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Interaction type and intimacy structure networks between forestdwelling organisms and their host trees

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Highlights

- Species interaction type may determine their network structure.
- We investigate whether trophically different organisms show also different networks.
- We found higher modularity for heterotrophs reflecting their trophism.
- We found stronger nestedness for autotrophs reflecting their commensalism.
- We conclude that the type of interaction defines the properties of each network.

Abstract

Species interact in many ways. Potentially, the type of interaction, i.e. mutualistic or antagonistic, determines the structure of interaction networks, but this remains poorly tested. Here we investigate whether epiphytes and wood decomposers, having different types of interaction with their host trees, show different network properties. We also test whether the traits of host trees affect network architecture. We recorded presence/absence of organisms colonizing trees, and traits of host trees, in 102 forest plots. Epiphytic bryophytes (64 species) and lichens (119 species) were recorded on c. 2300 trees.

Similarly, wood-inhabiting fungi (193 species) were recorded on c. 900 dead wood items. We studied the patterns of species aggregation on host trees by comparing network metrics of species specialization, nestedness and modularity. Next, we tested whether the prevalence of interactions was influenced by host tree traits. We found non-random interaction patterns between host trees and the three ecological groups (bryophytes, lichens and fungi), with nested and modular structures associated with high host specificity. A higher modularity and number of modules was found for fungi than for epiphytes, which is likely related to their trophic relationship with the host plant, whilst the stronger nestedness for epiphytes is likely reflecting the commensalistic nature of their interactions. For all three groups, the difference in prevalence of interaction across modules was determined by a gradient in interaction intimacy (i.e. host tree specialization), driven by host trees' traits. We conclude that the type of interaction with host trees defines the properties of each network: while autotrophic epiphyte networks show similar properties to mutualistic networks, the heterotrophic wood decomposers show similarity with antagonistic networks.

Keywords: Autotrophs; Commensalists; Heterotrophs; Modularity; Mutualists; Nestedness.

Introduction

The analysis of emergent structural properties of ecological networks provides a valuable tool to understand the processes underlying species interactions in communities. Ecological networks have different properties reflecting diverse underlying mechanisms and the types of interactions, i.e. antagonistic or mutualistic. Specifically, consumers and their resources have an antagonistic relationship, as it is the case for predator-prey and host-parasitoid food webs, whilst mutualistic interactions occur in plant-pollinator, plant-seed disperser and mycorrhizal networks (Mittelbach 2012). Thébault and Fountaine (2010) found that, in plant-animal systems, the network structure is defined by the type of interaction, i.e. mutualistic or antagonistic. However, other ecological networks lie in a "grey zone", in which interaction types are less easy to characterize. This is the case of the interactions occurring in plantplant and fungi-plant networks, like the non-vascular epiphyte-host tree and fungi-host tree networks that we analyze in the present paper. The network properties for comparable communities have been preliminarily defined only in recent times, as for vascular epiphytes (Laube & Zotz 2006, Burns 2007; Burns & Zotz 2010; Sáyago et al. 2013), and parasitic (Vacher, Piou, & Desprez-Loustau 2008), ectomycorrhizal (Bahram, Harend, & Tedersoo 2014) and endomycorrhizal (Encinas-Viso et al. 2016)

fungi. All these networks share more intimate and long-lasting relationships with their hosts (sensu Guimarães et al. 2007) than the often-studied antagonistic and mutualistic networks, but they also show differences among each other. Pires and Guimarães (2013) define interaction intimacy as the degree of biological integration between interacting individuals, and suggest that this network property shapes the way interactions are organized within communities, with an increasing role in organizing antagonistic networks, characterized by greater complexity in their assembly rules (see also Ollerton 2006).

Here we compare for the first time the structures of three networks formed by host trees with nonvascular epiphytic bryophytes and lichens and wood-inhabiting fungi. While these groups share a physical dependency from their host tree during their whole life time, their different trophic roles are likely to determine differences in the arrangement and intensity of their interactions. The heterotrophic fungi initiate wood decay, and some species interact with living tree cells in the establishment phase (Boddy & Heilmann-Clausen 2008), and hence show a higher level of trophic dependency and physiological integration with the host tree than the free-living autotrophic epiphytes. Further, while fungi penetrate the wood with their hyphae and modify the wood structure via powerful enzymes (Stokland, Siitonen, & Jonsson 2012), epiphytes utilize the tree bark only as a growing substrate (Barkman 1958). For the heterotrophic wood-inhabiting fungi one could expect that the properties of their networks with host trees would be similar to the properties of antagonistic networks, while the autotrophism of bryophytes and lichens may show network properties more similar to mutualistic networks. Hence our dataset offers a unique opportunity to test whether these two contrasting types of interactions differ in network structure, according to the hypothesis of Thébault and Fountaine (2010), under controlled environmental variation as all three networks were collected form the same site.

