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COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Host plant environmental filtering drives foliar fungal community assembly in symptomatic leaves

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Abstract

Foliar fungi (defined as all fungal species in leaves after surface sterilization; hereafter, 'FF') are of great importance to host plant growth and health, and can also affect ecosystem functioning. Despite this importance, few studies have explicitly examined the role of host filtering in shaping local FF communities, and we know little about the differences of FF community assembly between symptomatic (caused by fungal pathogens) and asymptomatic leaves, and whether there is phylogenetic congruence between host plants and FF. We examined FF communities from 25 host plant species (for each species, symptomatic and asymptomatic leaves, respectively) in an alpine meadow of the Tibetan Plateau using MiSeq sequencing of ITS1 gene biomarkers. We evaluated the phylogenetic congruence of FF–plant interactions based on cophylogenetic analysis, and examined α- and β-phylogenetic diversity indices of the FF communities. We found strong support for phylogenetic congruence between host plants and FF for both asymptomatic and symptomatic leaves, and a host-caused filter appears to play a major role in shaping FF communities. Most importantly, we provided independent lines of evidence that host environmental filtering (caused by fungal infections) outweighs competitive exclusion in driving FF community assembly in symptomatic leaves. Our results help strengthen the foundation of FF community assembly by demonstrating the importance of host environmental filtering in driving FF community assembly.

Keywords Alpine meadow · Endophyte · Foliar fungal disease · Host filter · Phylogenetic congruence

Introduction

Foliar fungi (hereafter, 'FF', defined as all fungal species within leaves after surface sterilization, and not those living on leaf surfaces) of plants are a group of hyper-diverse taxa that can have a broad array of negative and positive effects on plant growth, fitness, and function (Waqas et al. 2012; Christian et al. 2019). Beyond such direct effects, FF can have a number of complex indirect effects, including reducing leaf damage caused by fungal pathogens (Busby et al. 2016; while the contrary can also occur, e.g., Busby et al. 2013), increasing host resistance to insect herbivores (Hartley et al. 2015), expanding habitats that plants can live in (Rodriguez et al. 2008, 2009), as well as profoundly affecting ecosystem functioning (Iqbal et al. 2012; Buckley et al. 2019). For example, FF reduced plant biomass by 22% in old field habitats (Seabloom et al. 2017). All known plant lineages harbor FF, and given the important functions of FF, it is critical to understand the mechanisms that influence FF richness and community composition.

The effects of abiotic environmental factors on FF presence and diversity have been studied extensively, and examples include climate (Zimmerman and Vitousek 2012), latitude (Arnold and Lutzoni 2007) and geographical distance between hosts (U'Ren et al. 2012). For instance, FF richness associated with *Betula ermanii* increased along elevation gradients in Changbai Mountain, China (Yang et al. 2016), and wildfires also increased FF diversity in Montane Forest Trees (Huang et al. 2016). Further, relationships between FF communities and attributes of host plants have also been frequently studied, with examples including host taxonomy (Vincent et al. 2016), host genotype (Bálint et al. 2015; Eusemann et al. 2016), and chemical traits (Yang et al.

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2016). FF communities could be shaped by both the host environment and biotic interactions among FF. However, the factors that influence FF community assembly processes at fine scales, especially how mechanisms such as host environmental filtering (e.g., colonization by compatible FF and exclusion of incompatible ones) and competition shape FF community diversity and composition across different plant species have not been adequately addressed.

It stands to reason that fungal community assembly and coexistence are strongly influenced by host tissues and defense compounds (Vályi et al. 2016). For example, host plant chemical traits such as the amount of phenols, tannin, lignin, and antifungal compounds (e.g., benzoxazinoids) influence the colonization success of FF within host tissues (Saunders and Kohn 2009; Saunders et al. 2010). Many studies have suggested that phylogenetically closely related plants share similar secondary metabolite compositions (e.g., Ellis et al. 2000; Ziemert et al. 2014; Piasecka et al. 2015), which means that plant communities with closely related species should predictably shape FF communities (Solis et al. 2016), leading to phylogenetic congruence between host plants and FF. Given that the host plant exerts such a strong selective filter on the FF community, it is possible that the resulting FF communities will be largely comprised of closely related species, resulting in phylogenetic clustering (Cavender-Bares et al. 2009). Since fungi alter a number of chemical traits in symptomatic leaves, we consider this host filtering as analogous to environmental filtering from the perspective of fungal communities (for brevity, we refer this filtering caused by fungal infections to 'host environmental filtering'), which is similar to the classic concept of environmental filtering that can alter community composition in complex ways by influence species fitness and competitive interactions (Cadotte and Tucker 2017).

In addition to the interactions between FF and the plant hosts, FF are likely to compete for limited space and nutrients with one another (Saunders et al. 2010), while facilitation between different FF species by ameliorating a toxic environment can also occur (e.g., Pan and May 2009; Saunders and Kohn 2009). FF biotic interactions that have been used in biological control of foliar fungal pathogens in agriculture provide a powerful example of competitive exclusion between FFs (for review see Cook 1993). For instance, the endophyte *Neotyphodium lolii* can reduce the total lengths of lesions on Perennial ryegrass (*Lolium perenne*) caused by the fungal pathogen *Alternaria alternata* (Tian et al. 2008). Further, early colonizing fungal species can prevent the subsequent colonization of FF by inducing a 'priming effect' in host defense systems or producing antifungal compounds per se (e.g., Djonović et al. 2007; Minerdi et al. 2009), leading to strong priority effects during FF community assembly (e.g., Fukami 2015). However, the FF being deterred are more likely to be closely related species since plant–fungus interactions are relatively conserved and closely related FF are most likely to compete for the same tissues (Gilbert and Parker 2016). For example, the strains of nonpathogenic *Fusarium oxysporum* were used in biological control of soil-borne Fusarium wilt (Mandeel and Baker 1991). Such limiting similarity may also occur among FF and in other plant parts beyond roots. For instance, *Aspergillus fumigatus* showed an inhibitory effect on powdery mildew disease (caused by *Erysiphe cichoracearum*) in *Cucurbita maxima* (Srivastava and Bisht 1986). Furthermore, competition for infection targets between closely related FF is thought to be the important drivers of phylogenetic overdispersion as a consequence of competitive exclusion (Cavender-Bares et al. 2009).

