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Review

Analysis of fungal networks

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ABSTRACT

Mycelial fungi grow as indeterminate adaptive networks that have to forage for scarce resources in a patchy and unpredictable environment under constant onslaught from mycophagous animals. Development of contrast-independent network extraction algorithms has dramatically improved our ability to characterise these dynamic macroscopic networks and promises to bridge the gap between experiments in realistic experimental microcosms and graph-theoretic network analysis, greatly facilitating quantitative description of their complex behaviour. Furthermore, using digitised networks as inputs, empirically-based minimal biophysical mass-flow models already provide a high degree of explanation for patterns of long-distance radiolabel movement, and hint at global control mechanisms emerging naturally as a consequence of the intrinsic hydraulic connectivity. Network resilience is also critical to survival and can be explored both *in silico* by removing links in the digitised networks according to particular rules, or *in vivo* by allowing different mycophagous invertebrates to graze on the networks. Survival depends on both the intrinsic architecture adopted by each species and the ability to reconnect following damage. It is hoped that a comparative approach may yield useful insights into not just fungal ecology, but also biologically inspired rules governing the combinatorial trade-off between cost, transport efficiency, resilience and control complexity for self-organised adaptive networks in other domains.

1. Network development in mycelial fungi

Mycelial fungi and plasmodial slime moulds (myxomycetes) form elaborate interconnected networks that are highly responsive to local environmental conditions. Unlike other biological transport networks such as plant or animal vascular systems, the network formed by these organisms is not *part of* the organism, it *is* the organism. These networks develop as

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the organism forages for new resources in a patchy resource environment and must both transport nutrients between spatially separated source and sink regions, and also maintain the network integrity in the face of predation or random damage (Selosse *et al.*, 2006; Bebber *et al.*, 2007b; Boddy and Jones, 2007; Fricker *et al.*, 2008a, 2009; Boddy *et al.*, 2009). Filamentous fungi grow by apical extension of slender hyphae that then branch apically or sub-apically (Harris, 2008) to form a fractal, tree-like mycelium (Boddy and Donnelly, 2008). Ascomycetes have septa with pores which allow cytoplasmic continuity and organelle movement across the intact mycelium (Lew, 2011), but which can be quickly blocked by Woronin bodies or organelles following damage (Jedd, 2011). Likewise, septa in basidiomycetes are perforated by a central pore so the cytosol can flow between adjacent cells, but the dolipore structure prevents nuclear movement. Extensions of the ER forming the septal pore cap (SPC) can cover the pore to restrict leakage following damage (Jedd, 2011). The combination of tip growth and branching allows fungi to explore complex physical environments using a range of efficient, but species-specific space searching algorithms (Hanson *et al.*, 2006; Held *et al.*, 2011). In ascomycetes and basidiomycetes, tangential hyphal fusions or anastomoses occur behind the colony margin to form an interconnected microscopic mycelial network (Rayner *et al.*, 1994, 1999; Read *et al.*, 2009, 2010).

In the larger, more persistent saprotrophic and ectomycorrhizal basidiomycetes that are able to grow out into soil from colonised food sources, the network architecture develops further with the formation of specialised high-conductivity organs, termed cords or rhizomorphs (Cairney, 1992, 2005). The extent to which mature cords have a clear structure varies between species, but the internal anatomy has similar features across the basidiomycetes (Rayner *et al.*, 1985; Cairney, 1992). The central inner medulla contains numerous rigid, hollow vessel hyphae with diameters from 10 to 15 μm and few septal pores that form a pathway for translocation (Cairney, 1992, 2005). In contrast, the outer medulla contains loosely packed hyphae which may be as little as 2 μm wide.

Cords are covered by an outer rind that inhibits exchange of water and solutes. As some regions of the mycelium expand and mature, other regions regress (Fig. 1), and the process of autophagy appears to be critical to enable fungi to forage effectively through recycling redundant material to support new growth (Olsson, 2001; Falconer *et al.*, 2005, 2007; Fricker *et al.*, 2007). This is particularly noticeable when new resources are encountered; cords interconnecting resources thicken, whilst mycelium emanating in other directions

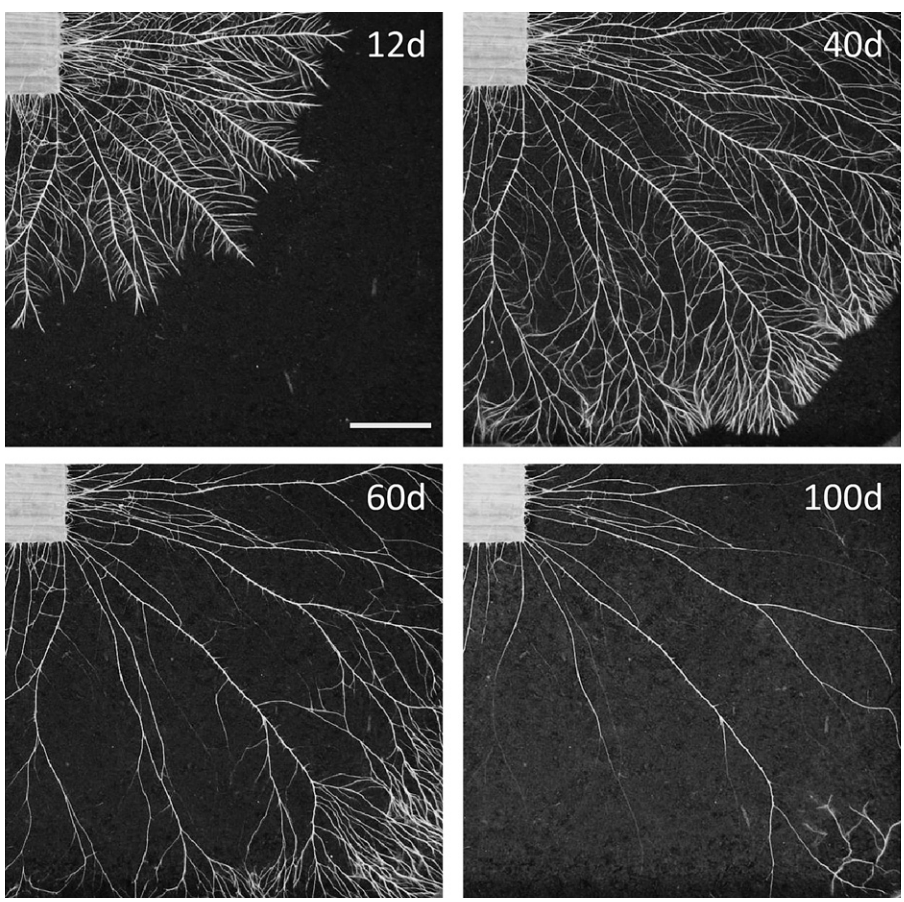

Fig. 1 – Development of *Phallus impudicus* growing from 2 × 2 × 1 cm beech wood block inocula across the surface of non-sterile soil at 18 °C at the times indicated. Initial colony growth forms a densely cross-linked network. Further expansion is fuelled in part by recycling of redundant material, eventually thinning out to a minimal skeleton as the resource is consumed. Scale bar = 2 cm. Images courtesy of AD A'Bear.

from the original resource regresses, to an extent depending on the species and the relative sizes of the old and new resources (Boddy, 1999; Fricker et al., 2008b). The corded systems resulting from these different processes form visible networks interconnecting food resources on a scale of centimetres in laboratory microcosms to metres in undisturbed woodland (Fricker et al., 2008a). Indeed, mycelial fungi form the most extensive biological networks so far characterised, popularly known as the Wood Wide Web (Smith et al., 1992; Simard and Durall, 2004; Southworth et al., 2005; Selosse et al., 2006; Lamour et al., 2007; Whitfield, 2007; Boddy and Donnelly, 2008; Beiler et al., 2010; Simard et al., 2012).