Patterns of aggregation formed by interacting organisms can result in distinct structures: (1) "random aggregation", when species are assigned without any pattern to different resources, (2) "nested structure", when more specialized, less frequent species tend to interact with subsets of more generalist species, which form the core of common hosts with many interactions (Bascompte et al. 2003), (3) "modular structure", when clusters of organisms are aggregated on subsets of resources/ hosts and interact more rarely with species exploiting other subsets of resources (Lewinsohn *et. al.* 2006; Vázquez, Chacoff, & Cagnolo 2009).

The bio-environmental drivers structuring the communities are different for epiphytes and woodinhabiting fungi. Sáyago et al. (2013) showed that modularity may emerge in vascular epiphytes if some species show strong dependency on certain host trees, while nestedness may reflect a passive sampling effect

and higher niche diversity related to host size, with larger trees acting as substrate for a higher number of interactions than small trees and scrubs. For epiphytes, nestedness may also be a consequence of environmental filtering; for bryophytes in particular, high nestedness can result from the dependency on high and stable levels of humidity as affected by bark roughness (Löbel, Snäll, & Rydin 2006; Burns 2007). Instead, bark acidity is likely to play an important role in species aggregation for all epiphytes (Sáyago et al. 2013; Woods, Cardelús, & DeWalt 2015). For wood-inhabiting fungi a modular network structure may be driven by their specificity for susceptible host plants (Ferrer & Gilbert 2003; Dahlberg & Stokland 2004; Gilbert, Gorospe, & Ryvarden 2008), affected by biotic interactions of early decay stage specialists establishing on living hosts (Boddy & Heilmann-Clausen 2008). For fungi, a high degree of specialization for the host, i.e. host intimacy, is likely to produce networks with low nestedness and high levels of modularity (cf., Pires & Guimarães 2013; Heilmann-Clausen et al. 2016). Here we tested whether all these bio-environmental drivers play a different role in structuring the networks. More specifically, we tested whether substrate availability and bark characteristics differently affect the prevalence of interactions across the three networks. For prevalence of interactions we mean the proportion of sample plots with individuals of a given host tree genus occupied by a given colonizer in the networks' modules.

We compared network structures between three organism groups connected by their interaction with common host trees, asking: (1) Do autotrophic epiphytes and heterotrophic wood-inhabiting fungi have non-random patterns of interactions, in terms of generality, network specialization, specialization asymmetry, modularity and nestedness? (2) Are the gradients in nestedness and modularity related to gradients in metrics of interaction intimacy with the host trees? (3) Do host trees' traits, such as substrate availability and bark characteristics play a role in structuring networks of epiphytes and wood-decomposing fungi? We expect that wood-inhabiting fungi, which use the tree not only as substrate but also as a resource, show higher degree of host specialisation and produce more modular networks than epiphytes. Moreover, we expect that the narrower association of epiphytes with host trees' traits results in higher levels of network nestedness for bryophytes- and lichens- than fungi-tree networks.

Materials and methods

Study area

Our study area was the forest of Lille Vildmose nature reserve, one of the largest protected terrestrial areas (76 km²) in Denmark, located in North Jutland (56°509 N, 10°159 E, see Fig. 1A). The study area consisted of two neighboring forests (maximum distance between sites was 10 km): Tofte Skov and Høstemark Skov (Fig. 1B). Lille Vildmose includes conifer plantations, grasslands, lakes, moors, an intact raised bog and several old-growth forests remnants, aggregated in two more coherent forest complexes (maximum distance between sites 10 km): Tofte Skov (500 ha) and Høstemark Skov (133 ha) (Fig. 1B). The area is a Natura 2000 site and subject to a European Union LIFE+ Nature and Biodiversity program, which ran from 2011 to 2016, aiming to restore the natural water levels in the area (Anonymous 2011). For this study, mixed broadleaved forest, almost pure beech (*Fagus sylvatica*), and mixed deciduous-coniferous forests were included. Due to grazing and browsing of red deer, the forests are relatively open, with good light conditions for epiphytes.

