant increase its growth but have different growth effects on native plants. Biological Invasions 18: 231–243.
Dickie IA, St John MG, Yeates GW, Morse CW, Bonner KI, Orwin K, Peltzer DA 2014. Belowground legacies of *Pinus contorta* invasion and removal result in multiple mechanisms of invasional meltdown. AoB Plants 6: plu056.
Dickie IA, Bufford JL, Cobb RC, Desprez-Loustau M-L, Grelet G, Hulme PE, Klironomos J, Makiola A, Nuñez M, Pringle A, Thrall PH, Tourtellot SG, Waller L, Williams NM 2017a. The emerging science of linked plant-fungal invasions. New Phytologist 215: 1314–1332.
Dickie IA, Cooper JA, Bufford JL, Hulme PE, Bates ST 2017b. Loss of functional diversity and network modularity in introduced plant-fungal symbioses. AoB Plants 9: plw084.
Diez JM, Dickie I, Edwards G, Hulme PE, Sullivan JJ, Duncan RP 2010. Negative soil feedbacks accumulate over time for non-native plant species. Ecology Letters 13: 803–809.
Dissanayake AJ, Purahong W, Wubet T, Hyde KD, Zhang W, Xu H, Zhang G, Fu C, Liu M, Xing Q, Li X, Yan J 2018. Direct comparison of culture-dependent and culture-independent molecular approaches reveal the diversity of fungal endophytic communities in stems of grapevine (*Vitis vinifera*). Fungal Diversity 90: 85–107.
Dormann CF, Fruend J, Gruber B 2008. Introducing the bipartite package: analysing ecological networks. R News 8: 8–11.
Elton CS 1958. The ecology of invasions by animals and plants. London, Methuen. 181 p.
Fávaro LC, Sebastianes FL, Araújo WL 2012. *Epicoccum nigrum* P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. PLoS ONE 7: e36826.
Flory SL, Clay K 2013. Pathogen accumulation and long-term dynamics of plant invasions. Journal of Ecology 101: 607–613.
Flory SL, Alba C, Clay K, Holt RD, Goss EM 2017. Emerging pathogens can suppress invaders and promote native species recovery. Biological Invasions 20: 5–8.
Ford KA, Glenny D, James T 2007. NZ Grass Key – key to the grasses of New Zealand. http://www.landcareresearch.co.nz/resources/identification/plants/grass-key (Accessed 14 January 2020).
Gatehouse HAW 2008. Ecology of the naturalisation and geographic distribution of the non-indigenous seed plant species of New Zealand. Lincoln University, unpublished Ph.D. thesis.
Gilbert GS, Webb CO 2007. Phylogenetic signal in plant pathogen-host range. Proceedings of the National Academy of Sciences of the United States of America 104: 4979–4983.
Gilbert GS, Parker IM 2016. The evolutionary ecology of plant disease: a phylogenetic perspective. Annual Review of Phytopathology 54: 549–578.
Gómez JM, Verdú M, Perfectti F 2010. Ecological interactions are evolutionary conserved across the entire tree of life. Nature 465: 918–921.
Hiscox J, Savoury M, Müller C, Lindahl BD, Rogers HJ, Boddy L 2015. Priority effects during fungal community establishment in beech wood. The ISME Journal 9: 2246–2260.
Huelsenbeck JP, Ronquist F 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754-755.
Hume DE, Stewart AV, Simpson WR, Johnson RD 2020. *Epichloë* fungal endophytes play a fundamental role in New Zealand grassland. Journal of the Royal Society of New Zealand 50: 279–298.
Janzen D 1970. Herbivores and the number of tree species in tropical forests. The American Naturalist 104: 501–528.
Johansen RB, Johnston P, Mieczkowski P, Perry GL, Robeson MS, Vilgalys R, Burns BR 2017. Scattered far and wide: A broadly distributed temperate dune grass finds familiar fungal root associates in its invasive range. Soil Biology and Biochemistry 112: 177–190.
Johnston PR, Park D, Smissen RD 2017. Comparing diversity of fungi from living leaves using culturing and high-throughput environmental sequencing. Mycologia 109: 643–654.
Katoh K, Toh H 2010. Recent developments in the MAFFT

multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298.
Keane RM, Crawley MJ 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17: 164–170.
Kennedy PG, Bruns TD 2005. Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings. New Phytologist 166: 631–638.
Koyama A, Maherali H, Antunes PM 2019. Plant geographic origin and phylogeny as potential drivers of community structure in root-inhabiting fungi. Journal of Ecology 107: 1720–1736.
Lekberg Y, Gibbons SM, Rosendahl S, Ramsey PW 2013. Severe plant invasions can increase mycorrhizal fungal abundance and diversity. The ISME Journal 7: 1424–1433.
Li D, Trotta L, Marx HE, Allen JM, Sun M, Soltis DE, Soltis PS, Guralnick RP, Baiser B 2019. For common community phylogenetic analyses, go ahead and use synthesis phylogenies. Ecology 100: e02788.
Liu J, Zhao J, Wang G, Chen J 2019. Host identity and phylogeny shape the foliar endophytic fungal assemblages of *Ficus*. Ecology and Evolution 9: 10472-10482.
Mangla S, Inderjit, Callaway RM 2008. Exotic invasive plant accumulates native soil pathogens which inhibit native plants. Journal of Ecology 96: 58–67.
Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sandchez EI, Bever JD 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466: 752–755.
Maron JL, Laney Smith A, Ortega YK, Pearson DE, Callaway RM 2016. Negative plant-soil feedbacks increase with plant abundance, and are unchanged by competition. Ecology 97: 2055–2063.
Ministry for the Environment & Statistics New Zealand 2015. New Zealand's Environmental Reporting Series: Environment Aotearoa 2015. Available from www.mfe.govt.nz and www.stats.govt.nz. Accessed 24 May 2020.
Mitchell CE, Power AG 2003. Release of invasive plants from fungal and viral pathogens. Nature 421: 625–627.
Mitchell CE, Blumenthal D, Jarošík V, Puckett EE, Pyšek P 2010. Controls on pathogen species richness in plants' introduced and native ranges: roles of residence time, range size and host traits. Ecology Letters 13: 1525–1535.
Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20: 241–248.
Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H 2019. vegan: community ecology package. R Package Version 2.5-5. https://CRAN.R-project.org/package=vegan.
Parker JD, Burkepile DE, Hay ME 2006. Opposing effects of native and exotic herbivores on plant invasions. Science 311: 1459–1461.
Parker IM, Saunders M, Bontrager M, Weitz AP, Hendricks R, Magarey R, Suiter K, Gilbert GS 2015. Phylogenetic structure and host abundance drive disease pressure in communities. Nature 520: 542–544.
Peralta G 2016. Merging evolutionary history into species interaction networks. Functional Ecology 30: 1917–1925.
R Core Team 2019. R: A language and environment for statistical computing. Version 3.6.1. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from. https://www.R-project.org.
Reinhart KO, Callaway RM 2006. Soil biota and invasive plants. New Phytologist 170: 445–457.
Reinhart KO, Lekberg Y, Klironomos J, Maherali H 2017. Does responsiveness to arbuscular mycorrhizal fungi depend on plant invasive status? Ecology and Evolution 7: 6482–6492.
Rodriguez RJ, White Jr. JF, Arnold AE, Redman RS 2009. Fungal endophytes: diversity and functional roles. New Phytologist 182: 314–330.
Schroeder JW, Martin JT, Angulo DF, Razo IA-D, Barbosa JM, Perea R, Sebastián-González E, Dirzo R 2019. Host plant phylogeny and abundance predict root-associated fungal community composition and diversity of mutualists and pathogens. Journal of Ecology 107: 1557–1566.
Sikes BA, Bufford JL, Hulme PE, Cooper JA, Johnston PR, Duncan RP 2018. Import volumes and biosecurity interventions shape the arrival rate of fungal pathogens. PLoS Biology 16: e2006025.
Suryanarayanan TS 2013. Endophyte research: going beyond isolation and metabolite documentation. Fungal Ecology 6: 561–568.
Vacher C, Daudin J-J, Piou D, Desprez-Loustau M-L 2010. Ecological integration of alien species into a tree–parasitic fungus network. Biological Invasions 12: 3249–3259.
van Bael SA, Estrada C, Arnold AE 2017. Foliar endophyte communities and leaf traits in tropical trees. In: Dighton J, White JF eds. The fungal community: its organisation and role in the ecosystem. Boca Raton, CRC Press, Taylor and Francis. Pp 79–94.
Vincent JB, Weiblen GD, May G 2016. Host associations and beta diversity of fungal endophyte communities in New Guinea rainforest trees. Molecular Ecology 25: 825–841.
Wang J, Chen C, Ye Z, Li J, Feng Y, Lu Q 2018. Relationships between fungal and plant communities differ between desert and grassland in a typical dryland region of northwest China. Frontiers in Microbiology 9: 2327.
Webb CO, Donoghue MJ 2005. Phylomatic: tree assembly for applied phylogenetics. Molecular Ecology Resources 5: 181–183.
White TJ, Bruns T, Lee S, Taylor JW 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ eds. PCR protocols: a guide to methods and applications. New York, Academic Press. Pp 315–322.
Williamson B, Tudzynski B, Tudzynski P, van Kan JA 2007. *Botrytis cinerea*: the cause of grey mould disease. Molecular Plant Pathology 8: 561–580.
Wood JR, Holdaway RJ, Orwin KH, Morse C, Bonner KI, Davis C, Bolstridge N, Dickie IA 2017. No single driver of biodiversity: divergent responses of multiple taxa across land use types. Ecosphere 8: e01997.
Young LM, Norton DA, Lambert MT 2016. One hundred years of vegetation change at Cass, eastern South Island high country. New Zealand Journal of Ecology 40: 289–301.
Yu G, Smith DK, Zhu H, Guan Y, Lam TT-Y 2017. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods in Ecology and Evolution 8: 28–36.

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Supplementary material

Additional supporting information may be found in the supplementary material file for this article:

Appendix S1. Fungal Operational Taxonomic Units (OTUs) isolated and identified from leaves of ten grass species at Cass Mountain Research Area, Canterbury, Aotearoa New Zealand in January 2020.

Appendix S2. Taxa accumulation (a) and rarefaction curves (b) for foliar fungi cultured from ten grass species at Cass Mountain Research Area, Canterbury, Aotearoa New Zealand.

Appendix S3. Ordination showing principal coordinates analyses of Bray-Curtis dissimilarities among the foliar fungal isolated from individual grasses at Cass Mountain Research Area, Canterbury, Aotearoa New Zealand. Each point represents the cultured foliar fungal community of an individual grass, with different colours indicating the different grass species. Individual grasses closer together in ordination space hosted more similar fungal communities.

Appendix S4. Foliar fungal diversity metrics for each grass species surveyed at the Cass Mountain Research Area, Canterbury, Aotearoa New Zealand.

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