2. Visualisation of network structure and network extraction

Early measures of macroscopic mycelial organisation focussed on fractal dimension as a useful tool to capture aspects of the network structure as a metric (Boddy and Donnelly, 2008). However, such a single summary statistic only expresses a small fraction of the characteristics of the mycelial architecture. Tools from graph theory allow more detailed characterisation of the network structure and dynamics further and allow exploration of the underlying mechanism leading to network optimisation (see Section 3). This approach needs all branching points and linking cords to be digitised which, because of the large number of interconnections, requires (semi-)automated network extraction techniques.

Semi-automated image processing approaches that extract networks have been developed for mycelium grown on substrates, such as cellophane (Crawford et al., 1993; Hitchcock et al., 1996; Ritz et al., 1996), or nitrocellulose (Barry et al., 2009) to facilitate acquisition of high-contrast images. The subsequent processing procedure typically involves noise reduction, using median or band-pass filters, intensity-based thresholding, with manual or automated threshold selection, followed by morphological processing to achieve an improved skeletonisation of the colony structure e.g. (Tucker et al., 1992; Barry et al., 2009; Barry and Williams, 2011). However, these methods require rather specific culture conditions far removed from those of the natural environment, and illumination regimes that cannot readily be adapted for macroscopic networks grown in soil microcosms. Using the latter is crucial to explore the natural behavioural capability of these organisms and the full range of their ecological responses (Boddy, 1999). In addition, cords are multi-hyphal aggregates and show considerable variation in contrast as the individual cords span several orders of magnitude in diameter from μm to mm within a single colony, and have to be segmented from the background of compressed soil which is non-homogeneous in texture and reflectivity. To date, delineation of the network architecture from the larger soil-based systems has only been possibly manually (Bebber et al., 2007a; Fricker et al., 2007; Lamour et al., 2007; Rotheray et al., 2008; Boddy et al., 2010). As a result, the total number of macroscopic fungal networks analysed so far is relatively low.

Recently, Obara et al. (2012, in press, submitted for publication) developed a high-throughput automated image analysis approach to detect and characterise large complex fungal networks grown under realistic conditions. The first step in segmenting network structures is often to use matched filters based on the spatial properties of the object to locate its position from the ridges and ravines in the image intensity. The shape of the matched filter are often based on the second derivative of a Gaussian through the calculation of the local Hessian matrix. To detect features at different orientations, the filter is often rotated through a reasonable set of angles and the maximum response from the filter bank is calculated. In general, a tensor representation of an image, such as the Hessian, gives information about how much the image varies along and orthogonal to the dominant orientations within a certain neighbourhood (Knutsson et al., 2011). Inclusion of different weightings for the orthogonal directions and intensity components give measures such as vesselness (Frangi et al., 1998) or neuriteness (Meijering et al., 2004), that have been used to improve network-specific feature enhancement.

While tensor representations can be built on purely intensity-based filters, these are sensitive to changes in absolute intensity and image contrast. To reduce this problem, methods based on local phase have been proposed as a contrast-independent alternative to detect edges (Kovesi, 1999). Salient features have similar values of local phase when observed at different scales, thus phase congruency values are high in the direction perpendicular to the cords, while they remain close to zero in the direction parallel to the cords. More importantly, the values of phase congruency are minimally affected by contrast changes. Thus the phase congruency tensor (PCT) can be used to improve significantly on intensity-based tensors to give PCT-vesselness or PCT-neuriteness enhancement algorithms (Obara et al., 2012, in press).

Following the selective enhancement arising from the PCT approach (e.g. Fig. 2), the network can be segmented rapidly using a watershed transform. Normally the basins in the watershed image represent the objects of interest. However, in the case of fungal networks the watershed boundaries themselves delineate ridges in the image that correspond to the network branches of interest, and also guarantee extraction of a fully-connected network as they form closed loops. The resulting skeleton is then pruned on the basis of local cost functions that incorporate both intensity and tensor direction information (Fig. 2). A graph representation of the network is constructed from the pruned skeleton with link weights based on the Euclidean lengths and the link diameter derived from sampling the local intensity, with appropriate calibration, to give each link a weight that depends on its length (l) and cross-sectional area (a). Each cord is modelled as a cylinder packed with identical hyphae, rather than a single tube that increases in diameter, although the internal structure of cords can be much more complex (Rayner et al., 1985). This approach provides a rapid, robust and extremely effective means to extract fungal networks with up to 10^6 links from even low-contrast, noisy images compared to $<10^4$ links possible with manual digitisation (Obara et al., submitted for publication) (Fig. 2).

3. Characterisation of the network

Networks extracted using image analysis are strictly a subsample of the full network. The improved image analysis

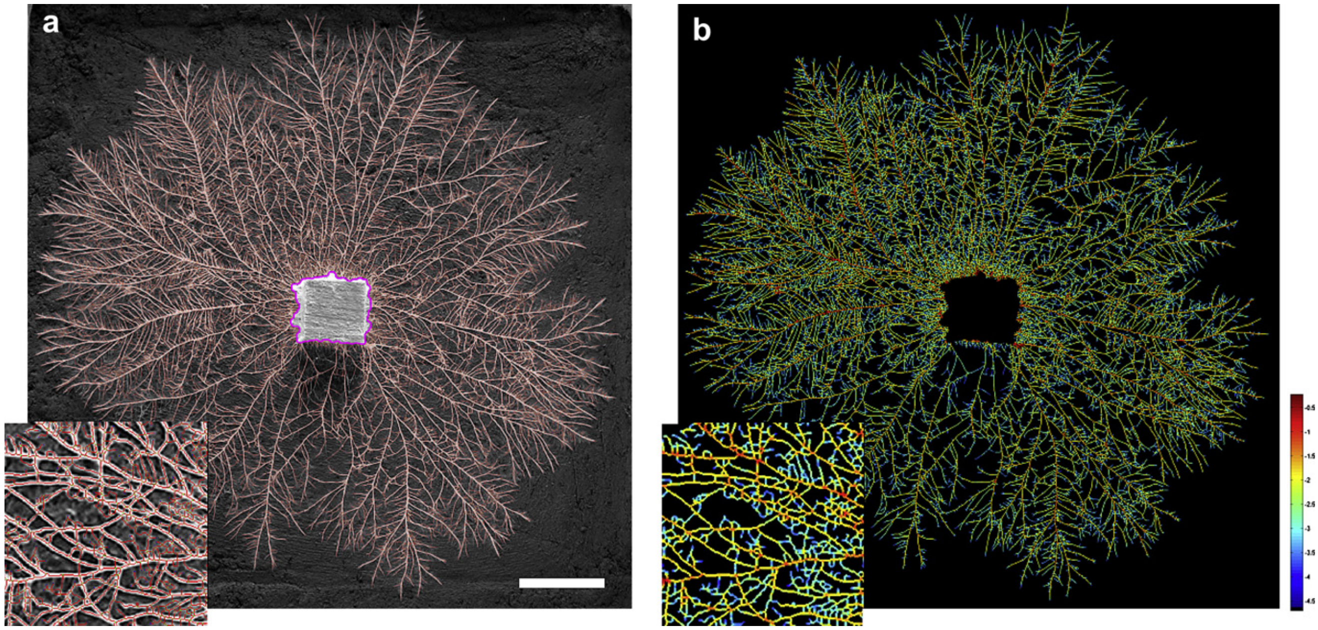

Fig. 2 – Automated network extraction from a colony of *Phallus impudicus* grown on compressed soil from a wood-block inoculum after 20 d. (a) Extracted network and the source of food overlaid on the input image with the centre line (red), branching points (green), endpoints (blue), and food source boundary (magenta). (b) Network with $\sim 10^6$ links pseudo-colour coded for cord thickness. Scale bar corresponds to 2 cm.

techniques described above yield more complete sampling of the network architecture down to the level of relatively minor cords, but still cannot resolve individual fine hyphae. It is know that topological properties of networks and their samples can differ (Stumpf *et al*., 2005). There are geometrical reasons to expect that subsamples of planar graphs will inherit more of the properties of the full graph, nevertheless potential sampling issues still have to be borne in mind when considering the results described below.

