The Interplay between Structure and Function in Fungal Networks

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Abstract

Saprotrophic woodland fungi form self-organised transport networks as they forage for resources across the forest floor. These networks adapt during development by selective reinforcement of major transport routes and recycling of the intervening redundant mycelium to support further extension. The predicted transport performance of the resulting weighted networks show improved efficiency in comparison to evenly weighted networks with the same topology, or standard reference networks. Experimental measurement of nutrient movement using radiotracers and scintillation imaging show that fluxes are more dynamic, with synchronised oscillations and switching between different pre-existing routes. The same structures that confer good transport efficiency also show good resilience to both simulated damage and experimental attack by grazing insects, with persistence of a centrally connected core. We argue that fungi grow as self-organised planar spatial networks, honed by evolution, which may exemplify potential solutions to real-world compromises between search strategy, transport efficiency, resilience and cost.

Keywords: fungal mycelial network, weighted network efficiency, biological network resilience

1. Introduction

Transport networks, including those for rail [1], road [2], air [3], energy [4], and information [5], play a fundamental role in human economies, motivating research into how these systems function and in what way they may be improved. One approach is to draw inspiration from relevant biological systems as these have evolved through a process of natural selection, and are therefore likely to have achieved an appropriate balance between cost, efficiency and resilience. Conversely, application of the tools and techniques developed in the physical sciences to analyse biological systems may yield better understanding of the design constraints underpinning biological structures. Woodland fungi form some of the most extensive biological networks known [6, 7, 8, 9] as they scavenge the soil-litter interface for heterogeneous resources, such as wood and mineral nutrients [10, 11, 12]. Unlike many other biological networks so far described, the network formed is not part of the organism, it is the organism. The network develops without centralised control and must both transport nutrients between spatially separated source and sink regions, and also maintain its integrity in the face of continuous attack by mycophagous insects or random damage [12, 13]. The challenges that these conflicting demands place on the network organisation have strong parallels with those faced in the design of anthropogenic infrastructure networks. Thus, it is possible that the solutions found

by these biological systems will yield useful insights into the design of delocalised, robust infrastructure networks.

Although the concept of the networks is widespread in the mycological literature [14, 15, 16], there have been remarkably few attempts to characterise the network structure directly, nor to assess how the network structure impacts on function. At the microscopic scale, Ritz and co-workers used semi-automated image analysis to extract the network formed by *Trichoderma viride* growing on cellophane and visualise the spatial concentration of loops in response to a heterogeneous resource distribution [17]. At the other end of the spatial scale, Jeger and co-workers mapped the network formed by rhizomorphs of *Armillaria lutea* in 25 m² woodland plots, and concluded that the presence of numerous loops reduced the risk of disconnection of any part of the network, thereby increasing its robustness [18]. Here, we describe the architecture of networks formed by the saprotrophic woodland fungus, *Phanerochaete velutina*, that operates at an intermediate (cm-m) scale, to determine how these essentially planar, weighted spatial networks resolve the conflicting demands of exploration, exploitation, transport and resilience [19]. Like any organism, this requires efficient allocation of limited resources between competing requirements [20, 11, 13]. For example, construction of a very dense, highly connected network might confer high transport capacity and robustness to damage because of multiple transport pathways. However, such a network would incur a large material cost of construction per unit area of explored space, and would cover new ground slowly. Conversely, a sparse system could extend further for the same material cost, but would have fewer alternate routes and therefore lower resilience to disconnection. Individual species operate slightly different local growth and connection algorithms and form visually distinct network structures. Subtle shifts in the developmental process for different species may have effectively weighted the relative importance of each of these aspects to adapt to different spatial and temporal patterns of resource availability and environmental conditions, giving rise to a set of foraging strategies for each species [10]. We do not know what factors have been most significant in shaping each strategy in evolutionary terms [21] or how organisation and integration of such networks can emerge without centralised control [22, 23, 24]. However, we might envisage that the resulting networks represent an effective balance between cost, exploration, exploitation, transport efficiency and resilience to damage.