Data collection

In the two study forests of Lille Vildmose, we conducted a stratified random sampling (Fig. 1B). First, we defined 22 polygons (average size 19 ha) to represent various forest types and a variation in current and predicted drainage levels after restoration of hydrology (Riis, Møller, & Aaby 2009a,b) (Fig. 1B). In each polygon we randomly established five circular sample plots (radius 15 m), located at a minimum distance of 30 m and with a 15 m minimum distance from the forest edge (Fig. 1B). Eight of the 110 plots were located in recent or old clearings and were hence not monitored for epiphytes or wood-inhabiting fungi. In each plot, we recorded species incidence (presence-absence; for species lists see Tables in Appendix A) for each of three ecological groups, i.e. bryophytes, lichens and wood-inhabiting fungi, and the identity of their associated tree (living or dead) occurring in the plot in 2013. For the ease of comparability between fungi and epiphytes, tree identity at the genus level was used. However, all important genera are represented by one species only in the study area (Acer platanoides (0.01% of the basal area, BA), Alnus glutinosa (86.6% BA), Corylus avellana (0.1% BA), Crataegus monogyna (0.02% BA), Fagus sylvatica (4.7% BA), Fraxinus excelsior (0.1% BA), Juniperus communis (0.01% BA), Larix kaempferi (0.1% BA), Malus sylvestris (0.02% BA), Pinus sylvestris (0.02% BA), Populus tremula (0.1% BA), Sorbus aucuparia (0.3% BA), Tilia cordata (0.1% BA)), while a few tree genera are represented by two (Betula pubescens (3.4% BA) and B. pendula (0.2% BA); Picea abies (1.2% BA) and P. sitchensis

(0.3% BA); *Salix capraea* (0.005% BA) and *S. cinerea* (0.01% BA))) or three species (*Quercus petrea* (0.2% BA), *Q. robur* (2.0% BA) and *Q. rubra* 0.6% BA)), which are in all cases very similar in terms of traits (Appendix C). The following procedures were employed:

Dead wood: tree genus was recorded for all the 896 standing or lying dead wood items belonging to all the decay classes with a diameter ≥ 10 cm and length ≥ 1 m and for stumps larger than 20 cm in diameter, during fungal surveys in August;

Living trees: all living trees (2308 host tree individuals) with $DBH \ge 10$ cm were identified and their DBH was measured during vegetation surveys in August;

Fungi: fruit bodies of wood-inhabiting macrofungi, including agarics, polypores, steroid corticoids, jelly fungi, stromatic pyrenomycetes and discomycetes with fruit-bodies regularly larger than 5 mm (193 species recorded on 13 genera of host trees) were recorded per each standing and lying dead wood item in the plot, on two occasions (25-28 August, and 21-25 October) to cover the early as well as late aspects of fruiting. Fungal communities differ somewhat between standing dead trees, stumps and fallen logs (e.g. Sippola & Renvall 1999; Lindhe, Åsenblad, & Toresson 2004), but not in a way that is likely to influence network structure. Hence data for all dead wood types were aggregated for each tree genus at plot level.

Bryophytes and lichens: epiphytic bryophyte (64 species on 17 genera of host trees) and lichen species including crustose lichens (119 species recorded on 16 host tree genera) were recorded on all standing live and dead trees with DBH \geq 10 cm from 0 up to 2 m height in each plot. Stumps taller than 1.5 m were included, whereas lying wood (logs and branches) was excluded from the survey, as it typically hosts different communities, including epixylic and terrestrial species, than standing trees (Heilmann-Clausen et al. 2014). Epiphytic bryophytes and lichens were surveyed during three rounds: April, August and October. The bark and wood surface areas explored were of similar magnitude for deadwood (2.74 x 10⁷ cm²) as for living trees (2.63 x 10⁷ cm²), and the deadwood area was congruent with the living area across tree genera (Kendall's tau-b = 0.54, P = 0.01). Therefore we assume that the three networks were sampled with a similar sampling effort.

Species identification in all the surveys was carried out in the field if possible. The monitoring data on wood-inhabiting fungi are stored in svampeatlas.dk, while data on bryophytes and lichens are in the Natural History Museum of Denmark (Copenhagen).

