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Research

Host preference and network properties in biotrophic plant–fungal associations

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Summary

- Analytical methods can offer insights into the structure of biological networks, but mechanisms that determine the structure of these networks remain unclear. We conducted a synthesis based on 111 previously published datasets to assess a range of ecological and evolutionary mechanisms that may influence the plant-associated fungal interaction networks.
- We calculated the relative host effect on fungal community composition and compared nestedness and modularity among different mycorrhizal types and endophytic fungal guilds. We also assessed how plant–fungal network structure was related to host phylogeny, environmental and sampling properties.
- Orchid mycorrhizal fungal communities responded most strongly to host identity, but the effect of host was similar among all other fungal guilds. Community nestedness, which did not differ among fungal guilds, declined significantly with increasing mean annual precipitation on a global scale. Orchid and ericoid mycorrhizal fungal communities were more modular than ectomycorrhizal and root endophytic communities, with arbuscular mycorrhizal fungi in an intermediate position.
- Network properties among a broad suite of plant-associated fungi were largely comparable and generally unrelated to phylogenetic distance among hosts. Instead, network metrics were predominantly affected by sampling and matrix properties, indicating the importance of study design in properly inferring ecological patterns.

Introduction

An understanding of biological complexity and the optimization of conservation planning in natural communities require knowledge on the interactions among species that form complex and often highly structured networks (Bascompte, 2010). Network analyses offer novel potential to shed light on the processes underpinning the ecological and coevolutionary dynamics of communities of symbiotic organisms (Proulx *et al.*, 2005). Nestedness is one of the main parameters to characterize the structure of ecological networks. The interactions between two groups of mutualist species have a nested structure, when specialist species, which have a few partners, interact with a subset of the numerous partners of more generalist species (Bascompte *et al.*, 2003). It has been shown that nested structure in plant–animal networks reduces interspecific competition and promotes community stability; hence, the examination of network properties may advance our understanding about the ecological drivers of biodiversity patterns (Bastolla *et al.*, 2009; Thébault & Fontaine, 2010). Another network metric, modularity, describes the degree of network compartmentalization, that is, the tendency of a network to be organized into distinct clusters, where species within a cluster tend to interact more frequently among themselves than with species from other clusters (Olesen *et al.*, 2007). Although nestedness is driven by varying degrees of association between specialist and generalist taxa, the extent to which species are organized into modules is attributable to partner selectivity and specialization asymmetry (Fortuna *et al.*, 2010).

Fungi are one of the most diverse kingdoms on Earth and govern both plant nutrition and disease outbreaks in most terrestrial

ecosystems (Dighton, 2016). Nonrandom associations with symbiotic partners are common phenomena in biotrophic fungi. This is an important mechanism that leads to niche partitioning (Dickie, 2007; Jacquemyn *et al.*, 2014), which may reflect ecological specialization, co-evolution, or both of these processes (Rochet *et al.*, 2011). Several studies involving multiple plant hosts have indicated that fungal specificity patterns display a host-associated phylogenetic signal (Jacquemyn *et al.*, 2011; Põlme *et al.*, 2013; Tedersoo *et al.*, 2013; Nguyen *et al.*, 2016; but see Veresoglou & Rillig, 2014). Alternatively, host–fungal compatibility may be influenced by environmental factors, a phenomenon referred to as ecological specificity (Molina *et al.*, 1992). In a broad sense, specificity simply refers to nonrandom host–symbiont associations between compatible partners, which is more commonly termed host preference when specificity is nonexclusive. In a strict sense, host specificity is defined as exclusive host–symbiont associations, which are probably governed by coevolutionary events (Paterson *et al.*, 2010).

Patterns of host preference have been commonly observed in multiple fungal guilds, including pathogens, saprotrophs and mutualists (Molina *et al.*, 1992; Zhou, 2001; van der Does & Rep, 2007). Differences in the magnitude of host preference and specificity among fungal guilds probably result from a complex set of factors, including the intimacy of association, phylogenetic and physiological differences among hosts, competitive interactions among fungi, mutualistic effects and preferential allocation of resources between symbionts (Molina & Horton, 2015; Heilmann-Clausen *et al.*, 2016). It has traditionally been thought that parasitic organisms exhibit greater host specificity than mutualists in order to avoid host defense mechanisms and to secure greater physiological compatibility (Borowicz & Juliano, 1991; Antonovics *et al.*, 2013), but there is little empirical evidence (Gómez *et al.*, 2010). Furthermore, it has been widely assumed that the extent of host preference varies greatly among mycorrhizal types that exhibit differences in soil nutrition and level of mutualism (van der Heijden *et al.*, 2015; Fig. 1).

