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Interaction type influences ecological network structure more than local abiotic conditions: evidence from endophytic and endolichenic fungi at a continental scale

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Abstract Understanding the factors that shape community assembly remains one of the most enduring and important questions in modern ecology. Network theory can reveal rules of community assembly within and across study systems and suggest novel hypotheses regarding the formation and stability of communities. However, such studies generally face the challenge of disentangling the relative influence of factors such as interaction type and environmental conditions on shaping communities and associated networks. Endophytic and endolichenic symbioses, characterized by microbial species that occur within healthy plants and lichen thalli, represent some of the most ubiquitous interactions in nature. Fungi that engage in these symbioses are hyperdiverse, often horizontally transmitted, and functionally beneficial in many cases, and they represent the diversification of multiple phylogenetic groups. We evaluated six measures of ecological network structure for >4100 isolates of endophytic and endolichenic fungi collected systematically from five sites across North America. Our comparison of these co-occurring interactions in biomes ranging from tundra to subtropical forest showed that the type of interactions (i.e., endophytic vs. endolichenic) had a much more pronounced influence on network structure than did environmental conditions. In particular, endophytic networks were less nested, less connected, and more modular than endolichenic networks in all sites. The consistency of the network structure within each interaction type, independent of site, is encouraging for current efforts devoted to gathering metadata on ecological network structure at a global scale. We discuss several mechanisms potentially responsible for such patterns and draw attention to knowledge gaps in our understanding of networks for diverse interaction types.

Keywords Biogeography · Ecological networks · Endolichenic fungi · Endophytic fungi · Symbiosis

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Introduction

Over recent decades there has been a rising interest to describe ecological communities as networks of interacting species (e.g., Cohen 1978; Jordano 1987; Pimm et al. 1991; Bascompte et al. 2003). The high enthusiasm for this approach has arisen in part because network theory can reveal rules of community assembly within and across study systems, and in part because network patterns can generate novel hypotheses regarding the structure and resilience of particular bipartite assemblages (i.e., two guilds of organisms interacting together in a community context, such as plants and pollinators; Chagnon et al. 2012).

One major challenge when studying bipartite networks is to disentangle the relative influence of the various forces

which shape community assembly (Vázquez et al. 2009). For example, some have argued that the nature of the interaction itself will strongly impact network structure (e.g., Thompson 2005; Guimarães et al. 2007; Thébault and Fontaine 2010; Fontaine et al. 2011; Martos et al. 2012; Elias et al. 2013; Wardhaugh et al. 2015). Mutualistic interactions involving sustained, close interactions (i.e., with high codependence) are often suggested to lead to compartmented networks, in which subgroups of species preferentially interact (e.g., Guimarães et al. 2007; see also Wardhaugh et al. 2015). In contrast, mutualistic interactions involving free-living species (i.e., with lower codependence) should lead to nested networks with a core of generalist partners that reciprocally interact and specialists that are preferentially linked to those generalists (Guimarães et al. 2007). Others have proposed that mutualistic communities should be nested due to the inherent stability of such networks, whereas antagonistic communities should feature compartmented structures that can, for example, limit trophic cascades (e.g., Thébault and Fontaine 2010).

Alternatively, environmental or ecological conditions may affect network properties in a manner independent of the nature of the interaction itself (e.g., Olesen and Jordano 2002; Albrecht et al. 2010). For example, Olesen and Jordano (2002) found that plant–pollinator networks in lowlands and at high latitudes show high connectance (i.e., a high fraction of potential interactions actually realized in the field). Likewise, Ramos-Jiliberto et al. (2010) found that nestedness (i.e., the tendency for a network to show little reciprocal specialization) systematically decrease in pollination networks at higher altitudes, while Albrecht et al. (2010) found higher nestedness in glacier forelands than in late successional communities.

Despite evidence that both interaction type and environmental conditions can influence the assembly and structure of ecological networks, such factors are typically considered apart from each other. As a result, our ability to assess their relative importance in community assembly has been hampered. Here, we address this issue by using network approaches to characterize co-occurring communities of plant- and lichen-affiliated fungi. Specifically, we examine two types of interaction networks for the first time—plants and their foliar endophytic fungi (i.e., Class 3 endophytes; see Rodriguez et al. 2009), and lichens and their endolichenic fungi (i.e., fungi that occur within healthy lichen thalli without causing symptoms; see Arnold et al. 2009)—using samples collected systematically in five sites with contrasting abiotic conditions at a continental scale (see U'Ren et al. 2012). Even though the interactions represent different hosts (i.e., plants and lichens), endophytic and endolichenic fungi share numerous ecological, morphological, and functional traits. For example, both groups of symbionts form intimate symbioses with a photosynthetic partner (i.e., green algae and/or cyanobacteria in lichens; photosynthetic tissues of plants). Moreover, both groups are horizontally transmitted, form highly localized, asymptomatic infections, and represent the same phylogenetic groups (Arnold et al. 2009). By examining closely related symbionts with similar interaction types in the same sites, which when considered together represent a wide gradient of abiotic conditions, we assess for the first time the relative importance of interaction type versus environmental conditions in shaping ecological network structure.