The network topology is defined by classifying junctions (branch points, anastomoses and the food sources) as nodes, and the cords between nodes as links. In general, during foraging the number of nodes, number of links and the total material in the network, increase through time. However, the local scale network evolution is also characterised by selective loss of connections and thinning out of the fine mycelium and weaker cords (Fig. 1). Thus, fungal networks progress from a radial 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 redundant material (Bebber *et al*., 2007a).

This shift can be quantified by the alpha coefficient (α) or meshedness (Haggett and Chorley, 1969; Buhl *et al*., 2004), that gives the number of closed loops or cycles present as a fraction of the maximum possible for a planar network with the same number of nodes, according to Euler's polyhedral formula (e.g. Barthelemy, 2010). The alpha coefficient measured for *Phanerochaete velutina* tends to increase from near 0, as expected for a branching tree, to around 10–20 % as systems become more cross-connected, depending on the resource environment (Bebber *et al*., 2007a). There is considerable variation in the values of α for different species, with values generally matching the observed qualitative level of cross-linking. However, this measure, like many other graph-theoretic network metrics, can be heavily skewed by parts of the network that are perhaps less relevant when considering the macroscopic architecture. Thus, the extent and level of detail from the peripheral growing region or fine mycelium that is included in the analysis can affect α. Nevertheless, values of α for fungi are similar to those for networks of tunnels in ant galleries (Buhl *et al*., 2004), *Physarum polycephalum* (Bebber, Nakagaki and Fricker, unpublished observations) and street networks in cities (Cardillo *et al*., 2006), suggesting that addition of up to 20 % of the maximum number of cross-links into a planar network maybe sufficient to achieve desirable network properties in a range of different scenarios.

Other topological network measures have not proved to be very informative for mycelia as they are heavily constrained by the developmental process and crowding effects restricting the maximum number of connections possible in a planar network (Hitchcock *et al*., 1996; Barrat *et al*., 2005; Bebber *et al*., 2007a; Fricker *et al*., 2007; Lamour *et al*., 2007). Thus, the possible degree (k, Table 1) of each node is limited to 1 for tips, 3 for branch points or fusion, or occasionally 4 for initially overlapping cords that then fuse. Likewise, the mean clustering coefficient, C, is of limited relevance for fungal networks, as their growth habit effectively precludes formation of triads. The frequency distribution of node strength (Table 1) shows more diversity than node degree alone, and follows an approximately log-normal distribution for *P. velutina* networks (Bebber *et al*., 2007a). However, we have not found evidence for power law relationships that have attracted so much attention in analyses of non-biological networks.

Table 1 – Definition of some network terms used

Topology measure	Definition
Node	Branch points, fusions or tips
Link	Connection between two nodes
Node degree	The number of links attached to a node
Clustering coefficient	A measure of the number of loops of length 3 (i.e. a triangle). Values between 0 and 1
Node strength	A measure of the importance of a node, determined by summing the weight of all links connected to the node
Link weight	A measure reflecting the size (e.g. length, cross-section area) or some other property relative to other links

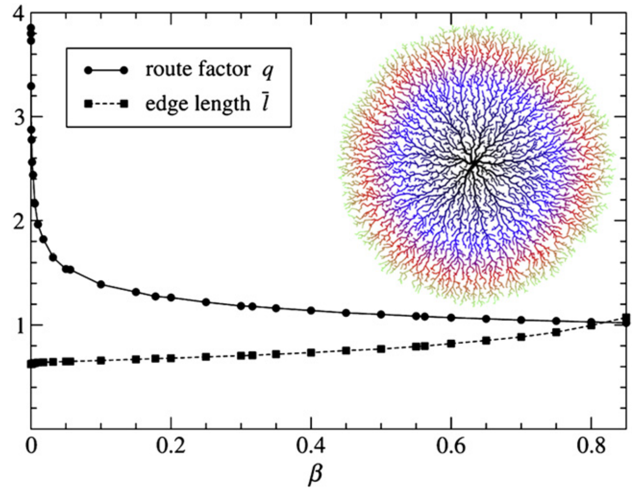

Fig. 3 – Theoretical optimal network structures that minimise route factor and total length. Networks can be close to optimal in terms of both route factor and total length. In Gastner and Newman's model (Gastner and Newman, 2006a) nodes are randomly distributed in two dimensional space with unit mean density, and node 0 is designated as the root or source for the network. The network grows over time, and at each time step a new link is added, connecting a previously unconnected node *i* to some node *j* that was already part of the network. The nodes i and *j* are chosen such that at each time step the cost $d_{ij} + \beta l_{j0}$ is minimised, where d_{ij} is the Euclidean distance between i and *j*, l_{j0} is length through the network from node *j* to the source node 0, and β is a tunable parameter. When $\beta = 0$, the shortest possible link is added at every time step. The resulting network will have the smallest possible mean link length $\bar{l}$, but it is likely that the mean route factor *q* will be large. By increasing β the network no longer grows by adding the shortest possible link, but the resulting networks have route factors which are remarkably close to 1 (the minimum possible). Each data point on the graph represents an example with 10,000 nodes, and the inset shows an example network with $\beta = 0.4$. From Gastner and Newman (2006a) with permission.

4. Predicted transport characteristics of the mycelial network

As fungal networks are embedded in Euclidean space, it is straightforward to measure the Euclidean distance between the nodes and compare it with the shortest path through the network between two nodes to give a route factor (Gastner and Newman, 2006a). Good distribution networks should be efficient in the sense that the paths from each node to the centre ought to be relatively short and the sum of the lengths of all links in the network should be low, so that the network is economical to build and maintain. These two criteria are often at odds with each other, extra links may be needed to reduce the route factor for some given node. Nevertheless, Gastner and Newman (2006b) have shown that there are solutions to the distribution problem that come remarkably close to being optimal in both senses. Interestingly, these theoretically optimal networks bear striking visual similarity to fungal networks in the absence of anastomoses (Fig. 3).

In general, it is important to consider the weights of links when considering transport efficiency. In most transport networks, the flux through a given link reflects the diameter of the pathway as well as just its length used in the calculation of the route factor, as longer, thinner cords have greater resistance to flow. In this case the diameter of a network can be kept small regardless of the number of nodes that need to be connected, or the Euclidean distance between them, as it is always possible to increase the weight of some transport backbone to provide a 'short' route from one side of the network to the other (Barrat *et al.*, 2005; Barthelemy, 2010). Indeed, much of the literature on optimal transport networks is concerned with the optimal distribution of weights for a given cost function (Murray, 1926; Sherman, 1981; Maritan *et al.*, 1996; West *et al.*, 1997; Rinaldo *et al.*, 2004; Durand, 2006, 2007; Bohn and Magnasco, 2007; Bernot *et al.*, 2008; Corson, 2010; Dodds, 2010; Katifori *et al.*, 2010).

An overall measure of transport is the average network efficiency (*E*), defined as the mean of the reciprocal of shortest path lengths for transport through the network (Latora and Marchiori, 2001, 2003). In isolation, *E* is not useful without some frame of reference. However, it is not straightforward to generate suitable reference models to test the extent that differential cord weighting improves the performance of the network. At present there are no suitable algorithms available to generate weighted planar networks with properties that mimic fungal networks, although some progress has been made with models of related systems such as leaf veination networks (Katifori *et al.*, 2010; Katifori and Magnasco, 2011). In other areas of network theory, comparisons are typically made with a reference network produced by random rewiring of the links or randomly reassigning the weights to different links. However, neither approach gives an intuitively satisfying model to test performance against, as they have no biological basis. We have previously used a two stage procedure to evaluate the performance of the fungal networks (Bebber *et al.*, 2007a). In the first step, the Euclidean fungal network, based on link length alone, is compared with model networks constructed using well defined neighbourhood graphs, including the minimum spanning tree (MST) as a lower bound for a low cost but vulnerable network, and the Delaunay triangulation (DT), giving an upper bound for a well-connected, robust, but expensive network (Buhl *et al.*, 2004; Cardillo *et al.*, 2006; Gastner and Newman, 2006b). 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, 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 to explore the consequences for transport if the fungus had allocated the same amount of resource evenly over the existing or model networks. This also allowed comparison with the real, differentially weighted network as the network measures were in comparable units.