Statistical analysis

Assembling the interaction networks

From presence-absence data, we assembled three matrices representing the three ecological networks: fungus-tree, bryophyte-tree and lichen-tree (Fig. 2; Tables in Appendix A and interaction matrices in Appendix D). In each matrix, each column (i) corresponds to a tree genus (i.e. the minimum identifiable unit for deadwood), each row (*j*) corresponds to a species, and each cell (a_{ij}) contains a measure of the prevalence of interaction $(aij \ge 0)$. The prevalence of interaction was defined as the percentage of plots in which a given interaction occurred in relation to the total number of plots in which the tree host was present. For instance, the prevalence of interaction was the highest (equal to 100%) when an interaction occurred in all the plots in which a host tree species was present. For fungi, we considered their interaction with deadwood items and for bryophytes and lichens with living trees. The three networks are represented in Fig. 2 with Gephi 0.9.1 (Bastian, Heymann, & Jacomi 2009). The layout algorithm was "Force Atlas" which attracts linked nodes each other while pushing apart non-linked nodes. Modules were chosen as color rank parameter for nodes. For the three networks we detected a considerable and congruent proportion of realized links: 57% of the expected links estimated by the Chao1 index (sensu Vizentin-Bugoni et al. 2016) for bryophytes, 69% for lichens and 60% for fungi (Table 1). The number of interactions was strongly positively correlated with the total surface area (dead or alive) covered by each tree genus in the sample plots (Kendall's tau-b \geq 0.65, P \leq 0.001). For all networks most interactions were recorded with the same tree genera (in decreasing proportion of interactions): Fagus (\geq 30% of the interactions for the three networks), Alnus (\geq 26%), Betula (\geq 12%), *Quercus* (\geq 8%) and *Sorbus* (\geq 3%) (Fig. 2).

Evaluating the network structure

In order to compare the structure of the three ecological networks, we calculated the following network metrics using the R-package *bipartite* (Dormann, Gruber, & Fründ 2008) (Table 1):

- *Number of links*, i.e. the total number of realized interactions between host trees and their associated species.

- *Nestedness* is measured by the wNODF index (Almeida-Neto & Ulrich 2011), which quantifies the nonoverlap and decreasing fill for both rows and columns of the quantitative matrix, ranging from 0 (minimum nestedness) to 100 (maximum nestedness).

- *Modularity* quantifies the presence of subsets of species interacting more among them than with other species in the networks. It is measured by the Q index by using the optimization algorithm QuanBiMo, ranging from 0 (minimum modularity) to 1 (maximum modularity) (Dormann & Strauss 2014). The higher the Q value, the stronger the data support the division of a network into modules. Due to the stochastic nature of this algorithm, the obtained values can be slightly different among runs, thus we chose the highest values from 20 independent runs set to 10^8 swaps for each network. As QuanBiMo is an optimization algorithm, the interpretation of module conformation was on the highest Q value found.

- Number of modules, i.e. the number of interactive subsets within each network.

- Weighted mean degree, i.e. the weighted mean number of interactions among patterns, i.e. of host tree genera per associated species (calculated from function *'generality'* sensu Bersier et al. 2002).

- *Network specialisation* (H'_2), a quantitative metric of network specialisation based on the standardized two-dimensional Shannon entropy (Blüthgen, Menzel, & Blüthgen 2006), ranging from 0 (highest generalization) to 1 (highest specialisation). It describes to which extent observed interactions deviate from those that would be expected given the species marginal totals. The more selective a species, the larger is H'_2 for the web.

- *Direction of interaction asymmetry*, the difference between a species' interaction strength on a host tree and the reciprocal interaction strength of a host on an associated species (Vázquez et al. 2007). The index takes values from 0 (high strength symmetry) to 1 (high asymmetry), with the sign indicating the direction of the asymmetry. The index is negative if a high proportion of the species' interactions occurs with the interacting hosts (experiences strong effects of its partner), and positive if the species represent a high proportion of the interactions of their host partner (exerts a strong effect).

To assess metrics significances and compare networks, we compared the observed metrics values to those obtained by 1000 null model randomizations. For modularity, we used fewer randomizations (100) due to large computational time required by the algorithm. For all metrics we used the *'vaznull'* null model, which generates null matrices with the same dimensions, connectance and marginal total than the observed matrix (Dormann, Gruber, & Fründ 2008).

In order to compare metrics across networks, we proceeded with a standardization based on nullmodel values, by calculating $z = (x - \bar{x}/\sigma)$, according to Ulrich, Almeida-Neto and Gotelli (2009), where x is the observed value and \bar{x} and σ are mean and standard deviation of the null model randomizations

respectively. Then the relative pairwise differences (Δ) in these standardised metrics were compared among the three networks. Standardized z values for the network metrics are reported in Table 1.

Explaining network structure with host tree traits

In order to determine which host tree traits structure each network, we evaluated the association between prevalence of interaction (*aij*) and host tree traits (median diameter, DBH; wood pH; bark surface structure, BSS; see later) via Kendall rank correlation coefficient (a distribution of 1,000 values was generated via bootstrapping) for the whole network and independently for each module (single effects and two-way interactions) (Fig. 3). Then, to find whether these traits play a role in determining species aggregation on the same host into network modules, we compared the values of the correlation coefficients for the whole network ("All" in Fig. 3) with the distribution of the coefficients generated (via bootstrapping) for each network module ("N_X" in Fig. 3).