2. Application of network analysis to fungal mycelia

Filamentous fungi grow out from a spore or an inoculum, by apical extension of slender hyphae (Fig. 1A) that then branch sub-apically to form a fractal, tree-like mycelium. In ascomycetes and basidiomycetes, tangential hyphal fusions or anastomoses occur as the colony develops to form an interconnected mycelial network [23, 24, 25, 26]. In the larger, more persistent saprotrophic and ectomycorrhizal basidiomycetes, the network architecture develops further as the colony grows with the formation of specialised high-conductivity channels, termed cords [27]. These form visible networks on a scale of centimetres in laboratory microcosms (Fig. 1B) to metres in undisturbed woodland (Fig. 1C). Cords are able to translocate nutrients between separate food resources or to and from the foraging margin [10, 14]. In *P. velutina*, cords form through aggregation and limited differentiation of hyphae (Fig. 1D) to give roughly parallel bundles (Fig. 1E) that we currently model as a cylinder packed with uniform pipes (Fig. 1F). At the same time as cords form, the intervening regions of the mycelium regress (Fig. 1G) and are recycled to support new growth [28]. The resulting structure and scale of the corded network vary between species, and give rise to qualitatively different, recognisable network architectures (Fig. 2).

The network topology is defined by classifying junctions (branch-points and anastomoses) as nodes, and the cords between nodes as links. At the microscopic

Fig. 1 Development of mycelial networks. (A) Bright-field image of hyphae of *Phanerochaete velutina* growing across agar. (B) Mycelial system of *P. velutina* grown from 4 cm³ beech wood inocula on a 24 × 24 cm tray of compressed, non-sterile soil after 39d. (C) A 75 × 75 cm portion of an extensive network of the saprotrophic fungus *Megacollybia platyphylla* interconnecting dead wood resources in Wytham Wood, Oxfordshire, UK. (D) Time lapse imaging of cord-formation through hyphal aggregation in a growing colony of *P. velutina* on compressed sand/soil. (E) Scanning electron micrograph showing the aggregation of hyphae in a cord. (F) Schematic representation of a cord illustrating the length (l) and area (a) measures used to weight each link. (G) Time lapse imaging of cord-regression and thinning-out of the network in a growing colony of *P. velutina*.

level, semi-automated image analysis routines have been employed successfully to extract the network [17]. However, at the macroscopic level, manual delineation of the network is currently required to resolve the numerous ambiguities in the complex structures analysed. This process is time consuming and adds a subjective component to the initial network structure. A validation step is routinely included in which the network is checked by a second individual.

As the thickness of the cords varied markedly with development of the network, the cord diameter, and hence cord area a , was estimated by image analysis. The internal structure was modelled as a cylinder packed with identical hyphae as a first approximation (Fig. 1F), although it is recognized that cord anatomy can be more complex [30]. The weighted networks were visualised as pseudo-colour representations to emphasize the change in diameter of particular cords over time. Reinforcement increased cord diameter and shifted the hue to the red end of the spectrum, whilst thinning out by regression shifted the hue towards blue (Figs. 3A and 3B). As there was considerable natural variation in the growth pattern of fungal net-

Fig. 2 Colony morphology and network architecture of different species of saprotrophic basidiomycetes. Mycelial systems of (A) *Resinicium bicolor*, (B) *Phane-rochaete velutina*, (C) *Hypholoma fasciculare*, (D) *Coprinus picaceous*, (E) and (F) *Phallus impudicus* growing from wood blocks of 2 cm side in 24×24 cm trays of compressed non-sterile soil. Photos courtesy of George Tordoff. Reproduced from [29] with permission.

works even under controlled conditions in microcosms, experiments were conducted at least in triplicate (Figs. 3B-D). These maps provide some indication of the general similarities between replicates, but also highlight the qualitative difference in hyphal density for example.

The material construction cost of each link was estimated from the volume ($l \times a$), i.e., longer, thicker cords were more costly to produce in terms of the mass of material required to build them. Similarly, the predicted transport flux through the network would be expected to increase with increasing cross-sectional area, but decrease with link length. The precise relationship between flow and area is less clear. Using the simplified model of the cord structure as a cylinder (Fig. 1F), flow would be expected to scale with the cord area, i.e., r^2 , rather than r^4 predicted by the Hagen-Poiseuille equation for a single tube of increasing radius.