The real weighted networks had much shorter physiological paths, especially in the central region, than their corresponding uniform networks (Bebber *et al.*, 2007a). More surprisingly, the weighted fungal network efficiency was greater than the uniform DT and the uniform MST when the predicted transport from just the inoculum (root) to all other nodes was considered. Although very well connected, the DT performed poorly, as distributing material across the large number of links present gave each one low cross-sectional area and consequent high resistance. Conversely, the MST performed better than the DT as it was populated with few, but extremely thick, links. The uniform fungal networks were similar in performance to the MST, although they clearly have a different architecture, but the fully weighted fungal network showed the best predicted transport behaviour. 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 (Bebber *et al.*, 2007a). The ability of fungal networks to modify link strengths in a dynamic way is therefore crucial to achieve a high transport capacity.

Subtle shifts in the predicted transport performance of the network as it grows can be identified by which links are carrying the greatest number of shortest paths and have a high shortest-path betweenness centrality (SPBC) (Latora and Marchiori, 2007). The relative importance of particular links between the inoculum and added resource, as judged by their SPBC, fluctuates in the early stage of growth with several cords competing before one thickens up sufficiently to achieve dominance (Fricker *et al.*, 2008b, 2009). Equally, one of the disadvantages of using shortest path analysis is that comparable parallel pathways that are only marginally longer do not feature in the analysis, but might be expected to participate in transport in a real system. This highlights one of the major problems with using shortest path based summary statistics. Shortest path metrics such as diameter, efficiency, or SPBC, are relatively easy to compute, and in each case there is a unique solution. However, such measures may be misleading, as in actual transport networks material moves along parallel flow paths, and not all of the material follows the shortest path. It is therefore important to make sure the theoretical analyses mirror the actual transport processes occurring within the fungal network itself.

5. Biological observations of network transport

Many species of fungi forage in heterogeneous environments where they have to grow through nutrient deficient regions to discover new resources and therefore require some kind of transport process to move the resources needed for growth from the source of nutrients, to the growing margin. Whilst direct uptake and intra-hyphal nutrient diffusion may be sufficient to sustain short-range local growth when resources are abundant (Olsson, 2001), long-distance translocation is required to deliver nutrients at a sufficient rate to growing tips, particularly in fungi that grow out extensively from organic resources and are therefore too large to distribute nutrients through diffusion alone (Wells and Boddy, 1995; Wells *et al.*, 1995; Davidson and Olsson, 2000; Boswell *et al.*, 2002, 2003, 2007). Remarkably little is known about the mechanism(s) underpinning such long-distance nutrient translocation, but the mechanisms proposed include vesicles moved by motor proteins (Steinberg, 2007), contractile elements (Jennings, 1987), diffusion through the vacuole system (Darrah *et al.*, 2006), carefully regulated osmotic gradients (Jennings, 1987; Cairney, 1992) and mass flows, discussed in detail below. There is also increasing evidence that a wide range of macromolecules can be translocated within the mycelium, including quantum dots (Whiteside *et al.*, 2009) and proteins (Woolston *et al.*, 2011). Conversely, water films on the surface of the network itself provide a highway for movement of bacteria, effectively bridging gaps between soil particles (Kohlmeier *et al.*, 2005; Wick *et al.*, 2010).

At the microscopic scale GFP tagged proteins, chemical stains and quantum dots (Jennings, 1987; Cairney, 1992; Lew, 2005; Whiteside *et al.*, 2009) suggest that hyphae contain both mass flow of fluid, and active transport mechanisms. For example, there is evidence that motor proteins and the cytoskeleton play a role in nuclear migration and positioning (Suelmann and Fischer, 2000), but live imaging of growing hyphae also indicates that mass flow of the cytoplasm is the dominant factor driving nuclear translocation (Lew, 2005; Ramos-García *et al.*, 2009). Additional direct evidence for mass flows within apical hyphae is through injection of oil droplets using a pressure probe to observe flow of the cytoplasm (Lew, 2005). These inert droplets move at the same rate ($\sim 0.2\ \mu m\ s^{-1}$) as the other contents of the hyphae, indicating that a mass flow of fluid is responsible for much of the observed motion, rather than motor proteins interacting with the cytoskeleton or other specific molecular interactions. At a larger scale, radio-labelled carbon and phosphate have been observed to move over distances and time scales that cannot be explained by diffusion alone (Jennings, 1987; Cairney, 1992; Wells and Boddy, 1995; Wells *et al.*, 1995; Olsson and Gray, 1998; Lindahl *et al.*, 2001; Fricker *et al.*, 2008b). For example, velocities in the range 4.4–9.8 $\mu m\ s^{-1}$ have been measured in *Armillaria mellea* (Jennings, 1987; Cairney, 1992), and velocities as large as 55–69 $\mu m\ s^{-1}$ have been measured in *Serpula lacrymans* (Jennings, 1987; Cairney, 1992). The extent of connectivity within the network has been demonstrated for rhizomorphs of *Armillaria* (Lamour *et al.*, 2007), but it is more difficult to establish whether the network can behave as a single contiguous entity for other species. In addition, it is clear that the fungi have the potential for sophisticated control of routing through regulated occlusion of septal pores (Jedd, 2011).

6. Modelling mass flows

We infer that mass flow (advection) is likely to be an important, if not the dominant, long-distance transport mechanism in larger network forming fungi. However, mass flow in fungi does not follow the paradigm for either plant or animal vascular systems. Plants drive mass flows in the xylem by transpiration from the leaves in an open system. They also actively maintain osmotic gradients along the phloem, inducing a flow of sap from sources, where water is drawn from the surrounding tissue into the sieve-tubes of the phloem, to sinks, where water leaves the phloem. Animals use hearts or contractile regions to circulate blood through hierarchical, fractal-like vascular systems that form a closed system (Sherman, 1981). In fungi, cords tend to be insulated from the environment by hydrophobic coatings and mass flow can only take place when water is able to exit the translocation pathway through either localised exudation (e.g. *Serpula lacrymans*), evaporation, or by growth itself. As fungal colonies form integrated hydraulic systems, the increase in volume that results from hyphal growth requires an equivalent uptake of water, or a reduction in the volume of another part of the mycelium that leads to growth-induced mass flow (e.g. Fig. 4; Heaton *et al*., 2010). This latter phenomenon is particularly significant in experimental microcosms which are run at high humidity thus restricting the potential for any transpirational mass flows.

With the advent of a fully digitised time-series of weighted networks (Bebber *et al*., 2007b; Fricker *et al*., 2007, 2008b, 2009; Rotheray *et al*., 2008; Boddy *et al*., 2010) it is possible to calculate the magnitude of growth-induced mass flow for individual colonies (Heaton *et al*., 2010). In essence, the empirically-determined weighted network is recast as an electrical circuit analogue, with conductances dependent on the cross-sectional area of the cords and inversely related to their length. The total current flowing through the network is estimated from change in the volume of all the cords in the network arising from thickening or thinning, or through new growth, between two time points (Fig. 4b). With the simplifying assumptions that the volume change represents water movement, the inoculum is the source of water (although the model accommodates uptake anywhere), and the flows minimise the work required to overcome viscous drag, the current flowing through every link can be calculated (Fig. 5; Heaton *et al*., 2010). If the total cross-sectional area of the growing front is much larger than the cross-sectional area of the supporting mycelium, our analysis suggests that because of the (effective) incompressibility of aqueous fluids, growth itself can drive mass flows which translocate resources towards the growing front at velocities that are much greater than the velocity of tip growth. The predicted distribution of velocities was heavy tailed, with many links carrying low velocity mass flows, and a few links carrying high velocity mass flows. Nevertheless, the velocities predicted for the major cords were in reasonable agreement with experimental