To understand which module characteristics (i.e. prevalence of interactions or traits) would be responsible for the significant correlations between prevalence of interactions and traits values, we compared the variability in the prevalence of interactions and in traits' values (response variables) across modules (N, categorical predictor) via Generalized Linear Models (GLMs) (observed values in Figs. 4-5; predicted values and model details for the prevalence of interaction in Appendix C in Tables C1-C2, for traits in Tables C3-C4-C5).

GLM specifications: In the GLMs where the response variable was the prevalence of interaction, *aij*, given its proportional nature, we assumed a gamma distribution of errors for the random part of the model and a log link function (systematic part) between the dependent variable and the predictor. The gamma distribution fits models with over-dispersed proportions equally well as the classical negative binomial distribution (Adell & De la Cal 1994). In the GLMs where one of the traits was the continuous response variable, i.e. median DBH, wood pH and BSS, we assumed a normal distribution of errors for the random part of the model and an identity function between the dependent variable and the predictor (systematic part). The means for each module of the GLM regression parameters for the response variable were calculated via the Wald test. In GLMs the homogeneity of variance does not need to be satisfied so we could make pairwise comparisons of trait values among all the modules (cf., McCullagh & Nelder 1989).

Kendall rank correlations (with relative bootstrapped values) and GLMs were estimated using SPSS 20.0 (IBM Corp. 2011).

Host traits: we selected the following host traits because they are considered bio-environmental filters explaining the prevalence of interaction: host tree median diameter (DBH, which is a surrogate for a trees' capacity to aggregate ("sample") more organisms, Sáyago et al. 2013), wood pH (Sáyago et al. 2013; Woods, Cardelús, & DeWalt 2015) and bark surface structure (BSS, Löbel, Snäll, & Rydin 2006; Burns 2007) (surrogates for substrate characteristics) (see in Appendix B Tables B1, B2 for details). Wood pH was used as a proxy of bark acidity for each tree genus (according to the references listed in Table B2, Appendix B). Bark surface structure followed a categorical scale ranging between 0-1 (= smooth) and 6 (= very coarse) texture, according to the categories defined in the TRY trait database (Kattge et al. 2011) and reported for each tree genus in Table B1, Appendix B.

Results

Network structures and characteristics

All three networks showed a higher level of specialization and were more nested and modular than expected by null models (Table 1). The three networks showed a descending gradient in the *z*-values of network specialization (*z*-H'₂) from lichens to fungi to bryophytes, reflected by a similar gradient in the number of links (Table 1). The differences in network specialization and number of links between lichens and fungi (Δz -H'₂ = 57.2; ΔL = 51.0) were smaller than the differences between lichens and bryophytes (Δz -H'₂ = 145.5; ΔL = 307) and between fungi and bryophytes (Δz -H'₂ = 88.4; ΔL = 256).

The values of nestedness (*z*-wNODF) showed a descending gradient from bryophytes to lichens and fungi (Table 1). In contrast the values of modularity (*z*-Q) descended from fungi, to bryophytes and lichens (Table 1). The differences in nestedness and modularity between bryophytes and lichens (Δz -WNODF = 1.0; Δz -Q = 2.8) were smaller than the differences between bryophytes and fungi (Δz -WNODF = 3.0; Δz -Q = 4.1) and between lichens and fungi (Δz -WNODF = 2.1; Δz -Q = 6.8). Finally, the number of modules was higher for fungi (7) than for epiphytes (5 modules both for bryophytes and lichens) (Table 1; for each network the allocation of the tree-associated species in the modules is reported in Fig. 2, in Tables in Appendix A and interaction matrices in Appendix D).

Other network metrics (i.e. weighted mean number of host tree genera and interaction symmetry) showed gradients corresponding to the ones found for nestedness and modularity. As for nestedness, *z*-values of weighted mean number of host tree genera per associated species decreased from bryophytes to lichens and

fungi (Table 1). The differences in the weighted mean hosts per species between lichens and fungi ($\Delta z =$ 19.0) were smaller than the differences between lichens and bryophytes ($\Delta z = 29.2$) and between fungi and bryophytes ($\Delta z = 48.2$). On the other hand, the high modularity of fungi can be associated with their low *z*-value of interaction asymmetry as compared to bryophytes and lichens (Table 1). The differences in the values of interaction asymmetry between bryophytes and lichens ($\Delta z = 65.4$) were smaller than the differences between bryophytes and between lichens and fungi ($\Delta z = 165.2$) (Table 1).