It might be appropriate to consider the links to be directed on the basis of their initial growth direction. However, in practice, the physiological direction of nutrient fluxes is more important and does not have to follow the developmental connection sequence. Unfortunately, we cannot predict *a priori* which direction the flux may move in and, indeed, we expect it to vary depending on the source-sink relationships within the network. Thus at this stage it is assumed that links are undirected and capable of moving nutrients in either direction. The resultant networks represent the maximum possible set of routes that nutrients could flow through the network. In practice, not all the links may be functional. In the case of the woodland *Armillaria* networks, functional connectivity was demonstrated for some rhizomorph segments by forcing air through the lumen of rhizomorphs [18]. In the *P. velutina* networks, there is no equivalent air conduit within cords, so functional connectivity is more difficult to assess. We return to this point later when considering experimentally measured transport within the network using radiotracers.

Fig. 3 Delineation of the weighted network for colonies of *P. velutina*. (A) Mycelial system of *P. velutina* growing from a 4 cm³ beech wood block (*I*) in a 24 × 24 cm tray of non-sterile soil in the presence of an additional wood resource (*R*) and photographed at intervals up to 39d. (Digital images were obtained from photographs taken by Rory Bolton). (B) Network representation of the same colony shown in (A) in which links are pseudo-colour coded by $\log_{10} a$ on a rainbow scale with red representing thick cords and blue representing thin cords. The positions of wood blocks are highlighted by white squares. (C and D) pseudo-colour coded networks from two other replicate experiments. Panel A and C reproduced from [19] with permission.

Fig. 4 Network measures for colonies of *P. velutina*. Mycelial systems of *P. velutina* were grown from 4 cm³ beech wood blocks in 24 × 24 cm trays of non-sterile soil in the presence (◇) and absence (○) of additional wood resources and photographed at intervals up to 39d. The weighted network was manually extracted from the digitised images and a range of network parameters calculated for three independent time-series. Each point represents the mean ± s.e.m. for (A) the number of nodes, (B) the total cost of the mycelium, (C) the mass density, taking into account the varying diameter of the cords, (D) the box-count mass fractal dimension, (E) the clustering coefficient, and (F) the alpha index measuring the proportion of fundamental cycles.

Early development in several species was characterized by initial diffuse growth and branching of individual hyphae, which then resolved into cords as the growing front moved outward (Fig. 3A). Thus global network size measures, such as number of nodes, number of links and the total material in the network, increased through time (Figs. 4A and 4B). However, the local scale network evolution was characterized by selective loss of connections and thinning out of the fine mycelium and weaker cords, which gave rise to a decrease in the network density (cost per unit area) with increasing time (Fig. 4C). This behaviour was also apparent in the box-count mass fractal dimension of these networks [31], which also showed a decrease as the

Fig. 5 Network evolution. Mycelial systems of *P. velutina* were grown from 4 cm³ beech wood blocks (*I*) in 24 × 24 cm trays of non-sterile soil in the presence of an additional wood resource (*R*) and photographed at intervals up to 39d. Images are shown for (A) 9d and (B) 39d. The link evolution (*LE*) was estimated from the growth trajectory of each link as the sum of the incremental change in area divided by the difference between maximum and minimum area for each cord. Results are plotted using a rainbow pseudo-colour scale in which consistent growth ($0 < LE < 1$) was coded red, varying through no change ($LE = 0$) coded green to a decrease ($-1 < LE < 0$), coded as blue.

networks thinned out (Fig. 4D).

The growth trajectory of each link was summarised by measures that captured the time-dependent changes, such as polynomial regression of link diameter over time. The behaviour of each link was then described relatively succinctly by the polynomial coefficients. Alternatively, a single measure of link evolution was defined as the sum of the incremental changes in diameter divided by the difference between the maximum and minimum for each link ($\sum \Delta D / (D_{\max} - D_{\min})$) to give a simple normalised measure of whether links were growing consistently ($LE = 1$), remaining constant ($LE = 0$), or regressing ($LE = -1$). In the example shown in Fig. 5, most of the dense growth present at the first time point (Fig. 5A) declined and was therefore coded blue. A limited number of cords from this stage persisted with little change in size, and were therefore coded green, whilst the new growth (Fig. 5B) was coded yellow to red.