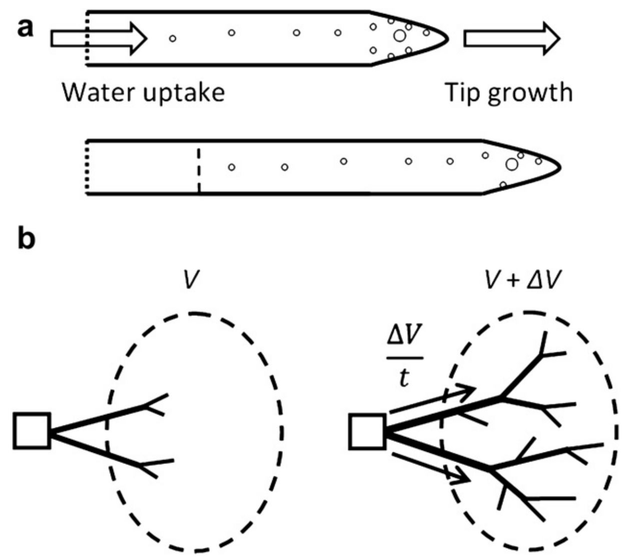

Fig. 4 – Physical principles of growth-induced mass flow. (a) Turgor pressure is induced by an osmotic gradient at the site of water uptake. Vesicles (circles) have to deliver material to the tip and must move towards the tip faster than it grows, while the cytosol behind the growing tip moves forward at the rate of tip growth (Lew, 2005). Conservation of volume dictates that as the tip expands, fluid flows towards the tips from the site of water uptake creating a mass flow. (b) Suppose that a fungus grows out of an inoculum (square) and into a region (oval). Some of the material that becomes part of the fungi may come from within the oval region. The rest of the material must have travelled along the cords (links) that cross the region's boundary. If the volume of fungi within the region increases by ΔV over a period of time t, and none of the material is drawn from within the region, it follows that the average current flowing into the region is $\Delta V/t$. Furthermore, if the total cross-sectional area of the boundary crossing cords is a, the mean velocity of flow will be $\Delta V/at$ (Heaton *et al*., 2010).

data for radiolabel transport, and the pressure gradients needed to produce these flows are small. Furthermore, cords that were predicted to carry fast-moving or large currents were significantly more likely to increase in size than cords with slow-moving or small currents (Heaton *et al*., 2010).

We do not yet have methods to measure water movement directly in these networks. However, we can image nutrient movement by mapping the distribution of the amino-acid analogue, ^{14}C-amino isobutyrate (^{14}C-AIB) using photon-counting scintillation imaging (Tlalka *et al*., 2002, 2007, 2008). ^{14}C-AIB accumulates in the free amino acid pool and is not metabolised in a range of woodland fungi so far examined, as judged by the lack of incorporation of ^{14}C in other metabolites or released as $^{14}CO_2$, (Watkinson, 1984; Olsson and Gray, 1998). This allows it to be used as a proxy for nitrogen translocation (Watkinson, 1984) and provides an opportunity to compare the predictions made by the theoretical network analysis to the actual pattern of nutrient movement in the same microcosms (Fricker *et al*., 2008c, 2009; Heaton *et al*., submitted for publication). This requires a significant extension

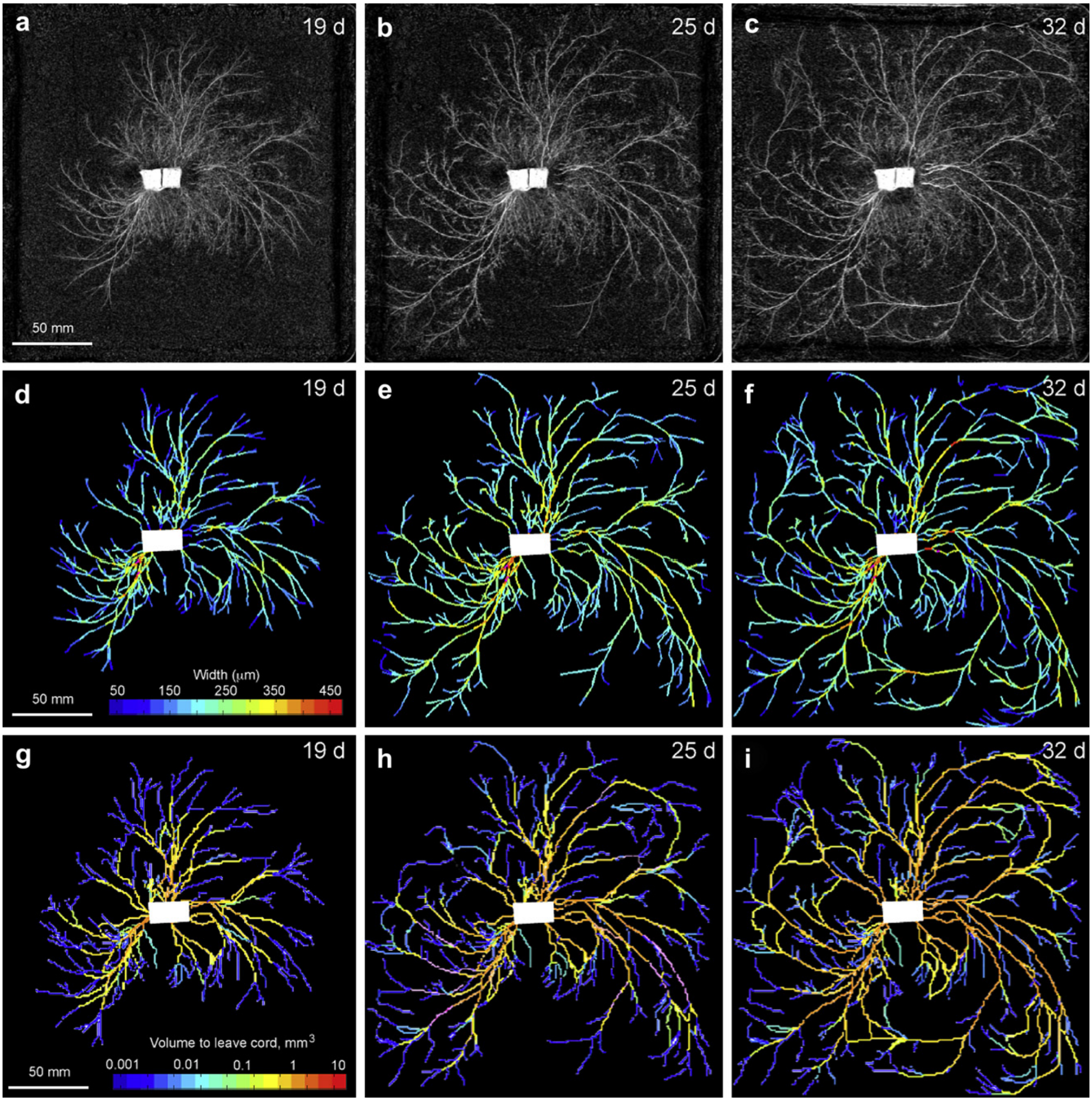

Fig. 5 – Network development and predicted currents in *Phanerochaete velutina* based on a growth-induced mass flow model. Images (a–c) show network development after 19 d, 25 d and 32 d respectively. The image intensity of cords was used to estimate their thickness, enabling the production of weighted, digitised networks (d–f). These are colour coded to show the estimated thicknesses of all sections of all links, while the white block represents the inoculum. Images (g–i) are colour coded according to the total volume that has passed through each cord, as calculated from a growth-induced mass flow model (Heaton *et al.*, 2010).

of the growth-induced mass flow model to incorporate the fate of a nutrient loaded into the transport pathway which will also become dispersed by diffusion (Lew, 2011), and potentially taken out of the pathway during transit to maintain the network itself (Heaton *et al.*, submitted for publication).