Materials and methods

Field sampling and molecular analyses

Fresh, mature, and apparently healthy foliage and lichen thalli were collected from five sites representing distinct environmental conditions, biological communities, and biogeographic regions: Beringian tundra and boreal forest in the Seward Peninsula ecoregion of western Alaska (AKN); inland, subalpine tundra in the Interior Highlands of east-central Alaska (AKE); the Appalachian Mountains of western North Carolina (NCH); the Madrean Sky Island Archipelago of southeastern Arizona (AZC); and subtropical scrub forest in Florida (FLA) [Electronic Supplementary Material (ESM) Table S1; see U'Ren et al. 2012 for detailed descriptions of the sites]. Phylogenetically diverse hosts were collected in each site, including plant species representing Bryophyta, Lycopodiophyta, Pteridophyta, Pinophyta, and Angiospermae (ESM Table S2; U'Ren et al. 2012) and lichen species encompassing diverse mycobionts, growth forms (i.e., foliose, fruticose, crustose), substrates (epiphytic, terricolous, saxicolous), and photobionts (multiple lineages of green algae and some lichens containing cyanobacteria; ESM Table S3; U'Ren et al. 2012). In each sampling site, host collections were made in three replicate microsites located approximately 30 m apart along a ±100-m transect. In each microsite, three shoots (grasses, lycophytes, ferns, shrubs, or trees), one small mat (mosses; 4–9 cm^2), and at least one complete, mature lichen thallus per species were collected (U'Ren et al. 2012). Focal plants and lichens were typically within close proximity to one another (≪1–10 m apart). Sampling all host species or individuals in each site was beyond the scope of this study, but by selecting phylogenetically and functionally diverse hosts we captured representative samples of the plant and lichen diversity at each site (U'Ren et al. 2012).

Within 24–48 h of collection, host tissue was surface-sterilized by sequential immersion, with agitation, in 95 % ethanol for 30 s, 0.5 % NaOCl (diluted Clorox bleach) for 2 min, and 70 % ethanol for 2 min, following Arnold et al. (2007). Small pieces (approx. 1 × 2 mm) were incubated

on a general fungal culture medium [2 % malt extract agar (MEA)] for fungal isolations (see U'Ren et al. 2012).

The majority of fungal isolates remained sterile in culture. They were identified using molecular methods due to the lack of diagnostic morphological characters. DNA was extracted from each isolate, and the nuclear ribosomal internal transcribed spacers (ITS) and 5.8S gene (ITS rDNA; approx. 600 bp) and an adjacent portion of the nuclear ribosomal large subunit (approx. 500 bp) were amplified by PCR and sequenced (see U'Ren et al. 2012). Sequences were then grouped into operational taxonomic units (OTU) based on 95 % sequence similarity as a proxy for species (Arnold and Lutzoni 2007; Arnold et al. 2009; U'Ren et al. 2009, 2010, 2012). Species accumulation curves, in conjunction with bootstrap estimates of species richness, suggested that the majority of culturable fungi was recovered for each host type and site (see U'Ren et al. 2012), such that these data are appropriate for network inferences.

Overall, 4154 fungal isolates representing 359 OTU were evaluated in this study. Although all OTU were used in estimates of diversity and richness (U'Ren et al. 2012), singletons were removed prior to the analyses described here: singleton OTU would be perceived as strict specialists, but might instead represent rare generalists. A total of 267 nonsingleton OTU (comprising 4062 isolates) was used in the analyses described below. These were used to construct interaction matrices in which host species are represented as rows and fungal OTU as columns. Each cell corresponds to the number of times that a given OTU was found to be associated with the corresponding host species. All statistical analyses described below were coded in R using the packages 'vegan' (Oksanen et al. 2013) and 'bipartite' (Dormann et al. 2009).

Network metrics

Six aspects of network structure were evaluated for each interaction type (endophytic, endolichenic) in each of five sites ranging from tundra to subtropical forest: nestedness, the C-score index, power-law fit to degree distribution, betweenness centrality, modularity, and interaction strength asymmetry.