FF are typically composed of multiple ecological guilds, including symbiotic, neutral and pathogenic FFs. Plant infectious diseases, primarily fungal diseases, can strongly influence ecosystem function and the assembly of natural communities (Fisher et al. 2012), and may determine the composition of symbiotic and neutral FF in some way given the relatively high abundance of pathogens. When fungal pathogens intrude into the plant cells, the leaf tissues will receive continuous damage from pathogenic fungi and thus lead to a series of morphological and physiological changes (Berger et al. 2007), such as the reduced rate of photosynthesis, the rising content of phenols (Hahlbrock and Scheel 1989), or the plant–fungus interaction shifts through plant DAMP (damage-associated molecular pattern) and other responses (Zipfel 2014). The FF species richness and phylogenetic diversity might be thus lower in symptomatic leaves than those in asymptomatic ones, given the relatively harsh living environment for FF in symptomatic leaves. Similarly, FF community composition might differ between them, given the different selection regimes.

Here we propose two alternative mechanisms driving FF community assembly in symptomatic leaves. First, fungal infections change the micro-environment (e.g., chemical traits, including antifungal compounds) of leaves (Heil and Ton 2008). This results in the elimination of phylogenetically distantly related FF that are not well adapted to the changing conditions, and allows closely related FF to persist, leading to a more phylogenetically clustered FF community in symptomatic leaves compared to asymptomatic ones. Second, as strong competitors, fungal pathogens can either directly exclude phylogenetically closely related FF, or indirectly exacerbate competition for nutrients and infection sites between other closely related FF through competitive exclusion. This process thus allows distantly related FF to persist, leading to a more phylogenetically overdispersed community in symptomatic leaves compared to asymptomatic ones.

To address these knowledge gaps, we estimated foliar fungal communities from 25 host plant species in an alpine

meadow of the Tibetan Plateau. For each species, we sampled symptomatic and asymptomatic leaves. Given the high similarity in the phylogenetic positioning of fungal endophytes and fungal pathogens, in our study, foliar fungi include both endophytes and fungal pathogens. We focused on the differences of FF community assembly between symptomatic and asymptomatic leaves, especially, we investigated the following questions: (i) Is there phylogenetic concordance in plant–FF interactions? In other words, do phylogenetically closely related hosts harbor phylogenetically similar FF? (ii) Does the FF community composition differ between symptomatic and asymptomatic leaves, and are FF species richness and phylogenetic diversity lower in symptomatic leaves than those in asymptomatic ones? (iii) Do the FF communities of symptomatic and asymptomatic leaves show patterns of phylogenetic overdispersion or clustering? Specifically, does host environmental filtering drive changes in FF community after fungal infections and thereupon leading to phylogenetic clustering; or does competitive exclusion drive changes in FF community and thus resulting in phylogenetic overdispersion?

Materials and methods

Study site and field sample collection

We focused on the differences between FF communities in asymptomatic and symptomatic leaves from common herbaceous plants in an alpine meadow. The sampling site was located on the northeast part of the Qinghai–Tibetan plateau, in Maqu, Gansu Province, China (33°40′N, 101°52′E; 3500 m above sea level). The mean annual temperature is 1.2 °C with the lowest monthly average temperature of − 10.7 °C in January and the highest of 11.7 °C in July. Mean annual precipitation is 620 mm, occurring mainly between June and September (short growing season).

We selected 25 relatively abundant plant species in our study site (Liu et al. 2016a). For each host plant species, we randomly selected 10 mature individuals and randomly sampled one asymptomatic leaf and one symptomatic leaf (with approximately the same leaf area) from each individual on September 12th, 2016 (near the end of the growing season, after nearly five months growing). All the selected individuals were located within a rectangle of 200 × 150 m, while individuals from the same species were at least 10 m apart to minimize spatial autocorrelation in FF communities and exclude the effects of potentially gradients (e.g., solar aspect and soil moisture). For some species with small leaf area (e.g., *Medicago archiducis-nicolai*, *Euphorbia helioscopia*), we selected more than one leaf for samples. Symptomatic leaves are leaves with lesions, coloured moulds or powders caused by fungal pathogens, and all symptomatic leaves showed similar percentages of damage (disease severity = 30% ~ 50%). A full description of the protocols used to distinguish fungal diseases in our study site is provided in Liu et al. (2016a, 2017). Here, we regard all fungal taxa in leaves as the complete community (i.e., the high similarity in the phylogeny between pathogenic and endophytic fungi, and also the difficulties in separating pathogenic and true endophytic fungi in symptomatic leaves according to existing methods), and define 'foliar fungi' as all fungal species in leaves after surface sterilization, meaning that pathogenic fungi in symptomatic leaves were included. We collected 500 leaves in total (10 individuals × 2 leaves × 25 plant species), and all the sampled leaves were brought back to the laboratory in 24 h and stored with ice packs in transit.