The predictions made by such an Advection-Diffusion-Delivery (ADD) model provide an extraordinarily high level of explanatory power for the measured distribution of ^{14}C-AIB in networks of *P. velutina* (Fig. 6; Heaton *et al.*, submitted for publication). This is a marked improvement over our previous analysis of ^{14}C-AIB distribution patterns based on the static network architecture or shortest-path betweenness centrality, which only showed correlation in regions of the network where transport actually occurred (Fricker *et al.*, 2008b, 2009). The ADD model suggests that the minimum currents consistent with the observed growth

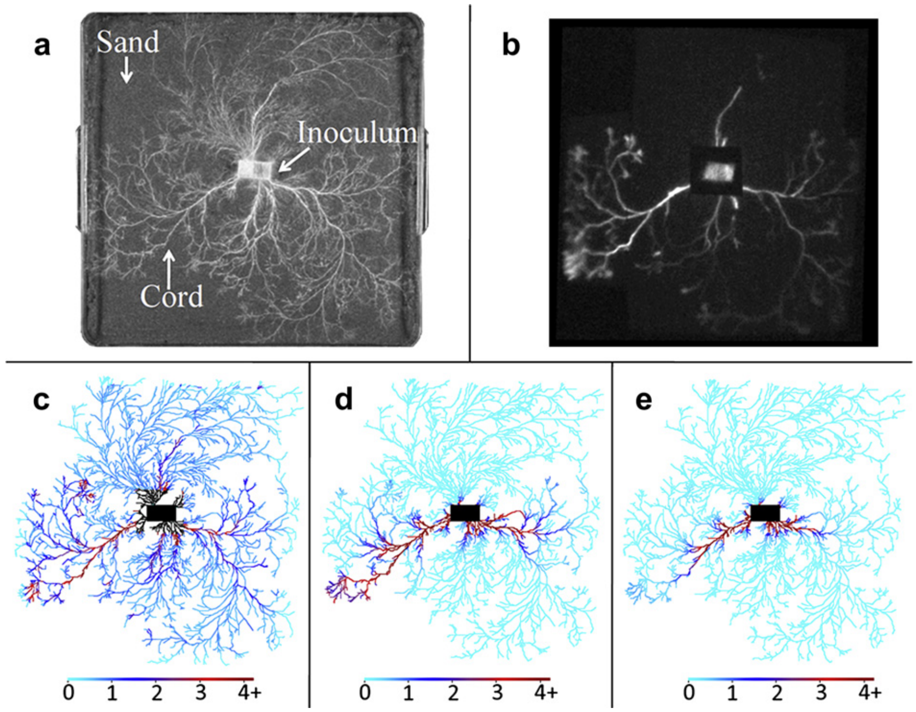

Fig. 6 – Measured and predicted patterns of ^{14}C-AIB distribution in networks of *Phanerochaete velutina*. (a) Mycelial network of *Phanerochaete velutina*, photographed just before ^{14}C-AIB was added to the inoculum. (b) Photon-counting scintillation image of ^{14}C-AIB distribution integrated over 32 h. The brightness of the image reflects the total number of photons emitted from each region. (c) Measurement of ^{14}C-AIB label from (b) using a superimposed manually digitised network, coloured to indicate the photon count. Links that were not covered by the scintillation screen are coloured black. (d) Predicted ^{14}C-AIB intensity from the ADD model (Heaton *et al.*, submitted for publication), with the assumption that AIB enters the network at the inoculum at a constant rate, each link in the final network continues to grow (or shrink) at the same rate that was observed over the final time step, and 10 % of each cord is occupied by transport vessels. (e) Predicted intensity under the same assumptions as (d), except that in this case 20 % of each cord is assumed to be occupied by transport vessels.

would effectively transport resource from the inoculum to the growing tips over the timescale of growth. Nevertheless, whilst advective mass flows carry resource over long distances from the inoculum out towards the growing tips (Jennings, 1987; Cairney, 1992; Olsson and Gray, 1998), diffusion and active transport mechanisms may be essential near the sites where the cell wall is expanding. This follows because the cytosol within the apical hyphae moves forward at the same rate as the growing tips (Lew, 2005), but to transport resource from the base of these hyphae to the growing tips, the resource has to move faster than the rate of growth.

7. Biophysical consequences of mass flows

The incompressibility of the fluids within fungi ensures that there is a rapid global response to local fluid movements. Furthermore, the velocity of fluid flow is a local signal that can convey quasi-global information about the role of a cord within the mycelium. There was a correlation between the thickening of cords and the speeds or flux densities predicted by the ADD model (Heaton *et al.*, submitted for publication). Similarly, there was a positive correlation between predicted current and the thickening of cords. This is consistent with the plausible assumption that *P. velutina* has evolved to reduce the work needed to overcome viscous drag, as significantly greater energy savings can be made by preferentially thickening the high current cords. The speeds predicted by the ADD model are consistent with experimental data. For example, a radio-labelled source of carbon has been measured moving at a velocity 7 $\mu m\ s^{-1}$ away from the inoculum. This is the same order of magnitude that the ADD model predicts for a major cord. The pressure gradients required to produce the predicted flows are very modest and unlike previous analyses (Jennings, 1987; Rayner, 1991; Lew *et al.*, 2004), it was suggested that intrahyphal concentration gradients are not strictly necessary for the production of mass flows.

In other vascular systems with nutrient distribution involving mass flows, a local adaptive response to wall shear stress is a key mechanism that enables the optimisation of the network (Kamiya *et al.*, 1984; Pries *et al.*, 2009). Flow velocities are of the order 100–1000 $\mu m\ s^{-1}$ in these systems and induce wall shear stresses of the order 0.1–1 Nm^{-2}. Flows in fungi are much lower than this, nevertheless, the wall shear stress will be greater in the septal pores, because at that point the same current that is passing through the hyphae or transport vessels must pass through a smaller channel, which means the local velocity of flow must be greater. If the mass flows also pass through the much smaller vessels that are found within cords, or if the shear wall stress is detected at the septal pores, fungi could plausibly detect velocities of the order 10 $\mu m\ s^{-1}$. It is less likely that fungi can detect the difference between currents with a mean velocity much smaller than this, as the corresponding changes in wall shear stress would be very small, even in the vessels whose diameter is only 2 μm.

As well as experiencing wall shear stress, the vessels within pressure driven vascular systems also experience intramural stress. This force per unit area is experienced throughout the vessel wall (and not just on the inside surface), as the vessel must resist the tendency to expand or burst. Hyphae and transport vessels are subject to several atmospheres of pressure (about 4–5 bar) (Money, 1990, 1997; Lew et al., 2004; Lew, 2005, 2011; Money, 2008) and the fluids within these structures flow at a very modest rate (Wells et al., 1995; Olsson and Gray, 1998; Tlalka et al., 2002, 2008; Lew, 2005; Fricker et al., 2008b). Consequently, the intramural stress will be orders of magnitude greater than the shear wall stress. Nevertheless, this does not mean that it is implausible that fungi are sensitive to changes in wall shear stress. The cell wall must be rigid enough to withstand the intramural stress (Money, 1997, 2008; Harold, 2002; Lew, 2011), but proteins embedded in the lipid membranes of tethered organelles, or in the septal pores (where the velocity of flow and the wall shear stress will be greatest) may be sensitive to the scale of flows within a fungal network.

The curvature of vessels can have important effects on the fluid flows within them (Truskey et al., 2010), particularly if flow rates are high, the vessel is large and the radius of curvature tight. Whilst this is an important issue for the major curved arteries of the human vascular system, in the case of fungal networks we estimate the effect of curvature on fluid flows is negligible.

The importance of localising water uptake

The efficiency of growth-induced mass flows as a means of transport requires that water uptake and growth are spatially separated. If water uptake occurs throughout the network, growth-induced mass flows will still occur, but the scale of advection will be significantly reduced (Fig. 7). For example, in a linear network, switching from water uptake at one end to water uptake throughout the link will halve the scale of mass-flow. In the case of a branching network the reduction in advection can be much greater. For example, if water uptake occurs throughout a branching network of unit cross-sectional area the velocity of mass flow would equal the velocity of tip growth v in every link, regardless of its generation (Fig. 7). This contrasts dramatically with the case where all the water uptake occurs at the inoculum. In the case where there are n generations in the tree and the link in question is part of generation i, the mean velocity is v^{2n-i} rather than v (Fig. 7).