Nestedness is a tendency for ecological networks to display asymmetric interactions, in which apparent specialists tend to interact mostly with generalist partners (Ulrich and Almeida-Neto 2012). This term was originally used to describe biogeographic patterns of species distribution (e.g., Patterson and Atmar 1986) and is now considered to be an insightful metric for understanding species interactions (e.g., Bascompte et al. 2003; Guimarães et al. 2006; Epps and Arnold 2010). Various metrics are available to quantify nestedness (e.g., Atmar and Patterson 1993; Almeida-Neto et al. 2008; Podani and Schmera 2012). We chose the NODF index (nestedness metric based on overlap and decreasing fill) because it is not sensitive to matrix shape or size and because it allows teasing apart the contribution of rows (hosts) versus columns (fungal OTU) to the overall nestedness of the matrix (Almeida-Neto et al. 2008). Briefly, this index calculates an overlap value for each species pair I and j where the number of partners of I is larger than that of j. This overlap value (O_{ij}) is calculated as $\frac{a_{ij}}{a_{ij}+b_{ij}}$, where a_{ij} is the number of partners shared by species i and j, and b_{ij} is the number of partners interacting with species i only. At the community level, NODF is reported as the mean of the calculated O_{ij}.

The C-score index has a relatively long history in community ecology. Similar to nestedness, its use was initially motivated by biogeographic questions. Diamond (1975) suggested that strong interspecific competition should lead to patterns of species pairs that never co-occur in islands/habitat patches. On a species-by-sites metacommunity matrix, this should translate into a "checkerboard" pattern, provided that the rows and columns are properly ordered. Later, Stone and Roberts (1990) developed a formal C-score index, or checkerboard index, to quantify this pattern. This index has been widely used in biogeography (e.g., Ulrich and Gotelli 2013), as well as for the study of ecological networks (e.g., Gotelli and Rohde 2002; Wehner et al. 2014). Although the original idea was to infer interspecific competition from species distribution patterns, many other factors can generate a checkerboard pattern (e.g., environmental filtering and habitat heterogeneity; Connor et al. 2013). In a network, the C-score is calculated at the species pairwise level as $(r_I - s_{ij})(r_j - s_{ij})$, where r_i and r_j are the number of interactions for species i and j, respectively, and s_{ij} is the number of partners shared by these species.

The cumulative frequency distribution of the number of interactions per species (i.e., degree distribution) is often heterogeneous, with many specialist species and a few generalists (e.g., Jordano et al. 2003). This pattern has often been reported to fit a power law (e.g., Beiler et al. 2010), where the probability P of having x links in the network is equal to $x^{(-\gamma)}$, γ being a constant. Using 'bipartite' (function degreedistr), we determined the power law constant γ for each network, including both hosts and fungal symbionts. The higher the value of this constant, the more heterogeneous the degree distribution: very few species achieve high generalism, and most species are specialists. Such heterogeneity could be expected, for example, if the number of interactions of a species is proportional to its abundance, and if hosts and fungal communities show a typical species abundance distribution with many rare species and few very abundant ones (e.g., McGill et al. 2007). Thus, the value of the power law constant can suggest potential

constraints to being a generalist (e.g., dispersal limitation, phenotypic mismatch, or other factors).

Because the number of interactions does not fully describe the centrality and importance of a species in a network, we computed betweenness centrality for all species in our networks. The betweenness centrality of species i corresponds to the number of shortest paths between any two species in the network that pass through species i (Freeman 1977).

Bipartite modularity is defined as the strength of subdivision of a network into subunits, or modules, of preferentially interacting species (i.e., species that interact frequently together, but rarely or not at all with species outside their own module). This pattern may be caused by partner selection or by habitat heterogeneity and environmental filtering (e.g., Lewinsohn et al. 2006; Chagnon et al. 2012). To quantify modularity in our networks, we used a simulated annealing procedure to maximize Barber's modularity index (Barber 2007), as implemented in the C++ executable MODULAR (Marquitti et al. 2014). This modularity index is calculated as $Q = \sum_{i=1}^{N_M} \left[\frac{E_i}{E} - \left(\frac{k_i^F k_i^P}{E^2} \right) \right]$, where N_M is the number of modules identified, E_i is the number of interactions in module i, E is the total number of interactions in the whole network, k_i^F is the sum of the degrees for species of fungi belonging to the ith module, and k_i^P is the sum of the degrees for photosynthetic partner species belonging to the ith module. The optimization procedure for this modularity index followed a simulated annealing optimization approach. Briefly, we started with a random partition of species within a certain number of modules and calculated the modularity index. Swappings were then made to the partition that can change species' module affiliation and/or create or delete modules. The modularity index was recalculated, and the modification was accepted if it increased modularity. It also was accepted if it reduced modularity, but only in the earlier stages of the optimization process. This latter particularity to simulated annealing allows exploration of many network configurations during the optimization process, thereby avoiding entrapment by local (but not global) maxima.