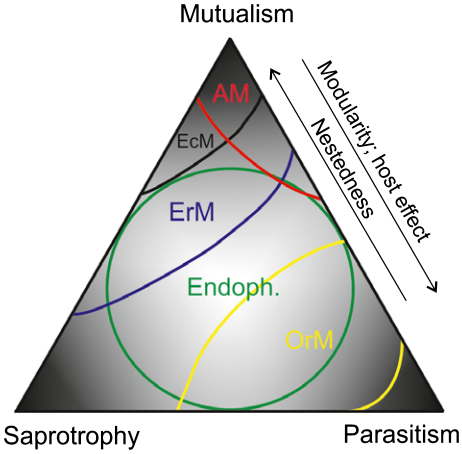

Fig. 1 Proposed relationship among host specificity, nestedness and modularity in associations between plants and different fungal guilds (AM, arbuscular mycorrhiza; EcM, ectomycorrhiza; ErM, ericoid mycorrhiza; Endoph., endophytes; OrM, orchid mycorrhiza).

In the orchid mycorrhizal (OrM) symbiosis, plants exploit their fungal partners during the period of germination and seedling establishment, sometimes maintaining a partial or fully mycoheterotrophic lifestyle at the adult stage (Gebauer *et al.*, 2016). Accordingly, a relatively high level of specificity has been reported among OrM fungi compared with other mycorrhizal fungal guilds (Dearnaley *et al.*, 2012). Ectomycorrhizal (EcM) symbiosis involves a high variety of obligately mutualistic fungal taxa with independent evolutionary origins (Tedersoo & Smith, 2013) and a number of specific cases of unipartite or reciprocal specialization (Bruns *et al.*, 2002). For example, the tree genus *Alnus* hosts distinct EcM fungal assemblages at the global scale (Põlme *et al.*, 2013), whereas the EcM fungus *Cenococcum geophilum* serves as an extreme example of the absence of host preference (Dickie, 2007). Despite their ubiquity and ancient origin, only *c.* 300 species of obligately symbiotic arbuscular mycorrhizal (AM) fungi have been described to date within the subphylum Glomeromycotina (Öpik & Davison, 2016). Although many of these taxa are widely distributed globally (Davison *et al.*, 2015), a certain level of host preference is common on a local scale (Vandenkoornhuyse *et al.*, 2002; Veresoglou & Rillig, 2014; Davison *et al.*, 2016). Local scale host preference of AM fungi has been interpreted through host ecological groups (habitat specialists and generalists: Öpik *et al.*, 2009; Davison *et al.*, 2011; Koorem *et al.*, 2017), whereas, at the global scale, AM fungi appear to respond to higher level plant phylogeny (Davison *et al.*, 2015). In addition, some evidence emphasizes the importance of stochastic processes in structuring the chance of encountering partners in AM plant–fungal associations (Davison *et al.*, 2016; Encinas-Viso *et al.*, 2016). Ericoid mycorrhizal (ErM) symbiosis involves the crown group of the Ericaceae plant family and a diverse range of ascomycete and some basidiomycete fungal lineages that are common soil saprotrophs and root endophytes (Kohout, 2017). In spite of occurring in only 1% of angiosperm species, ErM symbiosis has a nearly global distribution with higher abundance in habitats in which harsh conditions limit decomposition and plant nutrient uptake. So far, the ecological and evolutionary factors driving ErM fungal community composition are poorly understood (Leopold, 2016). Recently, Toju *et al.* (2016) documented a highly modular and anti-nested architecture of an ErM plant–fungal network indicating high host preference in the ErM host plant–mycobiont interaction. A similar result was also observed by Bougoure *et al.* (2007), who reported differences in ErM fungal community composition between *Calluna vulgaris* and *Vaccinium myrtillus* hosts. However, other studies have recovered no host effect on ErM fungal communities (Kjøller *et al.*, 2010; Walker *et al.*, 2011). In addition to mycorrhizal fungi, vegetative tissues of all living plants are colonized by fungal endophytes, which do not form specialized structures for nutrient exchange, but may alter plant receptiveness and response to diseases and stress (Rodriguez *et al.*, 2009). Previous studies have suggested varying degrees of host preference and organ/tissue specificity among endophytes (Arnold, 2007).

This study aims to assess the generality of organizational patterns in biotrophic plant–fungal symbioses. We hypothesized that fungi of different ecological guilds would differ in the extent

of their host preference, as well as in network properties. Specifically, based on local plant–fungal species richness ratios and relative positions in the mutualism–parasitism continuum, we hypothesized that host preference and modularity would be greatest in the OrM symbiosis, followed by EcM, ErM and endophytes, and lowest in the AM symbiosis (van der Heijden *et al.*, 2015; Fig. 1). To test these hypotheses and to control for the effect of host phylogeny, climate, sampling and community matrix properties, these variables were simultaneously integrated into a data synthesis. This approach allowed direct comparisons to be made of host preference and interaction network structure across a diverse suite of fungal guilds and plants, whilst accounting for sampling and community matrix properties.