3. The utility of topological network measures

The degree distributions for nodes in fungal networks are not very informative [17, 19, 18], as with other spatial networks [32], because of the constraints imposed by the developmental process and crowding effects restricting the maximum number of possible connections. Thus hyphal tips are $k = 1$ and branch points are typically $k = 3$, because the growth processes forming the network tend to give a single branch or a single fusion at each point. Cords that initially overlap will often subsequently fuse, which generates $k = 4$ nodes. It is unlikely that there will be any loops where a link curls back round on itself to re-join the same node, although multiple parallel links between two nodes are possible. As the fine structure of the mycelium within a food resource cannot be resolved, each of these is represented as a node with many links, resembling a hub in other network systems. During digitisation, additional temporary nodes are required to track curved segments. These temporary $k = 2$ nodes are stripped out in the subsequent analysis, whilst retaining the intervening cord lengths, predicted transport resistance and cost. In weighted networks, the weighted degree distribution or node strength, measured as the sum of link cross-sectional areas per node, is regarded as a more informative measure than node

degree, to take into account the varying importance of the connections to each node [33, 34]. For the *P. velutina* networks, the frequency distribution of node strength followed an approximately log-normal distribution [19].

The next level to consider after individual nodes and their summary statistics, is the properties of their local neighbourhood. There are several measures that describe how well each node and its neighbours are interconnected. The mean clustering coefficient, C [35] was not particularly appropriate for fungal networks, as with other planar network, as their construction effectively precludes formation of triads (Fig. 4E) [36, 37, 38]. In practice, the fungal network formed an interconnected reticulate system with many 4-, 5- or 6-node rings spreading away from the central inoculum. More general measures have been proposed to capture the structure of larger cycles [39, 40]. The meshedness or alpha coefficient [1] provides a simple measure for planar graphs and has recently been used to characterise networks of ant galleries and road networks [36, 37, 38]. The shift from essentially a radial tree during early growth to a more reticulate network with increased cross-linking was reflected in an increasing value of the alpha index (Fig. 4F). Thus these fungal networks progressed from a branching tree to a weakly connected lattice-like network behind the growing margin, through a process of fusion and reinforcement to form loops, and selective removal and recycling of excess redundant material [19]. There was some evidence for slightly higher values of both the clustering coefficient and the alpha coefficient in networks with added resources, most probably as these networks appeared to thin out the redundant mycelium more rapidly (Figs. 4E and 4F). The values of the alpha index for *P. velutina* were similar to those for networks of tunnels in ant galleries [36] and street networks in cities [37, 38], suggesting that addition of around 20% of the maximum number of cross-links into a planar network may be sufficient to achieve desirable network properties in a range of different scenarios.

4. Predicted transport characteristics of the mycelial network

To investigate the transport capacity of the network, the resistance of each link was estimated, assuming that nutrient fluxes would be determined not only by the physical length of links in the path, but also by their cross-sectional area (a), with thick connections having lower net resistance than thin ones. Thus, the resistance of a link was defined as its length divided by its cross-sectional area (la^{-1}). The path of least resistance between each node in the functional network, was then calculated using a shortest path algorithm [41, 42], with the assumption that low node-to-node resistance indicated better connection and more efficient nutrient distribution.

Typical metrics used in network analysis are based on the shortest distance between any two nodes, and summarised as either the average, to give the average shortest path (or characteristic path length), through the network, or as the longest shortest path to give the network diameter. A small average shortest path or low diameter, imply that it is easy to transport material anywhere in the network. However, in weighted mycelial networks, the network diameter was a highly unstable measure of network size as it was extremely sensitive to thin links with very high resistance at the mycelial margin that were not biologically very significant. Thus, we have not found this a useful network statistic, even for comparison with networks from other domains.

Whilst the average shortest path provides a convenient metric to compare the performance of different networks, it is sensitive to disconnection of part of the network when the shortest path tends to $+\infty$. This problem is circumvented by the concept of average network efficiency (E), defined as the mean of the reciprocal of shortest path lengths [43, 44]. With this measure, the shortest path contribution from disconnected nodes is effectively zero. In isolation, neither the mean shortest path nor the average efficiency were very informative for fungal networks. However,

Fig. 6 Comparison of weighted fungal networks and neighbourhood graphs. *P. velutina* was grown from a wood block inoculum over compressed soil in the presence of an additional resource and the weighted network digitised at 9, 18, 25, and 31 d. (A) The weighted fungal network, in which line thickness and intensity indicate the relative cross-sectional area of each cord. (B) A simplified version of the network that retains nodes arising from branching or fusion, but not nodes simply required to trace the outline of each cord correctly. The amount of material present in the network is distributed evenly across all links to give a uniform network. The nodes present in the simplified graph were then connected according to well-defined rules to give (C) the minimum spanning tree, (D) the relative neighbourhood graph, (E) the Gabriel graph, or (F) the fully connected Delaunay triangulation.