DNA extraction and amplification

Before DNA extraction, all leaves were surface sterilized within 40 h after collection according to the protocols provided in Zimmerman and Vitousek (2012). In brief, the leaves were rinsed in deionized H_2O, ethanol, NaOCl, ethanol, and deionized H_2O in turn (Zimmerman and Vitousek 2012). The leaves were dried with sterile absorbent paper. Mortars and pestles were then used to disrupt leaves with liquid nitrogen. Each sample (10 leaves) was then divided into two subsamples: one for DNA extraction, and the other for permanent preservation. We extracted total genomic DNA from each sample using Qiagen Plant DNeasy kits (Qiagen, Hilden, Germany) according to the method modified by Zimmerman and Vitousek (2012). Then we purified the crude gDNA using PowerClean Pro Clean-Up DNA kits (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted DNA was evaluated by gel electrophoresis (1% agarose gel).

The internal transcribed spacer 1 (ITS1) region was amplified by polymerase chain reactions (PCR) using the forward primer ITS1-F (CTTGGTCATTTAGAGGAA GTAA) and the reverse primer ITS2 (GCTGCGTTCTTC ATCGATGC). We successfully amplified 46 samples, and PCR failed for 2 asymptomatic samples (*Lamiophlomis rotate* and *Saussurea stella*) and other 2 symptomatic samples (*Gentiana officinalis* and *Saussurea stella*). We removed host plant DNA sequences by gel extraction purification (1% agarose gel), and then, the fungal DNA sequences were purified by Agencourt AMPure XP (Beckman Coulter, USA) and quantified by NanoDrop 2000 (Thermo Fisher Scientific, USA).

Sequence processing and bioinformatics

Amplicon sequencing was performed on the paired-end 2 × 250 bp Illumina MiSeq Benchtop Sequencer (Illumina, San Diego, CA, USA) at Genesky Biotechnologies, Inc. (Shanghai, China), and all paired sequences (46 samples) were successfully assembled. The quality filtering of sequences was performed using the FASTX-Toolkit v. 0.0.13 (Gordon and Hannon 2010). Any sequences with a quality score < 20, length < 100 bp, or ambiguous bases were discarded. We further also discarded sequences with total error rates > 2 from Python (Python Software Foundation) scripts, leaving a total of 4,352,045 sequences. We identified and removed chimeric sequences and singletons using the UPARSE (Edgar 2013), and the fungal ITS1 sequences through quality control were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold. All sequences were deposited in the NCBI-SRA database (SRP107289).

Host plant phylogeny

A detailed description of the protocols used to build host plant phylogeny is provided by Liu et al. (2015). Briefly, we aligned *rbcL* and *matK* gene sequences by MUSCLE (Edgar 2004) and combined them into one supermatrix to estimate a phylogenetic tree using the maximum likelihood approach through PhyML 3.0 (Guindon et al. 2010). The best-fit nucleotide substitution model was GTR + I + G. We chose an early-diverging angiosperm lineage *Amborella trichopoda* to root phylogenies. We also compared our phylogeny to APG III megatree (R20120829) based on Phylomatic (Webb and Donoghue 2005) and found that they were the same in topological structure.

FF community phylogenetic measures

Faith's phylogenetic diversity (Faith's PD; i.e., the total branch length in a phylogeny linking all OTUs represented in a community), mean pairwise phylogenetic distance (MPD) and mean nearest taxon phylogenetic distance (MNTD), as well as their abundance (number of sequences)-weighted indices (i.e., MPD_{ab} and $MNTD_{ab}$) among FF in each sample, were calculated to evaluate phylogenetic community patterns (Webb et al. 2002; Table 1). MPD_{ab} and $MNTD_{ab}$ emphasize the phylogenetic relationship among dominant OTUs. These phylogenetic measures have different ecological significances. MPD represents the mean pairwise distance between all OTUs in each community, while MNTD reflects the mean distance separating each OTU in the community from its closest relative. The comprehensive use of multiple measures aids in understanding the nature of FF community assembly in symptomatic leaves, and confirm the robustness of the results. Moreover, we calculated the

Table 1 Definition of key terms used in text according to the order of occurrence

Terms	Description
FF	Foliar fungi
OTU	Operational taxonomic unit
SFFs	Symptomatic-specific foliar fungi. The OTUs found only in the symptomatic sample
AFFs	Asymptomatic-specific foliar fungi. The OTUs found only in the asymptomatic sample
BFFs	Non-specific foliar fungi. The OTUs found in both symptomatic and asymptomatic samples
MPD	Mean pairwise phylogenetic distance
MNTD	Mean nearest taxon phylogenetic distance
MPD_{ab}	Abundance (number of sequences)-weighted MPD
$MNTD_{ab}$	Abundance (number of sequences)-weighted MNTD
SES.MPD	The standardized effect sizes of MPD
SES.MNTD	The standardized effect sizes of MNTD
$SES.MPD_{ab}$	The standardized effect sizes of MPD_{ab}
$SES.MNTD_{ab}$	The standardized effect sizes of $MNTD_{ab}$
βMPD	Mean pairwise phylogenetic distance between each of two FF communities
βMNTD	Mean nearest taxon phylogenetic distance between each of two FF communities
$βMPD_{ab}$	Abundance (number of sequences)-weighted βMPD
$βMNTD_{ab}$	Abundance (number of sequences)-weighted βMNTD
SES.βMPD	The standardized effect sizes of βMPD
SES.βMNTD	The standardized effect sizes of βMNTD
$SES.βMPD_{ab}$	The standardized effect sizes of $βMPD_{ab}$
$SES.βMNTD_{ab}$	The standardized effect sizes of $βMNTD_{ab}$