When interaction frequencies are collected to build ecological networks, it is possible to examine interaction symmetry. An interaction between species i and j is said to be symmetric when both species share most of their interaction events together. Conversely, an interaction is considered to be asymmetric if most of interactions by species i are realized with species j, but most interactions by species j are realized with other species. Interaction symmetry is thought to have considerable impact on community dynamics and coevolution (e.g., Bascompte et al. 2006; Vázquez et al. 2007; Chagnon et al. 2012). We computed it as described by Vázquez et al. (2007): the effect of species i on species j(s_{ij}) is the proportion of interactions of species j that involve species i. Then, for this species pair, we calculated the difference between the reciprocal effects of species i and j on each other (i.e., $d_{ij} = s_{ij} - s_{ji}$). For a given species, its asymmetry (A) value is defined as the mean of its d values for all its partners. A_i is close to 1 if species i exerts strong effects on its partners while experiencing little reciprocal effects from them, and close to -1 in the reverse situation. Here, we evaluated whether hosts exerted consistently stronger or weaker effects on their fungal symbionts than the reverse and whether the pattern varied between endophytic and endolichenic associations. We did so by calculating the mean difference in A (i.e., $A_{host} - A_{symbiont}$) for all potential host–fungal species pairs in each network. A paired t test was used to compare those community-level mean A values between pairs of endophytic and endolichenic networks within each site.

Effects of interaction type versus abiotic conditions on network structure

Because network structure indices did not follow a multinormal distribution (multivariate Shapiro–Wilk test, $W = 0.43$, $P < 0.0001$), we used a PERMANOVA (permutational multivariate analysis of variance) to test the relative importance of interaction type (endophytic, endolichenic) and site identity on network structure. We also correlated network structure to abiotic conditions through a constrained ordination [redundancy analysis (RDA)] and tested statistical significance using permutation analyses.

Fungal frequency versus generalism

To verify whether fungal species that appeared to be generalists were simply those that were more frequently isolated, we performed Pearson's correlations between the number of isolates obtained for a given fungal species and the number of hosts from which it was isolated. Correlation coefficients were compared for endophytic and endolichenic fungi in each site using a paired t test.

Results

Overall, network structure differed significantly between endophytic and endolichenic fungi (PERMANOVA pseudo-$F_{1,9} = 27.6$, $P < 0.001$). Conversely, site identity had no effect on network structure (pseudo-$F_{4,5} = 0.21$, $P = 0.880$) (ESM Fig. S1). Accordingly, abiotic conditions were not associated with differences in network structure (RDA; pseudo-$F_{4,5} = 0.29$, $P = 0.850$). Endophytic and endolichenic networks from five sites, ranging from boreal to subtropical, are presented in ESM Fig. S2.

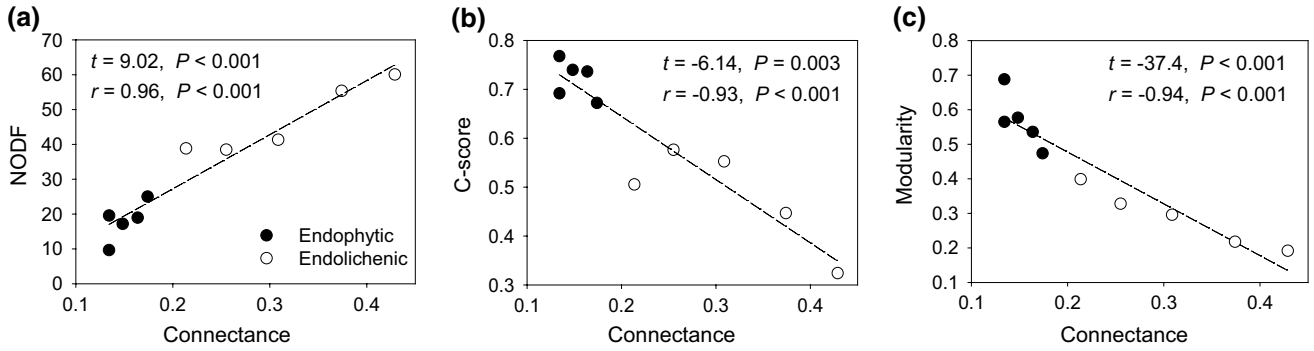

Fig. 1 Variation in nestedness metric based on overlap and decreasing fill (*NODF*) (**a**), C-score (**b**), and modularity (**c**) according to network connectance. The *t* values come from paired Student *t* tests; *r* values represent Pearson's correlation coefficients. Paired *t* tests revealed that endolichenic networks were more connected than co-occuring endophyte networks (paired $t = 5.10$, $df = 4$, $P = 0.007$). *Dashed lines* are linear models fitted to the data, *open circles* endolichenic networks, *filled circles* endophytic networks