Materials and Methods

Data sources

This global-scale data synthesis builds on individual case studies that were compiled from the Web of Science (as of 20 November 2016) by combining the search terms 'host specificity', 'host preference' and 'host effect' with 'mycorrhiza' and 'endophytes'. The analysis includes studies in which at least two host plant species were sampled in multiple replicates per study area and fungi were identified using either molecular or morphological methods (Supporting Information Table S1). If available, fungal species by host species community matrices were extracted from the supplementary materials of these studies. For 26% of cases, this information was not provided and therefore we contacted the first and/or senior authors for taxon distribution tables or raw data. In total, we were able to compile 67% datasets out of 73 studies that were regarded as suitable. Of 38 individual studies (including two unpublished datasets), 39% comprised more than one distinct study site, which were treated as independent sampling units in our analyses.

In most datasets, taxa were delimited using molecular methods and termed as operational taxonomic units (OTUs). OTUs were typically separated at 97% sequence similarity based on the internal transcribed spacer (ITS), large subunit (LSU) or small subunit (SSU) of the ribosomal RNA gene (the latter for studies focusing mainly on AM fungi). Datasets using both Sanger sequencing (68%) and high-throughput sequencing (HTS; 29%) were included. In a single study focused on root endophytes (including six sampling units), fungal symbionts were identified based on culture morphology.

The datasets of plant–fungal associations were categorized into the following guilds: AM, OrM, EcM, ErM, root endophytes and leaf endophytes. In the fungal datasets, we typically relied on trophic group annotations of the original authors. If these assignments were not performed or were unavailable, we assigned the identified data to broad functional groups (cf. Tedersoo *et al.*, 2014) and excluded taxa with unknown trophic status.

For each site, metadata on various geographic (latitude, longitude), floristic (number of host species sampled) and sampling (number of samples, molecular method and gene region, hereafter referred to as marker) variables were retrieved from the original publications. Approximate mean annual temperature (MAT) and precipitation (MAP) were retrieved from a high-resolution database of the Earth's surface climate (Hijmans *et al.*, 2005) using the software ArcMap 10.3 (ESRI, Redlands, CA, USA). This climate database represents a global model of the mean monthly surface climate features over all terrestrial areas with a raster size of 30″ latitude and longitude (*c.* 0.85 km^2 on the equator).

For each dataset, we calculated the average phylogenetic distance (APD) among hosts using the online phylogenetic query tool Phylomatic (http://phylodiversity.net/phylomatic/). We used 'supertree' as a source database, because it comprises unparalleled dated molecular phylogeny of 32 223 angiosperm species (Zanne *et al.*, 2014). The number of samples and fungal species and APD values were log-transformed before analyses to meet the assumptions of homoscedasticity.

Statistical analyses

To estimate the relative effect of the host plant on the community structure of symbiotic fungi in each sampling unit (i.e. dataset), we performed permutational ANOVA (PERMANOVA) as implemented in the adonis function of the vegan package in R (Oksanen *et al.*, 2012). Based on R^2, we calculated the adjusted determination coefficient (R^2_{adj}) of the host effect, which we interpreted as the proportion of explained variation in further analyses. We transformed all datasets into binary format, because many datasets were available only in this form. Singletons (i.e. taxa occurring only once per sampling unit) were removed before PERMANOVA to reduce the adverse effect of rare species on R^2_{adj}. For each dataset, the Sørensen dissimilarity metric was used to calculate the distance matrix of fungal communities. To assess whether differences in fungal communities were statistically significant, 999 permutations were used. As an input for subsequent analyses (see below), the R^2_{adj} values of each dataset were used as proxies for host effect *per se* and negative R^2_{adj} values were transformed to zeros.

To determine the effects of geographic (latitude, longitude), environmental (MAT and MAP) and sampling (matrix connectivity, number of samples, number of hosts and fungal species, APD, type of identification: morphology, Sanger sequencing, HTS) properties on relative host effect (R^2_{adj}), nestedness and modularity, we calculated linear regression models for each combination of response and predictor variables as implemented in the stats package of R. Variables were considered to be influential if they retained their significance after the effects of other significant predictors derived from linear regression models were partialled out in multiple regression models (Fig. S1).

To assess network properties, we calculated nestedness and modularity metrics based on plant–fungi co-occurrence matrices. In contrast with PERMANOVA, singletons were included in the network analysis. The modularity index of each dataset was calculated using a simulated annealing algorithm as implemented in Netcarto software (Guimerà & Amaral, 2005). We calculated the nestedness metric based on overlap and decreasing fill using the NODF function in the bipartite package of R. NODF

accounts for matrix dimensions as these may affect nestedness values (Almeida-Neto *et al.*, 2008) and are highly variable between our datasets. We did not include null models, because they are prone to type 1 or type 2 errors (Gotelli, 2000).