standardized effect sizes (SES) of MPD, MNTD, MPD_{ab}, and $MNTD_{ab}$ relative to the random communities drawn from whole species pool with 999 runs. By comparing these standardized effect sizes of indices with the value of zero, we can detect phylogenetic overdispersion or clustering directly. These indices (MPD, MNTD, MPD_{ab}, $MNTD_{ab}$, SES.MPD, SES.MNTD, SES.MPD_{ab}, and SES.$MNTD_{ab}$) are measures of α-diversity and thus describing the diversity within FF communities of symptomatic and asymptomatic leaves.

To understand the difference in FF community compositions from asymptomatic and symptomatic leaves, we defined a 'host FF species pool' as the OTUs found in both asymptomatic and symptomatic samples for each host plant species. For each host FF species pool, we designated three groups of fungal OTUs. First, we defined the OTUs found only in the symptomatic sample as 'symptomatic-specific foliar fungi' (hereafter, 'SFFs'). The second group was the OTUs found only in the asymptomatic sample as 'asymptomatic-specific foliar fungi' (hereafter, 'AFFs'). Finally, we designated the OTUs found in both symptomatic and asymptomatic samples (i.e., non-specific foliar fungi) as 'BFFs'. The rationale for separating the FF communities into subcommunities is to provide a better insight into foliar fungi community assembly in symptomatic leaves. We used the methods in Li et al. (2015) to quantify the phylogenetic dissimilarities between SFFs/AFFs to BFFs, and calculated SES.βMPD (incidence-based standardized effect sizes of βMPD), SES.βMNTD (incidence-based standardized effect sizes of βMNTD), SES.βMPD_{ab} (abundance-weighted SES.βMPD), and SES.$\beta MNTD_{ab}$ (abundance-weighted SES.βMNTD) for each host FF species pool. These are measures of β-diversity and thus describing how dissimilar AFF/SFF subcommunities are from the BFF subcommunities. To test whether the observed dissimilarities are greater or smaller than expected by chance, we used a null model that maintains the richness of SFFs and keeps BFFs unchanged, while randomly drawing the identities of SFFs from the whole species pool without replacements (with excluding the OTUs already appeared in the asymptomatic samples of this host species). Additionally, we kept the number of AFFs in each host species unchanged, and randomly drew the identities of AFFs from the OTUs that appeared in the asymptomatic samples of this host species (Li et al. 2015).

Statistical analyses

We employed Permutational Multivariate Analysis of Variance (PERMANOVA) to test the associations between plant species identity, disease status (asymptomatic/symptomatic) and FF community composition (without phylogenetic distances) using the *adonis* function based on Bray–Curtis dissimilarity in *vegan* package. Therein, statistical significance was assessed from 999 permutations for FF community composition in different samples. Moreover, given the modality of the FF community composition distribution, we employed the correspondence analysis (CA) plot based on Bray–Curtis dissimilarity with *cca* function to test the difference in FF community among asymptomatic and symptomatic samples.

To assess the strength of phylogenetic congruence in the associations and visualize the linkage between host plants and FF, we performed two cophylogenetic analyses, PACo (Hutchinson et al. 2017) and *Parafit* (Legendre et al. 2002), to test the hypothesis that plant and fungal phylogenies are nonrandomly associated and congruent, compared to 999 randomizations. We assessed the associations between host plant and FF phylogeny for (i) asymptomatic and symptomatic samples, respectively; (ii) SFFs, AFFs, and BFFs, respectively.

Plant phylogenetic distances and α-diversity of fungal communities (SES.MPD, SES.MNTD, and Faith's phylogenetic diversity) were calculated using the R package *picante* (Kembel et al. 2010). We set plant species as random effect, calculated 95% confidence interval (95% CI) of all α- and β-diversity indices of FF communities (SES.MPD, SES.MPD_{ab}, SES.MNTD, SES.$MNTD_{ab}$, SES.βMPD, SES.βMPD_{ab}, SES.βMNTD, and SES.$\beta MNTD_{ab}$), and illustrated the results with forest plots. For each diversity index, when their 95% CI does not include the null value, the diversity index of FF communities was significantly negative (<0; i.e., phylogenetic clustering) or positive (>0; i.e., phylogenetic overdispersion). Further, paired *t* tests were used to compare the differences in α-diversity indices of FF communities between asymptomatic and symptomatic leaves. In addition, independent-samples *t* tests were used to compare the differences in diversity indices (all α- and β-diversity indices) of FF communities (diversity indices of symptomatic samples minus diversity indices of corresponding asymptomatic samples).

Kraft et al. (2015) argued that phylogenetic clustering can also be caused by competitive exclusion (Mayfield and Levine 2010). To exclude this possibility, we assessed the phylogenetic signals in the presence and absence of individual fungus following Cadotte and Tucker's (2017) method as an independent line of evidence to confirm the role of environmental filtering in shaping FF community. Specifically, we measured phylogenetic signals with the *D* statistic (Fritz and Purvis 2010) using the *phylo.d* function in the *caper* package to test the phylogenetic conservatism of the presence and absence of FF OTUs in each host FF species pool that can be found in their symptomatic leaves.