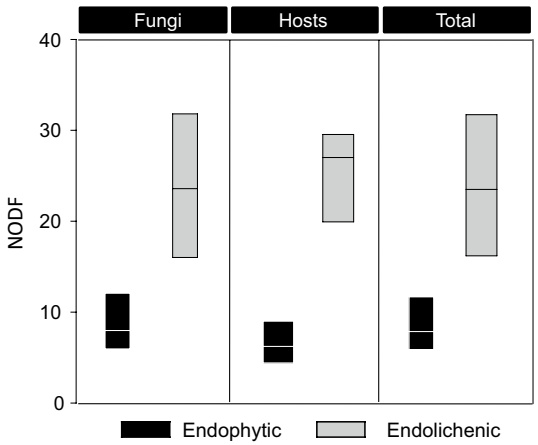

Fig. 2 Contributions of hosts and fungi to network-level nestedness (as measured by NODF). As outlined in Almeida-Neto et al. (2008), NODF has been dissected into *column* and *row* components (shown in the *left* and *center* panels). *Boxplots* in the right panel show overall network nestedness

Table 1 Variation in centrality of hosts and fungi in the networks, as expressed by the power-law constant fitted to the degree distribution[a] and betweenness centrality

Site[b]	Interaction type	Power-law fits		Cumulative betweenness centrality	
		Host	Symbiont	Host	Symbiont
AKN	Endophytic	0.48	NA[c]	2.54	2.42
AKE	Endophytic	0.42	1.5	1.51	5.41
NCH	Endophytic	0.63	1.8	2.47	3.11
AZC	Endophytic	0.65	NA[c]	2	4.01
FLA	Endophytic	0.92	1.65	4.13	5.30
AKN	Endolichenic	0.45	1.08	4.07	5.58
AKE	Endolichenic	0.45	0.58	7	7.42
NCH	Endolichenic	0.48	1.07	0	10.59
AZC	Endolichenic	1.21	0.96	0	9.63
FLA	Endolichenic	1.21	0.78	0	23.11

[a] See the "Network metrics" section for more details

[b] AKN, Alaska-Nome; AKE, Alaska-Eagle Summit; NCH, North Carolina-Highlands; AZC, Arizona-Chiricahua Mountains; FLA, Florida-Archbold Biological Station

[c] NA values report cases in which the number of interactions had an insufficient number of levels (i.e., ≤5) to fit a power-law using the degreedistr. function. For more information see ESM Table S1; for full site descriptions see U'Ren et al. (2012)

Nestedness, C-score, connectance, and modularity differed significantly between endophytic and endolichenic fungal networks (Figs. 1, 2). Endophytic networks were less nested, less connected, and more modular than endolichenic networks in all sites (Figs. 1, 2).

More modular networks featured higher C-scores ($r = 0.93$, $P = 0.0001$), corroborating that some fungal species interacted with different hosts (i.e., belonged to different interaction modules) and were thus less likely to co-occur within a given host (i.e., high C-score). Modules as defined by the simulated annealing algorithm are shown in ESM Fig S3.

Power law constants for fungal symbionts were consistently higher for endophytic networks than endolichenic networks (paired-$t = -14.8$, $df = 2$, $P = 0.004$). No such trend was found for hosts (i.e., plants vs. lichens; paired-$t = 1.09$, $df = 4$, $P = 0.330$; Table 1).

Betweenness centrality was marginally higher for fungi in endolichenic networks versus those in endophytic networks (paired-$t = 2.56$, $df = 2$, $P = 0.060$). For three of the five endolichenic networks (NCH, AZC, and FLA—the three most southern sites), one to two generalist fungi associated with all host species (ESM Fig. S2). Thus, hosts in those networks were fully connected, and betweenness centrality automatically takes a value of zero (Table 1) because