comparison of these metrics with reference networks was more valuable. Null models in other areas of network analysis are often generated by randomising links in the network. However, this approach is not appropriate to these types of planar fungal network as there is no biological meaning associated with a randomised fungal network. There is also currently no procedure to generate a randomised network that varies the link strength as well as the topology. We therefore developed a two stage

Fig. 7 Comparison of transport efficiency between weighted fungal and uniform model networks. (A) Plots of the global Euclidean efficiency (mm^{-1}) against network area (mm^2) for Delaunay Triangulation (cyan squares), fungal network (red diamonds) and Minimum Spanning Tree (green triangles). The fungal networks have intermediate global Euclidean efficiency. Data points are from six experiments including three with additional added wood resources. (B) Plots of the root Euclidean efficiency, measured from the inoculum to all other nodes. The fungal networks have similar efficiency to the well-connected Delaunay Triangulations. (C) Plots of the global functional efficiency (mm) against network area (mm^2) for the fully weighted fungal network (black circles) and the uniformly weighted fungal network (red squares), MST (green triangles) and DT (cyan squares). The Minimum Spanning Trees have the greatest functional efficiency. Fungal networks are intermediate. (D) Plots of the root functional efficiency, scales and symbols as in C. The fungal weighted networks have the greatest root functional efficiency. Redrawn from [19].

procedure to evaluate the performance of the fungal networks. In the first step, comparisons of the Euclidean fungal network (Fig. 6B), based on link length alone but excluding the effect of varying link area, were made with model networks constructed using well defined neighbourhood graphs, including the minimum spanning tree (MST: Fig. 6C) as a lower bound giving a low cost, but extremely vulnerable network, the relative neighbourhood graph (RNG: Fig. 6D), Gabriel graph (GAB: Fig. 6E) and the Delaunay triangulation (DT: Fig. 6F), giving an upper bound for a well-connected, robust, but rather expensive network [36, 45, 38, 46]. Visual inspection of the resultant networks suggested that the topology of the fungal network had some similarity to the RNG, in terms of the density of cross-linking outside of the inoculum itself (Fig. 6D). Quantitatively, the RNGs had an alpha coefficient of ~ 0.12 , slightly lower than the alpha coefficient of the fungal networks. It was also apparent that regression of some links triggered substantial re-arrangements in

the layout of the model networks, particularly for the MST, which showed dramatic alteration in the connections between neighbouring nodes over time (Fig. 6C).

In general, the ‘Euclidean’ efficiency declined as the colonies grew and covered a greater physical area (Fig. 7A). The fully connected DT performed the best, as it was maximally connected, whilst the MST performed the worst as it had no loops permitting shortcuts. In addition to comparison of the efficiency of the whole network, the local efficiency was determined just from the inoculum (or “root”) [46] to all other nodes for the different network configurations. Local efficiency is perhaps most useful in the early stages of development as the central inoculum is the biologically-relevant source of nutrients supplying the rest of the mycelium. Using this local efficiency measure, the fungal network performed as well as the DT, and markedly better than the MST (Fig. 7B). Thus, at this point we conclude that the organisation of the fungal network achieves the same predicted transport efficiency from the inoculum as a fully connected network would, but with lower cost.

In the second step of analysis, the effect of including a fixed amount of material in the network, equivalent to the total material in the real network (Fig. 6A), was examined. Thus, each link in the ‘uniform’ fungal and model networks was allocated a constant weight, such that the total construction cost was the same. Effectively we asked what the consequences for transport would be if the fungus had chosen to allocate the same amount of resource evenly over the existing or model networks, to determine the functional efficiency of the network. This also allowed comparison with the real, differentially weighted network (Fig. 6A) as the network measures were in comparable units. A very different picture emerged with these starting assumptions in comparison to the networks that just had Euclidean weighting. Across the whole colony, the DT now performed poorly, as distributing material across the large number of links present gave each link a low area and consequent high resistance (Fig. 7C). Conversely, the MST performed well as it was populated with few, but extremely thick, links (Fig. 7C). The uniform and real fungal networks were almost equivalent in performance and were again positioned between the two extremes.