Results

Characteristics of the FF communities in host plants

We successfully sequenced the FF ITS1 genes from 23 asymptomatic samples (PCR failed for *Lamiophlomis rotate*

and *Saussurea stella*) and 23 symptomatic samples (PCR failed for *Gentiana officinalis* and *Saussurea stella*) of the 25 host plant species, resulting in a total of 22 host FF species pools (*Gentiana officinalis*, *Lamiophlomis rotate* and *Saussurea stella* were removed). We detected a total of 4,308,193 quality-filtered sequences (Table S1), after quality control, OTU clustering, and singletons removal. In these detected sequences, we defined 1683 OTUs, 281 genera, 178 families, 85 orders, 70 classes as FF in this study (Fig. S1). We used FF communities in 22 host species successfully sequenced both in asymptomatic and symptomatic samples to allow a comparison for the following analysis. In addition, Faith's PD (3.29 ± 0.33) of FF in asymptomatic samples were higher than symptomatic samples (2.55 ± 0.22) based on paired *t* test comparisons ($P < 0.001$) (Table 2), while there was no significant difference in species richness, SES.MPD, SES.MPD_{ab}, SES.MNTD or SES.$MNTD_{ab}$ between asymptomatic and symptomatic samples.

Host filters play an important role in shaping FF community at local scale

Using PERMANOVA, we found that not only plant species identity ($F = 1.541$, partial $R^2 = 0.613$, $P = 0.001$), but also disease status ($F = 1.415$, partial $R^2 = 0.024$, $P = 0.009$), were significantly associated with FF community composition (Table 3; Fig. S2). This result indicates that there were significant differences in FF community composition between asymptomatic and symptomatic leaves after controlling the effect of species identity. In addition, host–fungi associations for FF were highly nonrandom based on both *Parafit* and PACo coevolution analyses for both asymptomatic ($P < 0.001$ for both coevolution tests) and symptomatic samples ($P < 0.001$ for both coevolution tests; Fig. S3). This means phylogenetically closely related hosts share phylogenetically similar FF, while related FF inhabited closely related plants, providing strong support for phylogenetic congruence between host plants and FF regardless of whether plants were symptomatic or not. Further, there was also strong support for phylogenetic congruence between host plants and AFFs ($P < 0.001$ for both coevolution tests), SFFs ($P < 0.001$ for both coevolution tests), and BFFs ($P < 0.001$ for both coevolution tests; Fig. 1).

Table 3 Results of permutational multivariate analysis of variance (PERMANOVA) for the associations between plant species identity, disease status and foliar fungi community composition

Term	*F*	Partial R^2	*P*
Plant species identity	1.541	0.613	0.001
Disease status	1.415	0.024	0.009
Residuals	–	0.363	–
Total	–	1.000	–

Statistical significance was assessed from 999 permutations for foliar fungi community composition in different samples. Shown are the *F* statistic, partial R^2 and *P* value

All four standardized effect sizes (SES) of α-phylogenetic diversity indices (SES.MPD, SES.MNTD, SES.MPD_{ab}, and SES.$MNTD_{ab}$) of FF communities for both asymptomatic and symptomatic samples exhibited significant phylogenetic clustering than expected by chance (Fig. 2a). This means the host filter appears to play a major role in shaping FF communities, although different host species varied in these four α-phylogenetic diversity indices of FF communities (Table S2).

Host environmental filtering appears to outweigh competitive exclusion in driving FF community assembly in symptomatic leaves

In general, the SES.βMPD, SES.βMNTD, SES.βMPD_{ab}, and SES.$\beta MNTD_{ab}$ of SFFs to BFFs were significantly negative (Table S3, Fig. 2b), indicating that SFFs were more closely related than expected by chance to BFFs. For

Table 2 The comparisons of α-diversity indices of foliar fungi (FF) communities in paired asymptomatic (Asy) and symptomatic (Sym) samples

Diversity indices	Pairs	Mean ± 95%CI (Asy)	Mean ± 95%CI (Sym)	T	*P*
Species richness	22	234.09 ± 34.80	255.26 ± 30.69	− 1.289	0.211
Faith's PD	22	3.29 ± 0.33	2.55 ± 0.22	3.851	< 0.001
SES.MPD	22	− 2.47 ± 1.15	− 1.99 ± 0.93	− 0.731	0.473
SES.MPD_{ab}	22	− 1.26 ± 0.74	− 1.04 ± 0.54	− 0.571	0.574
SES.MNTD	22	− 0.47 ± 0.43	− 0.72 ± 0.41	0.686	0.500
SES.$MNTD_{ab}$	22	− 0.41 ± 0.33	− 0.55 ± 0.13	0.811	0.427

Shown are the number of pairs (host species), mean ± 95% confidence interval (95%CI) of asymptomatic and symptomatic samples respectively, t statistics and *P* values of paired *t* test. Faith's PD is the total branch length in a phylogeny linking all OTUs represented in a community

SES.MPD standardized effect sizes of the mean pairwise phylogenetic distance, *SES.*MPD_{ab} standardized effect sizes of the abundance (number of sequences)-weighted MPD, *SES.MNTD* standardized effect sizes of mean nearest taxon phylogenetic distance, *SES.*$MNTD_{ab}$ standardized effect sizes of the abundance (number of sequences)-weighted MNTD