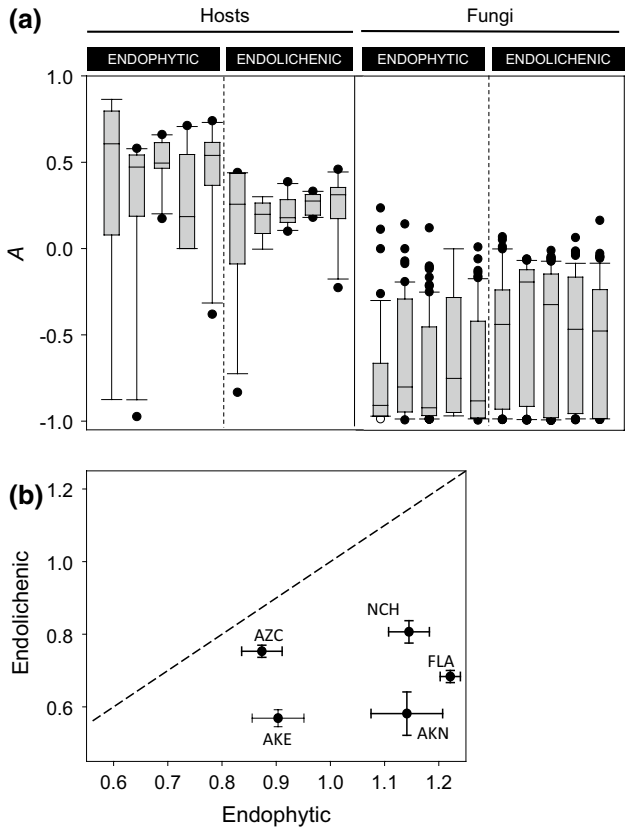

Fig. 3 **a** *Boxplots* of asymmetry (*A*) values for hosts and fungal symbionts in plant–endophytic vs. lichen–endolichenic networks. Each *boxplot* represents a single site, shown from *left to right* in each section as AKN, AKE, NCH, AZC, and FLA (see Table 1 for name of each site). **b** Differences in asymmetries between hosts and fungal associates as a function of interaction type. Each *data point* represents a single site. Host–symbiont species pairs were repeatedly resampled using a bootstrapping approach to compare their asymmetry scores. Values are given as the means and standard deviations based on 1000 bootstrap replicates. The *vertical dashed line* represents the 1:1 relationship

the shortest path from one host species to another is always the direct path between those two species (ESM Fig. S4). This made a statistical comparison of host betweenness centrality in endolichenic versus endophytic networks irrelevant as fully connected networks have the lowest host centrality.

Asymmetry of interactions

Interactions tended to be asymmetric, with hosts exerting a stronger effect on their fungal symbionts than vice-versa (Fig. 3a). This trend was stronger for endophytic networks than for endolichenic networks ($t = -4.72$, $df = 9$, $P = 0.009$) (Fig. 3b).

Fungal frequency vs. generalism

The number of interactions (i.e., degree of generalism) for endophytic and endolichenic fungi was positively correlated with their isolation frequency in eight of ten networks: interaction number (and apparent generalism) was strongly influenced by abundance in the field surveys (except for endophytic networks in sites AKN and NCH). Correlation coefficients were significantly higher for endolichenic versus endophytic networks (paired-$t = 2.80$, $df = 2$, $P = 0.040$), indicating that generalists were frequently isolated from each of their lichen hosts.

Endolichenic fungi typically were isolated more frequently than endophytic fungi (i.e., isolation frequency, defined as the proportion of tissue pieces yielding a fungal isolate in culture, was greater for lichens than for plants; see U'Ren et al. 2012). Therefore, we computed interaction accumulation curves to ensure that the patterns presented here are not methodological artifacts or spurious results based on underlying differences in isolation success (ESM Fig. S5). These show that if isolation frequency and resulting sample size are held constant between endophytic and endolichenic fungi, endophytic networks remain less connected than endolichenic networks across all sites (ESM Fig. S5).

Discussion

Understanding the factors that shape community assembly remains one of the most enduring and important questions in modern ecology. Network theory can reveal rules of community assembly within and across study systems and can suggest novel hypotheses regarding the formation and stability of bipartite communities. However, it is often challenging to disentangle the relative influence of factors such as interaction types and environmental conditions in shaping community assembly. Systematic sampling of two ecologically similar types of interactions (endophytic vs. endolichenic associations) contemporaneously and with the same methods in five sites across North America allowed us to demonstrate that in these fungal symbioses, the type of association (endophytic, endolichenic) had a much more pronounced influence on network structure than did environmental conditions. Those results came about even though there are marked contrasts in the five study sites in terms of abiotic conditions and associated biogeographic and biological history (i.e., from subalpine tundra in Alaska to subtropical forest in Florida; ESM Table S1 and U'Ren et al. 2012).

Interaction type

Although both endophytic and endolichenic fungi live within apparently healthy hosts, often represent the same phylogenetic lineages (and in many cases are congeneric; Arnold et al. 2009; U'Ren et al. 2012), were collected from spatially proximate hosts in replicate sampling in each study site, and could be isolated readily on the same media using culture-based methods, we found profound differences in the networks formed by each of these fungal types and their hosts. These differences were persistent even when we accounted for differences in isolation frequency (which we attribute to differences in tissue colonization rates rather than pervasive differences in cultivability between endophytic and endolichenic symbionts; U'Ren et al. 2012, 2014) and were persistent across the diverse environmental conditions represented by our study sites.