To test for differences among fungal guilds in host effect, nestedness and modularity, we applied a nonparametric multiple comparison procedure with an unbalanced one-way factorial design as implemented in the gao_cs function in the NPARCOMP package of R (Gao *et al.*, 2008). This computational procedure is the nonparametric equivalent of the sequential test procedure of Campbell & Skillings (1985). We modeled the proportion of variation explained by host (R^2_{adj}) from the above-described PERMANOVA models, with nestedness and modularity metrics as response variables. Climatic, phylogenetic, sampling-related and technical variables (i.e. matrix connectivity, number of host and fungal species), as well as identification method and barcoding region, were included in these models as predictors.

Because our results indicated high importance of technical variables and matrix properties, we sought to shed light onto the response of network properties to rare species and the number of plant taxa assessed. In particular, we tested the effect of rare fungal and plant species on the nestedness of AM fungal communities by subsampling the individual datasets of Davison *et al.* (2015), in which typically four individuals of four to seven hosts were sampled per study area. To estimate the effects of rare host and fungal species on nestedness, we removed singleton and doubleton fungal OTUs, or less abundant hosts (less than four individuals), and recalculated the nestedness metric. We also specifically addressed the number of hosts on network properties across all datasets. The datasets were divided into two categories: those with more than or equal to five host species, and those with less than five host species. We performed the nonparametric multiple comparison (Gao *et al.*, 2008) for both categories separately in order to examine whether the degree of nestedness and modularity differed.

Results

Our data synthesis covered 111 independent sampling units from 44 published studies and five unpublished datasets. AM, EcM, OrM, ErM, and root and leaf endophytic associations were represented by 48, 25, 12, 8, 11 and 7 datasets, respectively. Sanger sequencing, HTS and morphotyping were applied in 41, 64 and 6 datasets, respectively (Table S1).

When we took into account the effects of significant predictors derived from linear regression models (i.e. latitude, number of fungal species and matrix connectivity; Fig. S1) in multiple regression modelling, the type of plant–fungal association remained the only variable that significantly affected the relative host effect ($R^2_{adj} = 0.301$, $F_{5,105} = 10.46$, $P < 0.001$; Table S1). According to the pairwise nonparametric multiple comparison, OrM associations responded most strongly to host, with a significant difference from other associations except leaf endophytes (Fig. 2a; Table S1). No differences in the relative host effect were observed among any other plant–fungal association types.