Traits driving network structure

For the whole three ecological networks, the prevalence of interactions (*aij*) increased significantly with the median tree diameter ("All" boxplots in Fig. 3; in each case, P < 0.001). The prevalence of interactions was less affected by wood pH and bark surface structure (BSS), with a significant effect of wood pH on *aij* for fungi only ("All" boxplots in Fig. 3). All the pairwise interaction terms between the three host traits explained slightly better the prevalence of interactions, showing always significant ($P \le 0.01$) and positive but still weak correlations (+0.048 ≤ Kendall rank coefficient ≤ +0.326).

A positive correlation between *aij* and DBH was present in at least half of the modules in bryophytes, lichens and fungi ("N_X" boxplots in Fig. 3). These modules were generally characterized by trees with large DBH (*Acer, Alnus, Betula, Fagus, Fraxinus, Picea, Populus, Quercus, Sorbus*) and low prevalence of interactions (Figs. 4-5, Tables C3, C4, C5).

A positive correlation between prevalence of interactions and wood pH values was present in one module for each network ("N_X" boxplots in in Fig. 3). For both fungi and bryophytes the positive correlation was verified in modules characterized by high wood pH (i.e. *Acer, Alnus, Betula, Fagus, Fraxinus, Picea, Populus, Quercus* and *Sorbus*) and low prevalence of interactions (Figs. 4 and 5, Tables C3-C5) while for lichens there was a positive correlation in a module (in N = 4) with low bark pH (mean pH = 2.3 for *Crataegus, Malus* and *Salix*) and high prevalence of interactions (mean *aij* = 0.43; Figs. 4 and 5, Tables C3-C5).

Finally a lichen module and a fungal module showed positive correlation between prevalence of interactions and bark surface structure (BSS) ("N_X" boxplots in Fig. 3). Both modules were characterized by trees with generally smooth surface structure (i.e. *Alnus, Betula, Corylus, Fagus, Picea, Populus, Quercus, Sorbus* and *Tilia*) (Fig. 5). On the other hand, another lichen module (N = 5, Fig. 5) characterized

by trees with generally coarser surface structure (mean BSS = 2.75, *Betula, Juniperus, Larix, Pinus*) (Fig. 5), showed a negative correlation between bark surface and *aij* ("N_X" boxplots in Fig. 3). For bryophytes no module showed significant correlations between *aij* and BSS ("N_X" boxplots in Fig. 3).

Discussion

In this study, both epiphytes (bryophytes and lichens) and wood-inhabiting fungi showed non-random aggregation with their host trees, exhibiting high network specialization, and nested and modular topological structures. Structural differences in the networks followed a gradient in interaction intimacy and were influenced by host tree traits.

We documented convergence of the three networks towards specialization and structured aggregation (cf. Guimarães et al. 2007). These network features are likely due to intimate and long-lasting evolutionary relationships between species and their host trees. However, for the three networks the resulting nested structures and their segregation into modules were mostly associated with tree diameter and wood pH.

The larger differences in nestedness, modularity and interaction asymmetry between fungi vs epiphytes in comparison to bryophytes vs lichens likely reflect the different relationships of these species groups with their host trees. Interestingly, in our study, autotrophic epiphytes share the network properties previously reported for mutualistic networks, while heterotrophic fungi share the properties reported for antagonistic networks (Thébault & Fountaine 2010). Hereby our results extend the hypothesis suggested by Thébault and Fountaine (2010) for plant-animal systems, confirming that also in other networks the type of interaction, i.e. mutualistic or antagonistic, is reflected by the network properties.

In this study, we extend the knowledge obtained for the traits driving the aggregation of vascular epiphytes (Sáyago et al. 2013; Woods, Cardelús, & DeWalt 2015) to epiphytic bryophytes, epiphytic lichens and wood-inhabiting fungi. Substrate availability, i.e. the sampling effect of host tree trunks and branches, played an important role in aggregating all three ecological groups on their host trees, increasing the prevalence of species interactions on larger hosts (Sáyago et al. 2013). In other words, large host trees favour passive colonization by spores of tree-dwelling organisms by offering a larger available area for propagules to land and interact. Woods and collaborators (2015) pointed out the importance of microhabitat heterogeneity in structuring epiphytic metacommunities. Specifically, for our

groups we confirmed the findings for epiphytic non-vascular bryophytes and lichens (Löbel, Snäll, & Rydin 2006) and for vascular epiphytes (Burns 2007; Sáyago et al. 2013) showing that in fact microhabitat dimensions such as wood pH and bark surface structure are also determinants of the prevalence of interactions. For example, in some modules of bryophytes and fungi, we found the interaction prevalence to decrease for trees with acidic bark/wood, and increasing with less extreme (close to neutral) pH conditions, while in lichen modules there were more interactions in more acidic conditions, as observed for the response of species richness of these two groups to the two drivers (Löbel, Snäll, & Rydin 2006). For some modules of lichens and fungi we confirmed the findings for vascular epiphytes of Sáyago et al. (2013), who detected higher probabilities of interactions on trees with rougher bark texture. However, while wood pH and bark structure loosely increased as single traits the prevalence of interactions in the networks, we found that the synergistic presence in a certain host tree of more than one favourable trait had a stronger positive effect on the prevalence of interactions than any single trait. For example the complex bark structure of large trees provided also favourable microhabitats, in terms of wood pH and bark texture, for the establishment of interacting species (cf. Sáyago et al. 2013).