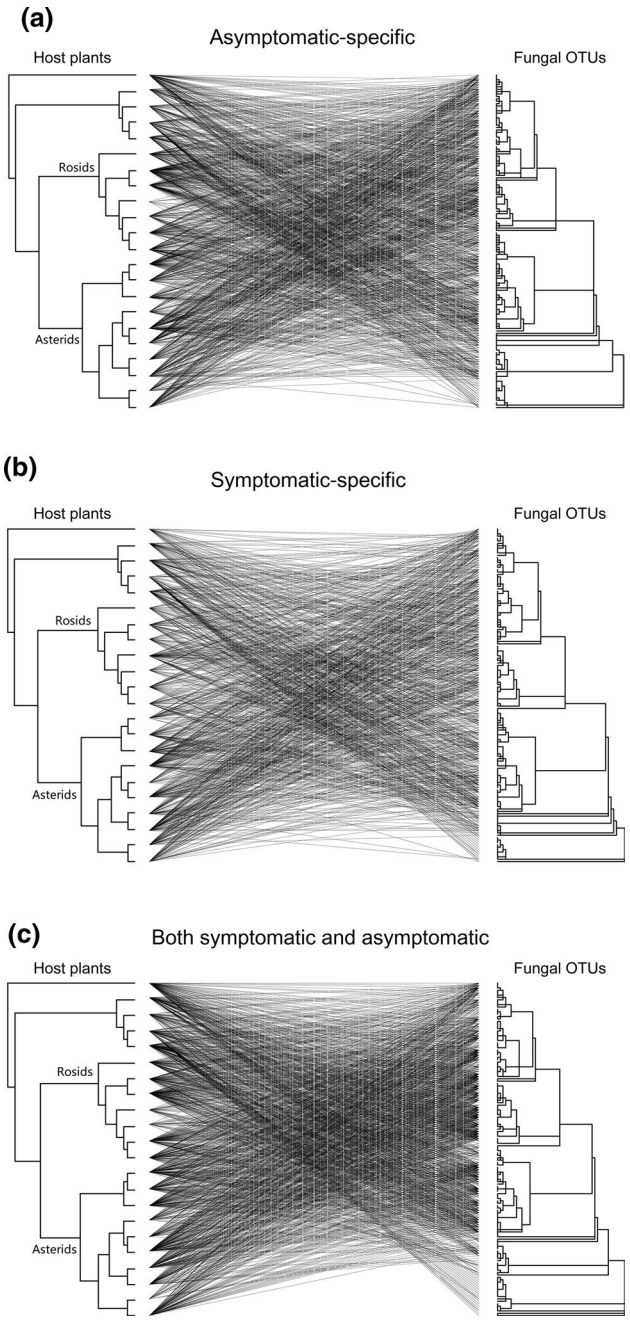

Fig. 1 Associations between plants and **a** asymptomatic-specific foliar fungal OTUs (AFFs; PACo test: $P < 0.001$; *Parafit test*: $P < 0.001$]; **b** symptomatic-specific foliar fungal OTUs (SFFs; PACo test: $P < 0.001$; *Parafit test*: $P < 0.001$] and c) the foliar fungal OTUs found in both symptomatic and asymptomatic samples (BFFs; PACo test: $P < 0.001$; *Parafit test*: $P < 0.001$]

the phylogenetic β-diversity of AFFs to BFFs, the values of SES.βMPD and SES.βMPD_{ab} of AFFs to BFFs were non-significant compared to zero, while the values of SES.βMNTD and SES.$\beta MNTD_{ab}$ of AFFs to BFFs were significantly positive (Table S3, Fig. 2b). This means that AFFs were not significantly different from BFFs on average, but the closest shared relative between AFFs and BFFs was phylogenetically less related than expected by chance. Our results showed that filtering by plant hosts, rather than competitive exclusion, shaped FF community in symptomatic leaves. Further, 21 of 22 (95.5%) FF communities showed significant but weak phylogenetic signals in the occurrence (presence/absence) of FF OTUs (Table 4), indicating that FF occurrence can be structured by host environmental filtering caused by fungal infections.

Discussion

Our study integrates within and among-host foliar fungal diversity patterns in asymptomatic and symptomatic leaves. We provide evidence that, filtering by plant hosts, rather than competitive exclusion, shapes FF community despite the influence of fungal infections. These results were in line with the results of phylogenetic congruence of host–FF associations, suggesting that the host filter appears to play the main role in shaping FF community.

Phylogenetic conservatism of host filters

We found that there was phylogenetic congruence between host plants and their FF, which might be caused by shared evolutionary history between them (Brem and Leuchtmann 2003; de Vienne et al. 2009; Hutchinson et al. 2017; Le Gac et al. 2007). Following Fahrenholz's (1913) rule in evolutionary biology, where 'parasite phylogeny mirrors that of its host', here we emphasize that interactions between hosts FF could result in simultaneous isolation/speciation, and lead to phylogenetic congruence (Brem and Leuchtmann 2003). Following speciation of FFs, host shifts between closely related hosts can also contribute to the phylogenetic congruence among host and fungal phylogenies (de Vienne et al. 2013). Besides, based on the Global Pest and Disease Database (USDA APHIS-PPQ), Gilbert et al. (2012) found a significant phylogenetic signal in host range of fungal pathogens. Latent fungal pathogens are important parts of FF, with a high degree of similarity in the spores and hyphae between latent pathogens and pathogenic fungus (Photita et al. 2004; Slippers and Wingfield 2007). Moreover, plant species could best explain the variation in FF community compositions, which indicates host affinity with FF and is in line with several previous studies (e.g., Liu et al. 2016b; Sarmiento et al. 2017), following Fahrenholz's rule. This is consistent with findings from other studies for not only fungal endophyte communities (Vincent et al. 2016) but also rhizosphere fungal communities (Becklin et al. 2012).