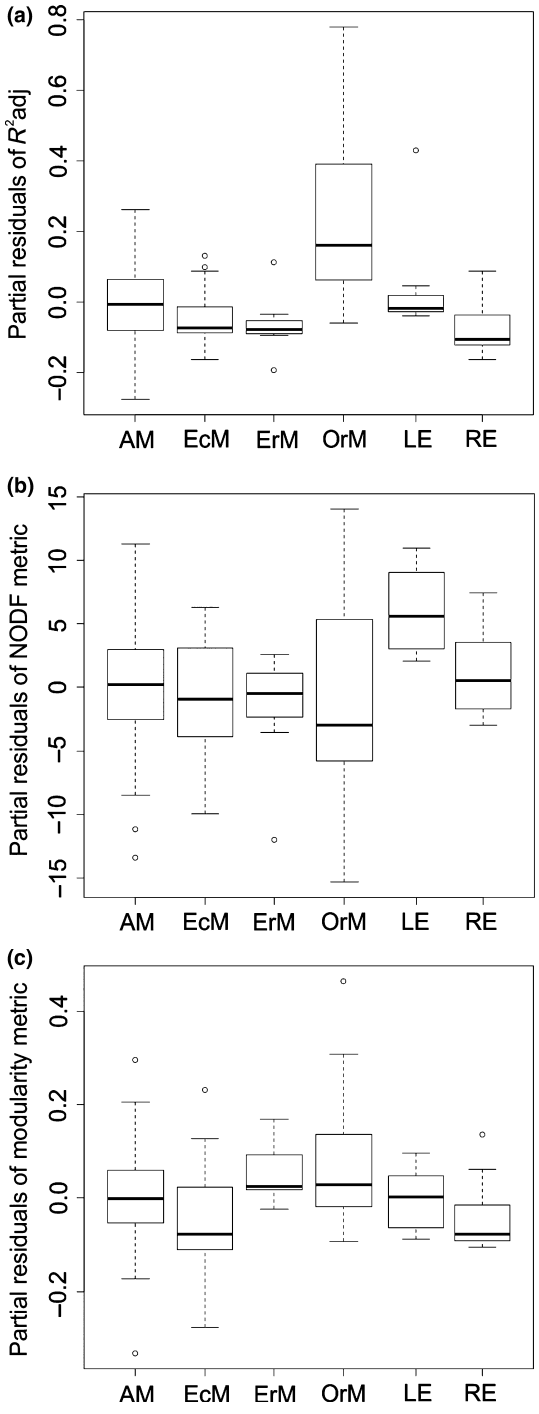

Fig. 2 Boxplots illustrating differences in group averages of fungal guilds in (a) R^2_{adj}, (b) nestedness and (c) modularity measure (as measured by Guimerà & Amaral, 2005). In each case, the effects of other significant predictors in the model have been accounted for. Bold horizontal lines represent mean values; box margins ± SE and vertical lines represent minimum and maximum values of the groups.

The nestedness metric was most strongly related to matrix connectivity (positive effect: $F_{1,105} = 21.8$, $R^2_{adj} = 0.164$, $P < 0.001$; Fig. 3a) and MAP (negative effect: $F_{1,105} = 11.1$, $R^2_{adj} = 0.087$, $P = 0.001$; Fig. 3b). However, MAP had no effect on nestedness when we tested different types of fungal associations separately,

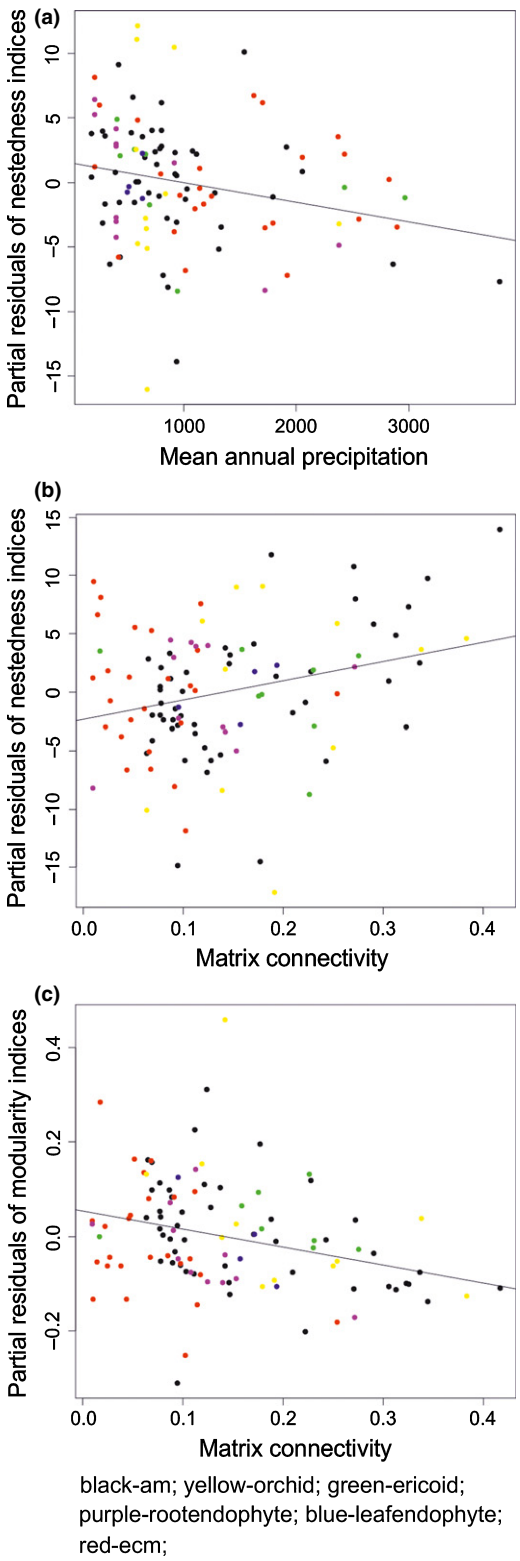

Fig. 3 The effect of (a) mean annual precipitation (MAP) and (b) matrix connectivity on residuals of nestedness indices; and (c) the effect of matrix connectivity on residuals of modularity indices. Black circles, arbuscular mycorrhizal fungal communities; red circles, ectomycorrhizal fungal communities; yellow circles, orchid mycorrhizal fungal communities; green circles, ericoid mycorrhizal fungal communities; blue circles, leaf endophyte fungal communities; purple circles, root endophyte fungal communities.

probably because of decreased analytical power in pairwise multiple comparisons. Leaf endophytes tended to exhibit the highest nestedness values among all fungal guilds, but significant differences were lost after the multiple testing false discovery rate stepwise adjustment procedure of Campbell & Skillings (1985) (Fig. 2b; Table S1).