Tree diameter and pH affected the prevalence of interactions in networks' modules in which the prevalence of interactions was low, likely because substrate availability and microhabitat characteristics are more likely to affect species interactions at low density. On the other hand, species at high prevalence of interactions already occupy most of the host trees and their occurrence is limited by other factors like competition for space (cf. Bowker, Soliveres, & Maestre 2010).

As observed for antagonistic and mutualistic organisms (Pires & Guimarães 2013; Toju et al. 2014), our results suggest that nestedness and modularity follow a gradient of interaction intimacy. From an evolutionary perspective, our findings suggest that the intimate interactions generated by co-evolutionary processes occurring among heterotrophic wood-inhabiting fungi and their host tree have led to greater reciprocal specialisation and modularity than the purely commensalistic interactions among free-living epiphytes (bryophytes and lichens) and the host tree. Interactions between living host cells and wood-decaying fungi are only evident in heart-rot agents, parasites and decomposers with a latent infection strategy (Boddy & Heilmann-Clausen 2008). The latter groups include many ascomycetes with tiny, inconspicuous or even lacking fruit bodies, which were not included in the present study. Hence, a sampling strategy utilising next generation molecular sampling of all fungi present as mycelia in wood (cf. Rajala et al. 2012) and focussing on wood in early stages of decay would most likely detect even

stronger modular community structures than found in the present study. For epiphytes, co-evolutionary processes are unlikely to be important in shaping their networks due to the lack of intimate interactions (cf. Wagner, Mendieta-Leiva, & Zotz 2015, discussing vascular epiphytes). For epiphytes, the relatively higher nestedness compared with fungi may be a consequence of their higher dependency on the micro-environmental conditions created by the host trees. These conditions include a humid microenvironment - affected by a gradient from smooth to coarse bark- and their high degree of association with different microhabitats (Barkman1958).

The differences in the number of links, network specialization and weighted mean degree between bryophytes and the lichenized vs non-lichenized fungal cluster could be explained by more recent historical factors. For instance, the study area is currently rather poor in bryophyte species specialized in old tree habitats, probably reflecting a depletion of this species pool. This depletion is likely due to regional old-growth forest continuity breaks in NW Europe (Heilmann-Clausen et al. 2014) and more local effects of high browsing pressure limiting the highly desiccation-sensitive epiphytic bryophytes in the study site (Mazziotta et al. 2016). This may cause the number of links and the level of specialization for host trees to be lower than under pristine conditions.

Peculiarities of the three networks

Stronger nestedness found for bryophytes would be explicable from their high dependency on humid micro-environments (Burns 2007). This is likely true also for lichens (cf. Barkman 1958), for which the observed network characteristics (high nestedness and relatively low modularity) could result from environmental filtering, including a higher demand for light than in bryophytes (Löbel, Snäll, & Rydin 2006). Smaller differences in nestedness and modularity for both epiphytic groups in comparison to fungi could also be explained by the balance between competition and facilitation within and between these groups (for mosses and lichens see Bowker, Soliveres, & Maestre 2010): competition for space and resource access on trees increases species segregation on the individual host trees, while facilitation favours biotic homogenization at community scale. For example, vertical segregation has been documented for epiphytic bryophytes and lichens (Fritz 2009).

Fungi show diverse interactions with different plant organs (e.g. Heilmann-Clausen et al. 2014). Here we found nested structures in their aboveground connections with dead trunks as wood-decayers, consistent with the findings by Vacher, Piou and Desprez-Loustau (2008) and Heilmann-Clausen et al.

(2016), but contrary to results found for mycorrhizal symbionts interacting with tree roots (cf. Toju et al. 2014). This could be due to a stronger trophic specialisation of aboveground fungi for the trunks of their host trees in comparison to belowground mutualistic fungi, i.e. mostly mycorrhizal fungi, which are thought to have generally evolved connections with wider ranges of host plants' roots (Toju et al. 2014). The modular structure of the tree/fungus network probably reflects ancient speciation events in seed plants (Vacher, Piou, & Desprez-Loustau 2008; Heilmann-Clausen et al. 2016).