We attribute the phylogenetic conservatism of host filters under both symptomatic and asymptomatic leaves to the evolutionarily conserved traits of both host plants and

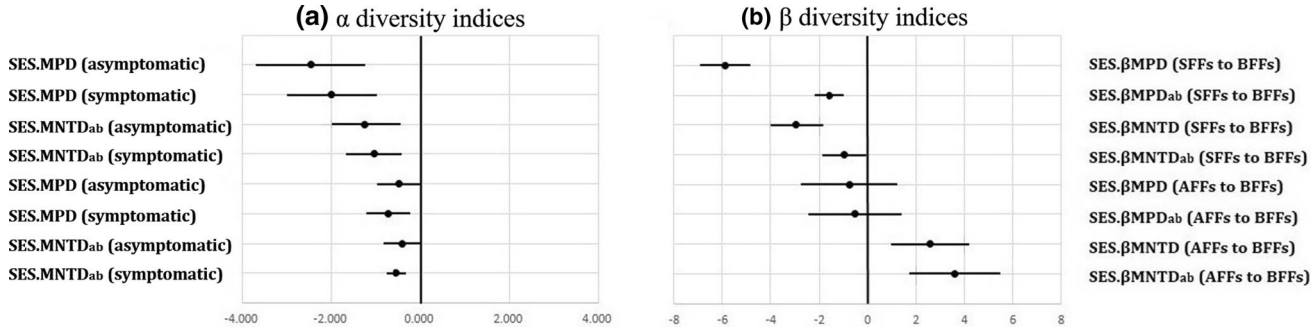

Fig. 2 95% confidence interval (95% CI) of α- and β-diversity indices of foliar fungi (FF) communities, with plant species as random effect. When their 95% CI does not include the null value, the FF community diversity index was significantly negative (< 0; i.e., phylogenetic clustering) or positive (> 0; i.e., phylogenetic overdispersion). **a** α-Diversity indices: SES.MPD, SES.MNTD, SES.MPD_{ab}, SES.$MNTD_{ab}$. **b** β-Diversity indices: SES.βMPD (SFFs to BFFs); SES.βMNTD (SFFs to BFFs); SES.βMPD (AFFs to BFFs); SES.βMNTD (AFFs to BFFs). These are measures of β-diversity and thus a positive value (> 0) means distantly related species among SFFs/AFFs and BFFs, while a negative value (< 0) means closely related species among SFFs/AFFs and BFFs

Table 4 Phylogenetic signal of whether each foliar fungi (FF) in each host FF species pool can be found in their symptomatic leaves (binary data: 0 for absence and 1 for presence)

Species	*N*	*D*	*P* (*D* < 1)	*P* (*D* > 0)
Anemone rivularis	283	0.842	< 0.001	< 0.001
Potentilla anserina	467	0.902	< 0.001	< 0.001
Saussurea pulchra	272	0.876	< 0.001	< 0.001
Anemone trullifolia	465	0.819	< 0.001	< 0.001
Medicago archiducis-nicolai	160	0.924	0.005	< 0.001
Saussurea nigrescens	308	0.844	< 0.001	< 0.001
Chamaesium paradoxum	242	0.830	< 0.001	< 0.001
Ligularia virgaurea	350	0.801	< 0.001	< 0.001
Herminium monorchis	433	0.891	< 0.001	< 0.001
Pedicularis kansuensis	355	0.877	< 0.001	< 0.001
Thermopsis lanceolala	247	0.788	< 0.001	< 0.001
Aster diplostephioides	294	0.837	< 0.001	< 0.001
Potentilla potaninii	503	0.819	< 0.001	< 0.001
Anemone obtusiloba	433	0.882	< 0.001	< 0.001
Saussurea leontodontoides	401	0.912	0.001	< 0.001
Tibetia himalaica	378	0.972	0.113	< 0.001
Thalictrum alpinum	321	0.857	< 0.001	< 0.001
Gentiana farreri	257	0.993	0.359	< 0.001
Oxytropis kansuensis	350	0.903	< 0.001	< 0.001
Euphorbia helioscopia	318	0.884	< 0.001	< 0.001
Veronica eriogyne	411	0.906	< 0.001	< 0.001
Daucus carota	308	0.831	< 0.001	< 0.001

Shown are the total number of OTUs found in a certain plant species (*N*), *D* statistic (*D*, a measure of phylogenetic signal in a binary trait) and *P* values for *D* < 1 and *D* > 0. Here *D* = 1 for a randomly distributed binary trait (i.e., no phylogenetic signal), while *D* = 0 for a phylogenetically conserved as expected under a Brownian threshold model

FF, as well as their interactions. Many studies showed strong phylogenetic conservatism of traits related to fungus colonization, such as the content of laccases (Dwivedi et al. 2011), monooxygenase (Jawallapersand et al. 2014), lyases, β-glucans from cell walls (Gururani et al. 2012), alkaloid and host-specific toxins (Kosentka et al. 2013); and morphological and reproductive traits, such as spore wall thickness, germ pore, shape (Bassler et al. 2015; Halbwachs et al. 2015), and septal pore apparatus (Celio et al. 2006). Moreover, the non-host resistance of plants, which can be induced through some ligand/receptor interactions, including plant DAMP, also shows strong phylogenetic conservatism among a wide range of fungi (for review see Gilbert and Parker 2016). So we expect that only some FF with specific traits can be able to tolerate the biotic environmental stress given by a certain host plant (host filter), leading to phylogenetic clustering of FF communities in our study.