In particular, our analyses revealed that endophytic networks were less nested, less connected, and more modular than endolichenic networks. Accordingly, the C-scores for endophytic networks were also higher than those for endolichenic networks. It is well known that nestedness (here quantified using the NODF index) is positively correlated to connectance (e.g., Almeida-Neto et al. 2008), leading us to evaluate the hypothesis that discrepancies between endophytic and endolichenic networks may simply reflect differences in connectance. Our analyses of degree distribution and centrality show that lichens tend to have a higher proportion of central, generalist fungi; these generalists increase network connectance and are likely to be important in shaping the nestedness of these network structures. Although endolichenic fungi are often host-generalists with regard to the lichens in which they occur, they are more closely related to endophytic symbionts than to saprotrophic fungi, suggesting that their associations with lichen thalli are not purely incidental (U'Ren et al. 2010, 2011). This observation is further illustrated by the occurrence of a minority of nonsingleton OTU in both plants and lichens (ESM Fig. S2).

The higher modularity of endophytic networks may suggest that plant hosts select more strongly than lichens for a specific subset of fungal partners. For example, fungal OTU found in both plants and lichens (including endolichenic generalists) are often restricted to a specific group of plants, such as bryophytes (see ESM Fig. S2; see also U'Ren et al. 2010). Plants surveyed by U'Ren et al. (2012) represented a much broader phylogenetic and phenotypic range (from Bryophyta to Angiospermae) than did the photobionts or mycobionts present in most lichen thalli (e.g., Miadlikowska et al. 2006). Such plant diversity encompasses major differences in structural and chemical defenses, mechanisms for nutrient transfer to fungi, and physiological traits. As for other fungal communities (Chagnon et al. 2013), a focus on particular functional traits may detect mechanisms that differ markedly among plant taxa and thus influence the assembly of endophyte communities (such as foliar chemistry, Arnold and Herre 2003), but differ less among lichen photobionts (with which endolichenic fungi preferentially associate more frequently than mycobiont components of the thallus; Arnold et al. 2009).

In turn, symbiotic fungi may face a trade-off between generalism and competitive ability within a host (e.g., Wilson et al. 2003). In particular, specialized fungal networks may represent species that are better competitors in particular hosts in which they are found—but those fungi may perform poorly on other hosts, with the ability to interact decreasing as phylogenetic distance between the original and new hosts increases (see Gilbert and Webb 2007).

Conversely, the apparently higher specialization of endophytic fungi may reflect dispersal limitation (but see Tedersoo et al. 2014) or low abundance in the sampled sites (i.e., niches that are realized, rather than fundamental). For endolichenic networks we found consistently strong and positive correlations between the frequency of isolation of each endolichenic fungal species and its number of host species; this pattern would better fit a realized niche scenario, where generalist symbionts are simply those that are widespread in the landscape. Conversely, in endophytic networks, such correlations were either weaker or non-significant, indicating that some symbionts were abundant in a small subset of host species (often in a single one) while being rare at the whole network level. This pattern would be consistent with a fundamental niche scenario, with some fungal species specializing on a few preferred hosts. In this case, phylogenetic distance and functional differences among hosts, which was greater among plants than among lichens in the communities sampled here, may play a key role.

Interpreting nestedness

Passive sampling can generate a nested pattern in interaction networks because interactions between rare species are inherently harder to sample (e.g., Blüthgen et al. 2008). In such cases, poorly sampled species have a set of interactions that are nested in those of more intensively sampled species (i.e., interactions with abundant, generalist partners). In our datasets, the sampling effort was always controlled for hosts, but it could not be controlled for fungi: all endophytes and endolichenic fungi that emerged in culture were isolated and enumerated. Given that NODF analysis can distinguish between nestedness of hosts versus fungal symbionts, we would expect fungi to increase overall network nestedness through an artifactual effect of passive sampling of their interactions, while hosts would not. Yet we observe similar trends of interaction nestedness for both

hosts and fungi, suggesting that mechanisms other than passive sampling yield nestedness in our system.

As suggested in previous biogeographic studies (Patterson and Atmar 1986), our results are consistent with the interpretation that co-occurring hosts may constitute a stress gradient for fungal symbionts: hosts with fewer partners may offer more stressful environments by being better defended against particular microbial invaders, limiting nutrient accessibility for symbionts, or investing less to the symbiosis. This mechanism has been referred to as selective extinction in the biogeography literature, as stressing agents selectively exclude intolerant species. Previous studies have revealed environmental effects on fungal symbionts in this context: for example, Verbruggen et al. (2012) reported that the distribution of arbuscular mycorrhizal fungi was nested along a land-use intensification gradient, in which agricultural intensification progressively sorted out species intolerant to agriculture-related stresses. Our results are consistent with a stress gradient that differs among hosts, rather than sites that differ in environmental conditions, and merits further study using culture-free approaches and quantitative evaluation of defenses, related functional traits, and host-microbial feedbacks (see Friesen et al. 2011).