Conclusions

Type of host tree interactions determined network properties for the three studied organism groups: autotrophic epiphytes showed higher levels of nestedness than wood-inhabiting fungi, which is typical for mutualistic networks, while heterotrophic fungi showed higher levels of aggregation into modules, which is typical for antagonistic networks. The structural similarity between the three networks is due to the decisive role of host trees in all of them, i.e. host trees provide the habitat for these specialized organisms. The differences in network properties are mainly determined by a gradient in interaction intimacy (i.e. degree of specialization to the host), maintained by various levels of co-evolutionary processes, and driven by traits of the host tree.

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Figure captions

Fig. 1. Study area. (A) Location of the Lille Vildmose reserve in Denmark; (B) aerial photograph showing the monitoring scheme in the two forest sites (above, Høstemark Skov, below, Tofte Skov) consisting of polygons (blue) and sample plots (pink) (scale 1:20000). Polygons in open areas do not contain any sample plot. The aerial photograph in Fig. 1B was based on Google Earth.

Fig. 2. The observed commensalistic networks of genera of tree hosts (nodes) interacting with (A) bryophyte, (B) lichen and (C) fungal species (numbered nodes represent species; see their ID, identity, associated module (N) and relative tree genera in Tables in Appendix A). Lines connect genera with species based on their interaction matrix. Highly connected genera and species occupy a central position in the graph. Host tree genera and their linked species sharing the same module are represented in the same color.

Fig. 3. Associations between prevalence of interaction (*aij*) and host tree traits (median diameter, DBH, wood pH, bark surface structure, BSS). The associations are evaluated via Kendall rank correlation coefficient (distribution, mean and interquartile range of 1,000 Kendall values generated via bootstrapping are reported in the boxplots) for the whole network (All) and independently for each module (N_1 – N_7) for which the variability in the trait of interest was not null (see Fig. 5). * = Significant Correlations at P < 0.05 level.

Fig. 4. Boxplots showing the distribution of values (mean, interquartile range, °outliers; \star extreme values) in the prevalence of interaction (*aij*) between species and host trees for each module of the three networks ((a) bryophytes, (b) lichens and (c) fungi). For each module (N) information is provided on the number of interactions (Int) and host tree genera

Fig. 5. Boxplots showing the distribution of values (mean, interquartile range, \star extreme values) of three traits (median tree diameter (DBH), wood pH and Bark Surface Structure (BSS)) for each module of the three networks (bryophytes, lichens and fungi). Letters are attributed via pairwise contrast in the distribution of values between modules (Table C3). The same letter (from *a* to *e*, with increasing modelled value, Table C7) characterizes modules with non-significantly different distribution values for a certain host trait at P < 0.05 level (see GLM details in Tables C2, C4 and C5).

Figures

Fig. 1.





Fig. 3.



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Fig. 4.







N	1	2	3	4	5
Int.	355	42	7	24	76
Free Genus	Alnus	Corylus	Acer	Crataegus	Betula
	Fagus	Populus		Malus	Juniperus
	Fraxinus	Tilia		Salix	Larix
	Quercus				Pinus
	Sorbus				



Sorbus

Fig. 5.



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Tables

Table 1. Properties of the three ecological networks from Lille Vildmose reserve (Denmark). The expected number of links estimated by the Chao1 index is reported in parentheses. In order to compare metrics between networks, their z-values (in parentheses) were calculated by standardization based on 1000 randomizations (100 for Q).

Notwork matrice	Networks			
Network metrics	Bryophytes-tree	Lichens-tree	Fungi-tree	
Number of links	153 (266)	460 (667)	409 (682)	
Nestedness (wNODF)	49.90 (22.02-	54.23 (38.78-43.64)	34.02 (21.82-	
	26.36) z =	z = 11.74	26.50) z =	
	12.70		9.66	
Modularity (Q)	0.338 (0.070-	0.313 (0.018-0.078)	0.393 (0.042-	
	0.116) z =	z = 15.25	0.091) z =	
	18.02		22.07	
Number of modules	5	5	7	
Weighted mean degree	9.5 (27.1-29.2)	27.2 (62.6-66.2)	37.8 (134.6-	
(host/species)	z = -57.00	z = -86.24	141.1) z	
			= -105.21	
Network specialization (H' ₂)	0.35 (0.012-	0.34 (0.013-0.018)	0.57 (0.023-	
	0.017) z =	z = 515.43	0.030) z	
	369.89		= 458.26	
Interaction asymmetry (species-	0.64 (0.11-0.18)	0.78 (0.22-0.26)	-0.001 (0.33-	
host)	z = 40.91	z = 106.34	0.36)	
			z = -58.90	