Removal of singletons from individual datasets of AM fungal communities (Davison *et al.*, 2015) had no effect on nestedness, whereas removal of doubletons increased the nestedness index by 27% on average (paired *t*-test: $t = -8.67$, df = 37, $P < 0.001$; Fig. S2). Excluding less abundant hosts (less than four individuals) from datasets reduced nestedness by 12.8% (paired *t*-test: $t = 7.65$, df = 24, $P < 0.001$; Fig. S2). A similar trend occurred when we excluded rare host species from studies addressing other guilds of fungi, where host individuals were sampled randomly (mainly the studies of Toju *et al.*, 2013, Toju *et al.*, 2016; results not shown).

The pairwise nonparametric multiple comparison among the datasets containing more than or equal to five host species did not show significant differences in nestedness and modularity values, probably because of insufficient analytical power (Fig. S3). Similarly, a comparison among the datasets with less than five host species did not detect significant differences in nestedness and modularity between fungal guilds.

There were significant differences in modularity among the different types of plant–fungal association ($F_{5,105} = 3.109$, $R^2_{adj} = 0.087$, $P = 0.011$; Fig. 2c). Multiple comparisons among fungal guilds revealed that OrM fungal communities were more modular than EcM fungal communities; ErM fungal communities were significantly more modular than EcM and root endophytic fungal communities, but there were no differences among other types of associations. In addition, the three LSU-based datasets displayed significantly lower modularity than datasets using SSU-based identification (Fig. S4). Furthermore, modularity was negatively related to matrix connectivity ($F_{1,109} = 13.1$, $R^2_{adj} = 0.099$, $P < 0.001$; Fig. 3c).

Discussion

Host effect on fungal community composition

Our data synthesis revealed that the magnitude of host effect differed surprisingly little among the types of plant–fungal associations, when the confounding environmental and sampling variables were taken into account. Nonetheless, OrM fungal communities clearly stood out from the other mycorrhizal and endophytic groups in the relative magnitude of host effect. Orchids fully rely on symbiotic fungi for carbon and nutrients at least during the very early developmental stages (Bidartondo & Read, 2008; Stöckel *et al.*, 2014), which necessitates a strong stimulus and thus higher specificity (Dearnaley *et al.*, 2012). The notable overlap of fungal symbiont communities of seedlings and adult orchids is also consistent with higher specificity (Rasmussen *et al.*, 2015; Waud *et al.*, 2017). Most orchid species develop an autotrophic habit in the adult stages, but many species remain partly or fully mycoheterotrophic (Gebauer *et al.*, 2016). In spite

of the fact that 11 of 12 datasets addressed adult photosynthetic orchids in our analysis, a stronger host effect compared with other biotrophic fungi was clearly evident. These differences persisted even after removal of the mycoheterotrophic dataset of Lee *et al.* (2015) (results not shown). Collectively, this high level of partner specificity suggests that mycorrhizal fungi may be important factors driving niche partitioning among coexisting orchid species (Jacquemyn *et al.*, 2014).

In spite of multiple examples of high unilateral or reciprocal specialization in EcM fungal symbiosis, such as *Alnus* and *Alnus*-associated fungi, Suillineae, *Leccinum* and *Gnetum* (Bruns *et al.*, 2002; Kennedy *et al.*, 2015), the average host effect on fungal community composition was comparable with that of root and leaf endophytes, as well as AM and ErM fungi, for which such examples of specificity are not known. We found that narrowly confined host associations were uncommon among EcM fungi, lowering the overall host effect among this group.

In contrast with EcM fungi, AM fungi are typically considered as host generalists, although patterns of partner preference have been clearly documented (e.g. Vandenkoornhuyse *et al.*, 2002; Davison *et al.*, 2011, 2016). Molecular taxonomic resolution in AM fungi was commonly coarser than in EcM groups, because several research groups used either LSU or SSU as molecular markers which may not distinguish all closely related species. However, our direct comparison of SSU-based and ITS-based studies indicated no significant differences among the recovered host effect. Similarly, Toju *et al.* (2014) reported that different cut-off thresholds of fungal OTUs did not result in qualitative changes in network properties. Interestingly, although it has been documented that AM fungi respond to phylum-level categorization of plants on a global scale (Davison *et al.*, 2015), we found that there was no overall evidence for a phylogenetic signal in plant–AM fungal associations.

Leaf and root endophytes represent a variety of plant-associated fungi with different evolutionary and trophic origin, and hence different functions (Rodriguez *et al.*, 2009). David *et al.* (2016) suggested that, because soil constitutes a stronger environmental filter, a host effect should be more evident in leaf endophytes than in root endophytes. Our results showed a similar tendency for specificity in above- and belowground endophytic communities, but there was no statistical support, possibly because of the small sample sizes. Systematic aboveground grass endophytes *Epichloë* and *Neotyphodium* are typically highly specific to certain Poaceae species, but this group is generally of low taxonomic richness (Saikkonen *et al.*, 2004).