Perspectives on null model approaches in this study system

When comparing network structure among sites, it is generally preferable to transform network structural indices as *z*-scores first (Ulrich et al. 2009). This can be done by comparing the structure of a single network to its structure when it is randomized following a given null model. This procedure eliminates the biases in network structure induced by matrix shape, fill, and symmetry (number of rows vs. number of columns). However, our networks had a very low connectance and a very high asymmetry (i.e., many more endophytic and endolichenic species than host taxa). In such conditions, a constrained null model (e.g., fixed row sums, column sums, or both) does not efficiently randomize interactions because those conditions considerably reduce null space. Accordingly, we observed that a null model with fixed row and columns sums (as suggested by Ulrich and Gotelli 2013) yielded endophytic networks with lower *z*-scores for modularity than the endolichenic networks, while they are clearly more modular (as each plant species almost has its own exclusive set of endophytic fungi). Thus, due to these low connectance values and high network asymmetry, we used crude network structure indices instead of *z*-scores: the latter provided counter-intuitive and misleading results.

Culture-based versus culture-independent approaches in host–fungal network characterization

We characterized endophytic or endolichenic fungal networks using an approach that is dependent upon the successful culture of these fungi under laboratory conditions. This approach is likely to capture only a subset of fungal taxa: for example, culture-based assays frequently miss lineages that are detected by PCR-based surveys (e.g., Basidiomycota; U'Ren et al. 2014). However, some fungi that are recovered in culture remain undetectable when culture-independent techniques are used (see Allen et al. 2003; Bougoure and Cairney 2005; Arnold et al. 2007; Higgins et al. 2011; U'Ren et al. 2014). Moreover, although inferences regarding the taxonomic composition of communities may change between culture-based and culture-independent approaches, major ecological conclusions often remain the same (Arnold et al. 2007; U'Ren et al. 2014). Culture-based studies provide isolate libraries that can be used in functional trait analyses or inoculation experiments to test hypotheses generated by network analyses. In future work, we recommend that network analyses consider endophyte and endolichenic communities using both culture-based and culture-free data sets for the same hosts, with the results of our study providing a first perspective on the structure of networks in these prevalent symbioses.

Interaction asymmetry and open questions

Relative to endolichenic networks, we show that endophytic networks have consistently more asymmetric interactions, with hosts exerting much stronger effect on their symbionts than vice-versa. Such network structure may have important consequences for coevolution, as high asymmetry should translate into strong selection by hosts on their symbionts. Thus, our work draws attention to several key knowledge gaps: (1) Can fungi that have not been isolated from a given host be good competitors in that host taxon under different conditions or at different ontogenetic or seasonal time points? (2) If hosts are confronted by a standard mixture of fungi in a controlled environment, do we still see the nested distribution of their interactions as in the field? (3) Are fungi that specialize on a given host better competitors than generalist fungi in that host species, and to what degree is that context-dependent? (4) Is there local adaptation of fungal populations to their host populations (e.g., Johnson et al. 2010), or do other populations of the same fungal species perform equally well on a given local host? (5) Do tropical environments favor an increasing number of generalists compared to temperate, boreal, and arctic environments, as suggested by survey data (e.g.,

Arnold and Lutzoni 2007; see also Tedersoo et al. 2014)? The interesting aspect of these host–fungal systems is that they are relatively amenable to experimentation in comparison to other study systems in which network studies began (e.g., plant–pollinators, plant–seed dispersers, fish–ectoparasites; see Chagnon et al. 2012). This offers new and exciting opportunities to disentangle ecological network assembly rules by empirically testing competing hypotheses and strengthening inferences (sensu Platt 1964), instead of relying on correlative/likelihood approaches alone.

Conclusion

We showed here that even for ecologically and phylogenetically similar symbionts, interaction type can have a much more pronounced effect than environmental conditions on network structure. This finding is encouraging for efforts devoted to gathering metadata of ecological networks at a global scale (e.g., http://www.santafe.edu/gevent/detail/science/1138/), where local abiotic conditions vary drastically from one network to another. It also supports earlier findings linking interaction type to network properties. Pioneering work in network approaches to community ecology tended to group interaction types into relatively coarse categories (e.g., mutualisms/antagonisms, high/low intimacy associations); here, we show that two similarly intimate symbiotic interactions involving phylogenetically related fungi contrast sharply in their network structure, with such contrasts repeated across environments that differ in the specific taxa present and the abiotic factors they experience. Such insights can complement previous work (e.g., Thompson 2005; Guimarães et al. 2007; Thébault and Fontaine 2010) to suggest new perspectives on how to organize interaction types for network-driven insights, especially with regard to finer scales that can untangle the drivers of network assembly and dynamics.

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