We calculated the relative global efficiency (E_{glob}) of the real network, uniform network and MST by normalising the efficiency to that of the fully connected DT [43, 44], and found values of 1.71 ± 0.11 , 1.59 ± 0.06 , and 2.97 ± 0.13 , respectively. Perhaps the most surprising result was when the local efficiency of the weighted networks from the inoculum to every other node was considered (Fig. 7D). The DT again performed badly, the uniform fungal network and MST were both about two-fold better, but the real network outperformed all of them by a considerable margin (Fig. 7D). Again, by normalising to the DT, the local efficiency (E_{loc}) of the real network, uniform network and MST were calculated as 4.4 ± 0.11 , 2.22 ± 0.07 , and 2.08 ± 0.12 , respectively. Thus, differential weighting of links in the real network gave a > 4 fold improvement in local efficiency in comparison to a fully connected uniform network constructed with the same total cost. We note that, although we have used a global measure to characterise the performance of the fungal network, the biological system achieves this level of performance without centralised control by implementation of local rules.

5. Centrality measures

Whilst calculating the local efficiency for a defined node, such as the inoculum, makes biological sense, there are several other measures to estimate the importance of any node or link to the overall transport. For example, the shortest-path betweenness centrality (SPBC) measures the number of shortest paths that pass through each node or link [47, 48]. Conversely, the (shortest-path) closeness centrality (SPCC), calculated as the inverse of the average shortest path to other nodes, reflects how easy it is to get from the chosen node to all others.

Fig. 8 Normalised shortest path betweenness centrality maps. The shortest path betweenness centrality (SPBC) for each link was measured for (A) the fully weighted network or (B) weighted by cord length alone, and plotted on a pseudo-colour scale normalised to the maximum betweenness centrality ($SPBC_{max}$) at each time point.

Maps of SPBC for each link (Fig. 8) revealed subtle shifts in the predicted transport performance of the network as it grew. For example, in the fully weighted network the relative importance of particular links between the inoculum and added resource fluctuated in the early stage of growth until a dominant pathway was established (Fig. 8A). Thus at 18d, two cords carried almost equal ($\sim 70,000$ – $90,000$) numbers of shortest paths between the inoculum and added resource. By 25d, the upper cord has become dominant nearest the inoculum and the lower cord dominant nearer the bait. After a further 6d, the upper cord dominates completely.

The effect of link weighting was also apparent from the difference between SPBC calculated using the cord areas in the weighted network (Fig. 8A) compared to the Euclidean case, based solely on cord length (Fig. 8B). Thus in the Euclidean network, shortest paths were distributed over a greater number of routes, reflected in the lower value of the maximum betweenness centrality measured for any individual link. Furthermore, the routes differed from those used in the fully weighted network. The SPBC maps also highlighted one consequence of using a shortest-path approach to predicting transport performance, namely comparable parallel pathways that were marginally longer did not feature prominently in the analysis. For example, there was a single cord between the inoculum and resource that carried the vast majority of the shortest-paths in the last two time points (Fig. 8A). However, there were at least two other parallel paths that were only marginally longer that might be expected to participate in transport in a real system.

The contribution of parallel pathways can be accommodated using centrality measures based on predicted flow through the network [49], which is effectively recast as an electrical circuit analogue [50]. With this in mind, we have started to use tools for solving current flow through complex resistor networks [51, 52]. The weighted adjacency matrix from the mycelial network was already in an appropriate form to be analysed as an electrical circuit analogue, with the additional constraint that flux (current) through the network can only be calculated if a driving force (voltage) was applied to certain points in the network. This allowed “what-if” type examination of the expected flux as source-sink patterns change. For example, a map of the predicted current flow from the inoculum to the hyphal tips was calculated when a voltage was applied at the inoculum and all the tips were grounded (Fig. 9). Biologically it may be more appropriate to set each tip as a constant current sink,

Fig. 9 Current-flow network model. Network representation of a *P. velutina* colony at 39d, in which links are pseudo-colour coded by the log of the current on a rainbow scale, with red representing high current and blue representing low current. Currents were calculated using Wu's algorithm [51], implemented by Hankin [52]. A fixed voltage was applied to the inoculum, and the tips ($k = 1$ nodes) were earthed.

representing a constant hyphal growth rate, and back calculate the current and potentials across the array. To calculate the current-flow betweenness centrality (CFBC) requires summation of the predicted flows across all node pairs [50, 48]. The equivalent current-flow closeness centrality [50] is identical to the earlier measure of information centrality [47, 49]. Recently the concept of delta centralities, that measure the change in network efficiency upon deletion of a node, have also been described as a measure of information centrality, although in this case they are based on shortest-path measures rather than current flow [48].