Nestedness

Networks of intimate interactions, such as plant–fungal associations, are often considered to be more compartmentalized and less nested than networks of interactions among free-living species, such as plant–animal networks (Guimarães *et al.*, 2007; Toju *et al.*, 2015). However, fundamental differences in matrix properties and underlying sampling design may render the direct comparison of absolute values of plant–animal and plant–fungal nestedness metrics unreliable. For example, studies of biotrophic fungi usually examine equal numbers of individuals of each host species (but see Toju *et al.*, 2015) and record multiple taxa per individual/sample. Conversely, sampling schemes of aboveground studies usually record incidence, which renders the abundance distribution of species of both partners log normal. Furthermore, HTS methods commonly applied in belowground studies are much more likely to encounter rare associations relative to aboveground observations. Most of the rare associations in HTS studies are regarded as technical artifacts. Moreover, the current state of knowledge does not enable us to distinguish between random links and ecologically meaningful plant–fungus interactions. To account for these sources of variation, we tested the effect of rare fungal and plant species on the nestedness of AM fungal communities by subsampling individual datasets of the study by Davison *et al.* (2015). Our results showed that the exclusion of rare associations (doubletons but not singletons) enhanced nestedness, whereas the removal of less abundant host species reduced nestedness. These results clearly indicate that, although general comparisons across different types of network are important for drawing larger ecological inferences, it must be emphasized that different sampling strategies can strongly influence the measured levels of nestedness.

We did not detect clear differences in nestedness values among fungal guilds. The relatively low nestedness within the OrM associations corroborates field observations that orchids typically associate with fungi in an unpredictable manner and mycoheterotrophic orchids specialize on fungal taxa that are not necessarily associated with photosynthetic sister species (Dearnaley *et al.*, 2012; Jacquemyn *et al.*, 2014). Low levels of nestedness as well as anti-nestedness patterns indicate that partner-specific plant species do not favor generalist fungal species over specialists and vice versa, that is, that reciprocal specialization is highly unexpected. The OrM symbiosis exhibited the greatest variation in nestedness values, suggesting that the structure of this association may strongly depend on local conditions or the selection of plant species for sampling.

Nestedness was the only network property that responded to climatic variables, exhibiting a negative relationship with MAP. Nestedness occurs when species are lost in consistent order; therefore, changes in nestedness may indicate whether a community is characterized by broadly similar or differing responses to environmental variation. When species respond differently to the major gradients associated with species richness (such as climatic variables for soil fungi: Tedersoo *et al.*, 2014), species loss in consistent order might be altered and the community as a whole will become less nested (Elmendorf & Harrison, 2009). We suggest that the negative relationship with MAP may be the result of a stronger environmental niche differentiation with increasing moisture.

Modularity

OrM and ErM fungal communities displayed the highest modularity, which differed significantly from that of root endophytes and EcM fungi. The high level of modularity of OrM fungi is consistent with the strong host effect, which is probably related

to the high dependence of orchids on symbiotic seed germination and early protocorm development, further reflected in fungal associations in adults. However, the high modularity in ErM associations contrasts with the relatively low level of host effect among this group. Such contrasts between modularity and host effect have been reported previously, possibly deriving from the sensitivity of the modularity measure to the total links in the dataset (Bahram *et al.*, 2014). Partner choice for mycorrhizal fungi also depends on habitat, especially soil moisture (orchids: Illyés *et al.*, 2012), suggesting that locally unmeasured microsite effects on a site scale may confound part of the host effect and network properties. In contrast with a recent study focusing on wood-inhabiting fungi at the regional scale (Heilmann-Clausen *et al.*, 2016), we found no relationship between network modularity and host phylogenetic distance within most individual datasets and across all studies.

Methodological considerations

Our data synthesis took advantage of previously published datasets, most of which addressed fungal specificity towards plant hosts, but very few were originally intended for network-based analyses. In concordance with previous studies, we demonstrate that the measures of nestedness and modularity are sensitive to sampling and matrix properties (see also Nielsen & Bascompte, 2007; Fortuna *et al.*, 2010), especially connectivity. We found that the level of matrix fill (i.e. the proportion of nonzero values) was by far the strongest predictor of nestedness, exhibiting a positive effect. Our results indicate that an increase in matrix connectivity increases nestedness and should be accounted for in other comparative studies as well. Fortuna *et al.* (2010) demonstrated that only matrices with low connectivity may simultaneously exhibit a nested and modular pattern. Although HTS analyses provide much larger datasets, we found that the method of fungal species identification *per se* was not an important factor. In particular, without considering connectivity in the models, data based on HTS still performed similarly to those obtained with other identification methods.