Host environmental filtering drives FF community assembly in symptomatic leaves

We attribute the shifts of FF community composition in symptomatic leaves to physiological changes in leaf tissues and the changes in plant–fungus interactions. On the one hand, fungal infections could directly reduce the rate of photosynthesis, and change the water absorption capacity, resulting in reduced rates of plant respiration (Hahlbrock and Scheel 1989). For example, after being infected by *Erysiphe pisi*, the cellular structure of palisade tissue in *Medicago sativa* changed, and resulted in the enhancement of fungal resistance (Zhang et al. 2015). On the other hand, fungal infections can also indirectly change plant–fungus interactions through plant DAMP and other noninfectious inflammatory responses (Zipfel 2014), and subsequently affect FF richness. Overall, it appears that the altered

micro-environment of symptomatic leaves will eliminate some specific FF that cannot tolerate these biotic changes, leading to shifts in FF community composition in symptomatic leaves.

Host environmental filtering shapes FF communities in symptomatic leaves by altering a number of facets of the internal environment. We observed that the β-phylogenetic diversity indices (i.e., SES. βMNTD and SES.$\beta MNTD_{ab}$) between AFF and BFF were significantly positive. This means that FF only occurring in asymptomatic leaves (i.e., AFF, the OTUs found only in the asymptomatic samples) were more phylogenetically distantly related to FF shared between symptomatic and asymptomatic leaves than expected by chance. This is what we expected to observe because of phylogenetic conservatism of FF traits (e.g., spore wall thickness, germ pore, shape). The significantly positive values of SES.βMNTD and SES.$\beta MNTD_{ab}$ between AFF and BFF suggest that the altered biotic environment directly excluded some distantly related FF (i.e., AFF) that were not able to tolerate specific internal environmental conditions. In contrast, only certain groups of close relatives with particular traits (e.g., the content of alkaloid) can tolerate the altered biotic environment caused by fungal infections (e.g., the reduced rate of photosynthesis, the rising content of phenols or plant antifungal compounds) and appeared in symptomatic leaves (symptomatic-specific foliar fungi), resulting in the significant negative SES.βMPD, SES.βMNTD, SES.βMPD_{ab}, and SES.$\beta MNTD_{ab}$ values of SFFs to BFFs. Collectively, these β-phylogenetic diversity indices of both symptomatic-specific foliar fungi to non-specific foliar fungi and asymptomatic-specific foliar fungi to non-specific foliar fungi consistently confirm that host environmental filtering drives changes in FF community after fungal infections and thereupon leads to phylogenetic clustering (Fig. 2b).

However, competitive exclusion between fungi seems to play a minor role in driving FF community assembly in symptomatic leaves compared to host environmental filtering. The probable reason is that FF still have sufficient space and resources to use, even in symptomatic leaves (Liu et al. 2016a). In addition, infectious diseases could weaken the competitive exclusion between FF through increasing leaf heterogeneity, given the areas of the symptomatic leaf that are damaged or undergoing necrosis.

There are often alternative hypotheses that should be excluded before definitive conclusions about assembly mechanisms can be made when inferring community assembly mechanism from phylogenetic patterns (Cavender-Bares et al. 2009; Mayfield and Levine 2010). As pointed out by Kraft et al. (2015), phylogenetic clustering might be insufficient to distinguish host environmental filtering from the outcome of FF interactions. In this study, following Cadotte and Tucker's (2017) method, we confirmed the robustness of the main conclusion by several independent evidence, including (i) plant–fungi phylogenetic congruence; (ii) α- and β-phylogenetic diversity indices of the FF communities; and (iii) phylogenetic signal of FF presence/absence. Consistently, these lines of independent evidence confirmed that filtering plays a significant role in shaping FF communities (Cadotte and Tucker 2017).

In addition, factors other than host environmental filtering and competitive exclusion (between fungi) can also contribute to the FF community composition. The effect of dispersal limitation (Donald et al. 2020), priority effects, and neutral processes can directly influence the FF community assembly and composition (Cordier et al. 2012). Moreover, bacteria are diverse and abundant in both asymptomatic and symptomatic leaves and are known to interact with FF communities (Griffin and Carson 2015; Jakuschkin et al. 2016), and bacteria can institute environmentally induced priority effects in microbial systems (Tucker and Fukami 2014). In addition, we sampled leaves with approximately the same leaf area, however, the leaf size might not be sufficient to determine leaf age. The leaf age is thought to affect the *Arabidopsis thaliana* phyllosphere bacterial community composition through stochastic events (i.e., priority effect) in early colonization, and coupled with dispersal limitation (Maignien et al. 2014). However, the phylogenetic analysis conducted in this study is not able to capture the strength of the above processes.

Our results help to strengthen the functional foundation of FF community assembly by demonstrating the importance of host environmental filtering in driving FF community assembly. The results indicate that the phylogenetic congruence between host plants and FFs can affect community assembly. Therefore, our study improves the knowledge at the interface of community assembly and disease ecology to explain the maintenance of the extremely high species richness in the alpine meadow.

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Author contribution statement XL, SZ and MWC conceived and designed the study. XL, CZ and FC performed the experiments. PJ, XL, MWC, SZ, XS, YW and JW analysed the data. XL, MWC, PJ and SZ wrote the manuscript and all authors approved the final manuscript.

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Data availability All sequences were deposited in the NCBI-SRA database (SRP107289).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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