The ADD model also helps to explain previously challenging experimental observations, most notably, the occurrence of sudden route switching (Fricker et al., 2008b), now interpreted as a change in relative distal growth, and the colony-wide coupling of polarised transport and growth arrest at sites remote from encounter with a new food resource (Tlalka et al., 2008). As growth, mass-flow and nutrient transport are coupled, there may be an interesting interaction between nutrient availability, control of branching and nutrient transport. It is well known that the rate of hyphal branching increases when tips encounter resource rich environments (Boswell et al., 2003, 2007; Falconer et al., 2005; Tlalka et al., 2008) which will give an increase in flux density in this region. If the rate of input from the inoculum remains similar, there has to be a concomitant reduction in fluxes elsewhere, in line with experimental observations (Tlalka et al., 2008).

By contrast, it is difficult to reconcile growth-induced mass flows, which might be expected to be directed apically, with observations of bi-directional radiolabel movement (Lindahl et al., 2001; Tlalka et al., 2007, 2008). At the growing margin, one of the potential roles of the tubular vacuolar system may be to provide an alternative pathway from the cytoplasm to allow basally directed diffusive transport of acquired solutes (Darrah et al., 2006). In regions more distal from the tip, bi-directional movement might involve establishing an anti-parallel circulation system within individual cords (Fricker et al., 2007). However, there is currently no direct evidence for such a system and it is still challenging to conceive how such loops would be able to re-configure themselves as the network architecture is remodelled.

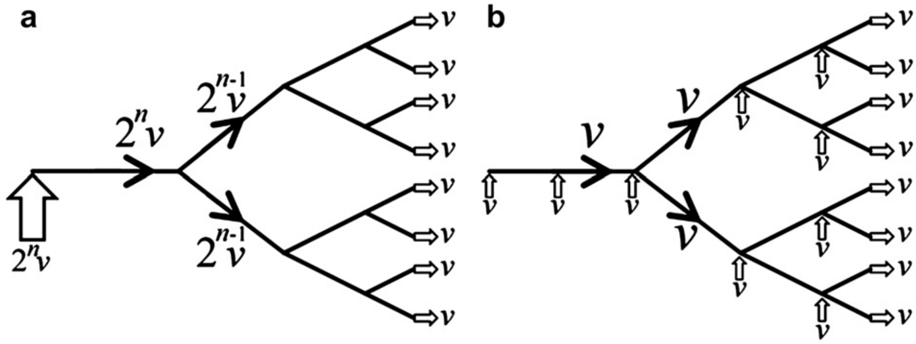

Fig. 7 – Velocities in a branching network with different patterns of water uptake. (a) If water uptake only occurs in the first link, the velocity of mass flow halves from one generation to the next. (b) If water uptake occurs evenly throughout the network, the velocity may be constant throughout.

8. Network robustness

High transport capacity and low construction cost could have come at the expense of other network properties, such as robustness to damage, as there is no *a priori* reason why link weight allocation for one feature necessarily enhances another. Robustness to damage from physical breakage or grazing by invertebrates (Harold *et al*., 2005; Bretherton *et al*., 2006; Wood *et al*., 2006; Boddy and Jones, 2007), is of major significance to long-lived mycelial systems.

This can be appreciated by examining the effects of breaking links in models of the fungal networks and assessing the impact on transport or overall connectivity (Bebber *et al*., 2007a; Lamour *et al*., 2007; Rotheray *et al*., 2008; Boddy *et al*., 2010). We note that cords (links) are the biologically relevant target for attack rather than nodes in non-biological systems. In natural systems, which links are broken depends on the agent causing damage. With invertebrate grazing, for example, different species graze in different ways: collembolan often target fine mycelium, millipedes graze arcs at growing fronts, and woodlice often devour mycelium in long straight paths (Crowther *et al*., 2011a, b, c, in press). These different grazing patterns have not yet been mimicked in artificial experiments on digitised networks *in silico*. Rather, links have been broken at random or by targeting critical connections (Fig. 8; Lamour *et al*., 2007), or in an order assuming that the probability of breakage increased with length and decreased with the thickness of the link (Bebber *et al*., 2007a). Robustness was measured by the size of the connected components remaining or the transport efficiency, and compared to standard networks such as the DT or MST (e.g. Fig. 8; Bebber *et al*., 2007a; Lamour *et al*., 2007). Having a large number of alternate pathways is important in this context, and the differential strengthening of links not only imparts high transport capacity but also robustness to damage (Bebber *et al*., 2007a).

A static analysis of the network represents a minimum estimate of the real network resilience in nature, as the network is also able to respond to local damage, by modification of adjacent link strength, and to regrow and reconnect. Thus, for example, local mechanical damage to a small region of the network promotes strengthening of distal circumferential connections (Fricker *et al*., 2009), whilst continuous collembolan grazing trims the network back to the reinforced core (Fig. 9; Rotheray *et al*., 2008; Boddy *et al*., 2010), in support of the *in silico* predictions, but also promotes an increase in tangential connections making the network more resilient, at the cost of a reduction in exploration (Fricker *et al*., 2007, 2009; Rotheray *et al*., 2008; Boddy *et al*., 2010). Since different species have different mycelial architecture, not surprisingly they have different resilience to damage depending on the extent of connectivity. In general, a high degree of connectivity confers greater resilience, but this comes at an increased cost in terms of more material in the network or a reduction in exploratory growth (e.g. Fig. 9).

9. Comparison with transport networks in other domains

The challenges that balancing the competing demands of cost, efficiency, resilience and control complexity place on the

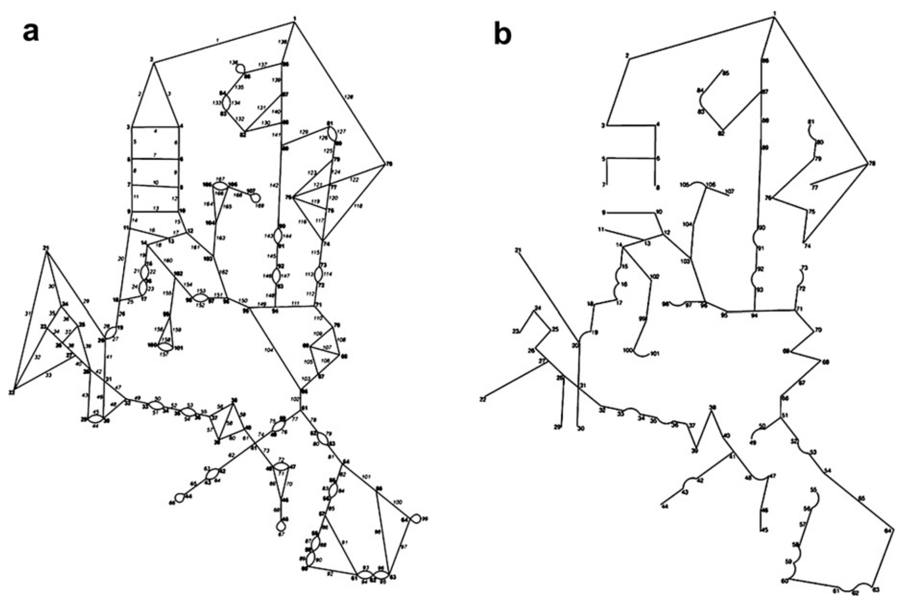

Fig. 8 – Rhizomorph network of *Armillaria lutea* growing over an area of 25 m^2 in a *Pinus nigra* plantation. (a) A manually extracted planar graph in which the 107 vertices (nodes) and 169 edges (links) have been numbered. (b) The minimal spanning tree for the same node positions as (a).
Disruption of two critical links (78 or 81) would lead to large parts (13 % and 11 %) being disconnected from the remainder of the mapped network. However, there is a low probability that amputation of a randomly chosen link would separate the network into two disconnected components. The high level of connectedness may enhance redistribution of nutrients and provide a robust rhizomorph structure, allowing *Armillaria* to respond opportunistically to spatially and temporally changing environments. From Lamour *et al.* (2007) with permission.