We emphasize that network metrics alone does not necessarily provide specific information about community assembly rules, because several alternative mechanisms may lead to similar patterns and the ecological interpretation should therefore be treated with caution (Chagnon, 2016). A nested structure of natural communities is often explained by greater stability of networks, but specific mechanisms underlying mycorrhizal plant–fungal assemblages may cause deviation of these networks from general assumptions. For example, differential mycorrhizal dependence among coexisting plant species (Graham *et al.*, 1997) might have a profound effect on network structure. In addition, network metrics do not account for abundance values, which may underestimate the effect of more abundant OTUs and give more weight to rare taxa, many of which may be artifactual in HTS studies. Furthermore, in observation-based co-occurrence datasets, including those of plant–fungal associations, positive relationships do not necessarily imply biotic interactions (Caruso *et al.*, 2012; Encinas-Viso *et al.*, 2016), and negative relationships may be artifacts of insufficient sampling effort or reflect niche differentiation to other environmental variables. The ongoing challenge is to distinguish between biologically meaningful interactions and occasional or artifactual co-occurrences. Researchers can distinguish functional interactions from artifacts by using previous information about these associations and recovering the same associations across space and time.

The identification of interacting organisms by means of sequencing, especially HTS, poses additional challenges and potential pitfalls for specificity and network analyses. In addition to analytical artifacts, such as chimeric sequences, OTUs may be defined at a phylogenetically inappropriate scale, which does not correspond to biological species or host-specific *forma speciales* in the case of pathogenic fungi. Our OTU delimitation followed traditional threshold values set by experts which should resemble species level as close as possible in each fungal guild, although species definition and ecological differentiation within the Glomeromycotinan taxa are heavily debated (Kivlin *et al.*, 2017). We anticipate that molecular markers vary substantially in taxonomic resolution and that optimal thresholds for species delimitation may differ across fungal phyla. In particular, SSU and LSU are often estimated to have coarser taxonomic resolution than other fungal barcoding markers (Lindahl *et al.*, 2013; Bruns & Taylor, 2016; but see Thiéry *et al.*, 2016). However, our results do not support systematic deviation of network structure among studies based on SSU, LSU, ITS or culture morphology. The only exceptions were LSU-based datasets which, despite a low number of replicates, displayed significantly lower modularity than datasets using SSU-based identification. The effect of taxonomic resolution of OTUs on network properties could be mediated by connectivity, as coarser resolution would underestimate the number of rare links. In future studies, the effect of barcoding markers and thresholds should be tested for the same samples.

We found that differences in the number of sampled host species affected the modularity measure and, potentially, also nestedness. A lower number of hosts led to weaker modularity within the subsampled datasets and across all datasets. This suggests that ecosystems with a lower number of sampled plant species may suffer from underestimation of modularity because of lower probability to include species belonging to different modules. Another issue is the estimation of modularity in ecosystems of low host diversity, such as northern temperate and boreal EcM and ErM plant–fungus associations, where much lower estimates of local modularity seem likely.

Conclusions

Overall, we found weak differences among biotrophic fungi in terms of host effect and network properties. OrM interaction networks represented a notable exception with regard to host identity effect, consistent with our hypothesis of greater specialization in more parasitic associations. The general lack of phylogenetic signal in host effect and network properties suggests that plant–fungal association patterns are either poorly influenced by host phylogeny, or that such analyses require the sampling of a much broader host range. Across all datasets, nestedness was negatively

related to mean annual precipitation, which may be linked to greater niche differentiation among plants and fungi with increasing moisture and associated greater taxonomic richness. Finally, our study indicates that sampling- and matrix properties-related variables need to be carefully accounted for when comparing network properties within and between below- and aboveground communities.

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Author contributions

L.T. and M.B. proposed the conceptual framework for the study. S.P. performed most of the necessary analyses and wrote the first draft of the manuscript. M.B., J.O. and P. Kohout provided statistical advice and M.B. helped to develop the necessary R codes. M.M., M.Ö. and P. Kohout performed OTU assignments in arbuscular mycorrhizal datasets. H.J., L.P. and J.O. performed OTU assignments in orchid mycorrhizal datasets. P. Kennedy, L.T. and S.P. performed OTU assignments in ectomycorrhizal datasets. All authors were involved in the data gathering process, provided original datasets and contributed substantially to revisions of the manuscript in their field of expertise.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Correlations between variables.

Fig. S2 Nestedness dependence on the number of fungal and host species.

Fig. S3 Boxplot depicting modularity and nestedness values of datasets of five or more and four or less host species.

Fig. S4 Boxplots depicting pairwise comparison of sequencing methods and markers in terms of R^2adj (derived from permutational ANOVA), nestedness and modularity values.

Table S1 Detailed sampling data and results

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