Given the biological transport system is likely to operate at least partially as a parallel flow system, the current-flow variants of betweenness and closeness centrality may well be the preferred metrics to capture the behaviour of the network, and will clearly be an important area for future research.

6. Comparison between predicted transport and experimental transport

In parallel to the theoretical network analysis, we have developed methods to image nutrient movement directly in these microcosms by tracking the movement of the non-metabolised amino-acid analogue, ^{14}C -amino isobutyrate (^{14}C -AIB) using photon-counting scintillation imaging (PCSI) [53, 54, 55, 56]. We were therefore interested to compare the predictions made by the theoretical network analysis to the actual pattern of nutrient movement in the same microcosms. Networks were allowed to develop in microcosms for $\sim 45\text{d}$ (Fig. 10A) and the weighted network digitised (Fig. 10B) and analysed to give the link evolution (Fig. 10C) and the SPBC (Fig. 10D). ^{14}C -AIB was added at the end of the growth period and imaged using PCSI to map nutrient movement (Fig. 10E). The pre-determined topological network was then superimposed on the ^{14}C -AIB image to extract the amount of AIB present in each link (Fig. 10F). The integrated ^{14}C -AIB intensity was extracted, representing a measure of the amount of ^{14}C -AIB present rather than the flux itself.

Fig. 10 Functional analysis of network transport. Bright-field images of *P. velutina* growing from a beech-block inoculum to a set of additional resource blocks over compressed soil were obtained at $\sim 3d$ intervals for 45d. A subset of the images is shown in (A). Branch points and anastomoses were manually coded as nodes connected by links, and the cord diameter estimated by image analysis, to give a weighted network (B) in which thick cords are represented in red and thin cords in blue. Various network parameters were calculated including link evolution, based on linear regression of the change in link weight with time and colour-coded by gradient of the regression equation (C) and shortest-path betweenness centrality, measured as the number of shortest paths passing through each link (D). To compare the predicted transport properties of the network with actual transport, ^{14}C -AIB movement was mapped by photon-counting scintillation imaging (PCSI) at the end of the time-series (E) and the amount of AIB present in each link extracted using the digitised network (F). The distribution of AIB was then compared with link area at the last time point (G), link evolution (H) or link betweenness centrality (I). Redrawn from [57].

Ideally we would like to calculate the total flux through each link using knowledge of the amount of ^{14}C -AIB appearing further downstream. However, this is challenging as it requires assumptions about the flow pathway to reallocate the AIB signal correctly. Nevertheless, as a first approximation we have compared ^{14}C -AIB maps with various network parameters such as final link weight (Fig. 10G), link evolution (Fig. 10H), based on linear regression of the change in link weight with time, and SPBC (Fig. 10I). A number of different populations of links were identified. The

most prominent were a cluster with high ^{14}C -AIB but low SPBC, corresponding to the tips where the ^{14}C -AIB accumulated. For the other links there was some degree of correlation between the AIB distribution and the network parameter. Equally, the AIB pattern did not always match expectations. For example, there was no obvious reason why there should be substantial accumulation on the right-hand side of the colony, or little apparent transport to the added resource or beyond (Fig. 10E) based on the final link weight (final panel of Fig. 10B), link evolution (Fig. 10C) or SPBC (Fig. 10D). There are clearly additional features governing the control of nutrient distribution that cannot be captured by simple predictions of flow based solely on network measures or shortest path calculation.

7. Predicted resilience of mycelial network

Whilst the architecture of the weighted network clearly improved transport efficiency in comparison to the even growth model or toy networks, in nature the same system also has to resist accidental damage or targeted attack by invertebrate grazers. The vulnerability of the network was examined by measuring the effect of link breakage on efficiency (E). This is more appropriate than just considering changes in the mean shortest path as it can handle disconnection of parts of the network. In other network analyses it is often the nodes that are attacked. However, for fungal mycelia, link breakage has more biological relevance (Fig. 11). We tested two models of link breakage. In the first, we assumed that the probability of breakage increased with link length, but was independent of cord thickness (Fig. 11A). This would correspond to damage or attack based solely on the probability of encounter. In the second, we assumed the probability of breakage decreased with the cord thickness as well as length, i.e., long, thin links were more likely to break than short, thick ones (Fig. 11B). As with the transport efficiency measures, we compared the robustness of the differentially weighted network to the uniform models, in which all links were assigned the same thickness to give a constant overall cost. The robustness was defined as the fraction of the total material cost of the network that remained connected to the inoculum when a certain fraction of the total link cross-sectional area was broken.

As expected, the MST fell apart very quickly, with 50% of the mass of the network becoming disconnected after $\sim 10\%$ of the links had been removed (Fig. 11C). Conversely, the fully connected DT was more robust than the MST and fungal networks when attack was proportional only to link length (Fig. 11C). However, the fungal networks were more robust to link removal than any of the uniform models, when link area was also taken into consideration (Fig. 11D). The real network therefore matched the DT until $\sim 40\%$ of link area had been removed, but then maintained a stronger connected core that was robust to damage following removal of a considerable proportion of link area (Fig. 11D). Thus, the differential strengthening of a sub-set of links that imparted high local transport capacity also conferred robustness, particularly to targeted attack in the developing mycelial network. This suggests that the same core architecture that gives the network good predicted transport properties also gives it good resilience. It is also important to note that part of the resilience of fungal networks lies not only in the architecture of the network prior to damage, but also in the ease and efficiency with which the network can reconnect itself following attack. In this respect, a self-organising spatial network may have considerable advantages over a random network in the cost, consistency and efficacy of the rewiring process needed to re-establish a functioning system.

8. Future prospects

Characterisation of mycelial networks is still in its infancy. However, the network approach provides a way of quantifying and analyzing complex fungal systems for

Fig. 11 Comparison of the resilience of the weighted fungal network in comparison to uniform model networks. (A) Illustration of the effect of removing links above a given length on the structure of the network. (B) Illustration of the effect of removing links above a given resistance on the structure of the network. (C) The amount of material remaining associated with the central inoculum (core size) was determined as links were removed in order of their length for the Delaunay Triangulations (cyan squares), fungal networks (red diamonds) and Minimum Spanning Trees (green triangles). Fungal networks had intermediate robustness to damage when only link length was taken into consideration. (D) The core size was determined when the probability of link deletion was proportional to the link length and inversely proportional to the link cross-sectional area for the real fungal network (black circles), the uniform fungal network (red squares), Delaunay Triangulations (cyan squares), and Minimum Spanning Trees (green triangles). When more than $\sim 30\%$ of total link area was broken, the weighted fungal networks maintained a greater connected core than other types of network. Panels C and D redrawn from [19].

the first time, and also makes it possible to link measurements in microcosms in the laboratory to observations of networks in the field. The simple models predicting transport through such networks, so far based on shortest path considerations through the weighted network, only capture part of the experimentally determined transport behaviour. Models that include parallel-flow pathways and evolution of the network should improve the match between simulation and experiment, and will benefit from the recent advances in fast algorithms to calculate the necessary metrics. The next conceptual advance will be to identify the rules that allow the network to iteratively refine its structure and transport behaviour to yield the net-

work architectures observed. It is conceivable that the identification of such rules will allow development of generic “fungal colony optimisation” algorithms similar to those that have evolved from the study of ant colony foraging patterns [58] in the emerging field of biologistics [59].

Acknowledgements

Research in the authors' laboratories has been supported by BBSRC (43/P19284), NERC (GR3/12946 & NER/A/S/2002/882), EPSRC (GR/S63090/01), EU Framework 6 (STREP No. 12999), Oxford University Research Infrastructure Fund and the University Dunston Bequest. We thank A. Ashford, K. Burton, P. R. Darrah, D. P. Donnelly, D. Eastwood, J. Efstathiou, J. Hynes, N. Johnson, F. Reed-Tsochas, M. Tlalka, G. M. Tordoff, S. C. Watkinson and members of CABDyN for stimulating discussions.

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