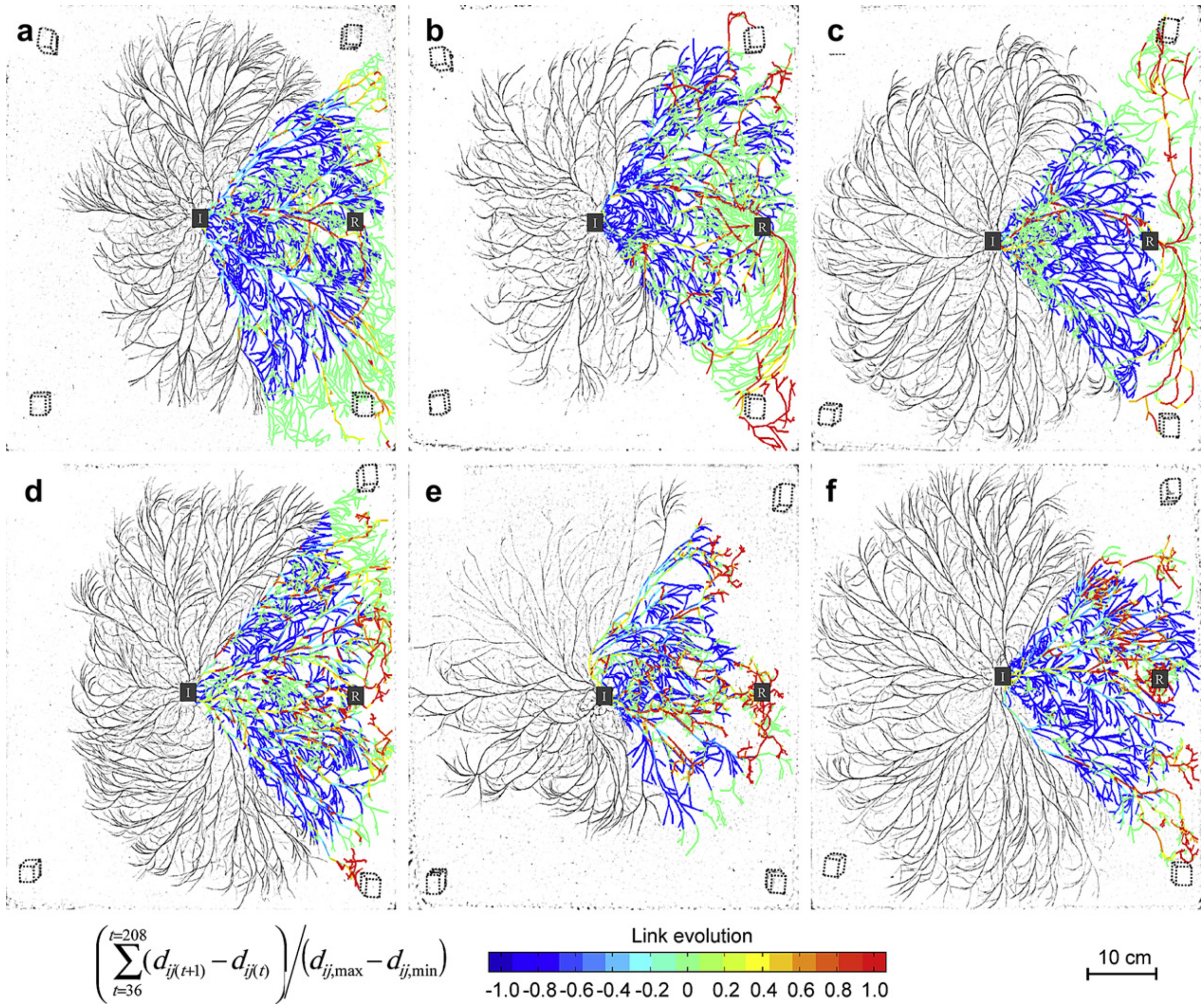

Fig. 9 – Link evolution in colonies of *Phanerochaete velutina* in response to grazing. Mycelial systems of *P. velutina* grown from beech wood blocks in trays (57 × 57 cm) of compressed non-sterile soil. For display, images were processed by background subtraction, contrast limited histogram equalisation, contrast stretching and look-up table inversion to give black-on-white representations of the colony morphology. Superimposed pseudo-colour display of the evolution of each link in networks with new resources added at 36 d (R) for three replicate ungrazed colonies (a–c) and three colonies with grazing Collembola added at 49 d (d–f). Link evolution was calculated as the ratio between the sum of the differences in link diameter between successive time-points and the maximum difference over the whole time period. Continuous growth is indicated by red, continuous regression of cords by blue and cords that remain constant throughout are indicated by green. The position of Perspex lid supports are indicated by dotted outlines. I = inoculum, scale bar = 10 cm. From Boddy *et al.* (2010) with permission.

network organisation have strong parallels with those faced in the design of anthropogenic infrastructure networks. We note, however, that the fluidic character of fungal networks makes them unusual since it allows information to spread across the system on very fast timescales. The solutions that fungi achieve may represent good compromises to such a combinatorial optimisation problem, and may yield useful insights into the design of delocalised, robust infrastructure networks that operate without central control. This presumes that solutions adopted by biological networks will exemplify useful generic theoretical principles, such as persistence, robustness, error-handling or appropriate redundancy, as they have been honed by evolution. The expectation is that the process of Darwinian natural selection based on variation, competition and survival has explored a significant range of possible network organisations and the resulting systems are likely to be well-adapted to survive and reproduce under particular biotic and abiotic conditions to solve certain ecological problems (Fricker *et al.*, 2009). A range of network architectures, development and dynamics can be found within the fungi and myxomycetes, suggesting a comparative approach may be instructive. However, the constraints imposed by the components used to construct the network (i.e. branching tubes) may have a profound effect on the possible network organisation and dynamics, so that any result can only be generalised to a very limited set of real-world problems.

Transport costs and optimal transport networks

The design of optimal distribution networks for water, electricity, telephone signals, etc is of great practical import in urban planning, and consequently aspects of this family of problems have been studied since antiquity. Over the last 15 y, a string of papers has explored the topic of transport networks that are optimal in some given sense. Some authors have pursued highly abstract models which assess the cost of different ways of connecting source nodes to sinks (with a variety ways of assessing the cost of any given pattern of flow) (Maritan et al., 1996; Banavar et al., 2000a, b, 2002, 2010; Dreyer, 2001; Dreyer and Puzio, 2001; Bohn and Magnasco, 2007; Bernot et al., 2008; Corson, 2010; Dodds, 2010), others have focussed on the general properties of networks with flows driven by potential differences (Sherman, 1981; Durand, 2006, 2007; Katifori et al., 2010), while West and colleagues consider the optimum network given a number of biologically inspired assumptions (West et al., 1997, 1999a, 2002; West and Brown, 2004; Savage et al., 2008). The papers by Banavar and West are particularly notable, as they played a key role in the development of the theory of allometric scaling, which attempts to explain the relationship between body size and metabolic rate.

Different studies use different definitions of a network, and the authors optimise different cost functions. For example, Durand (Durand, 2006, 2007) considers the optimal geometry and the relationship between the local geometry and the local topology of hydraulic networks whose currents derive from a potential, explicitly analogous to electrical networks, that are embedded in an ambient space. In contrast, Banavar et al. (2000a, b, 2002, 2010) propose a more abstract model where the graph is not assumed to be embedded in a target space, and the currents through the nodes are not explicitly constrained to derive from a potential difference.

A very general observation at the heart of the analysis by Banavar and colleagues is that any efficient flow pattern must be such that at every point, the flow moves materials away from the source. In other words, although there may be loops in the networks they consider, optimally efficient flows always transport materials in a directed manner. This feature of 'efficient' material flows is common to many definitions of 'efficient' (Sherman, 1981; Maritan et al., 1996; West et al., 1997, 1999a, 2002; Banavar et al., 1999, 2000a, b, 2002, 2010; Dreyer, 2001; Dreyer and Puzio, 2001; Colizza et al., 2004; Rinaldo et al., 2004; West and Brown, 2004; Durand, 2006, 2007; Bernot et al., 2008; Savage et al., 2008; Corson, 2010; Dodds, 2010; Katifori et al., 2010), and it is inevitable when the flows are driven by differences in potential.

Banavar and colleagues abstract approach enables the construction of formal proofs concerning optimal networks, but the proper physical interpretation of those results is somewhat elusive (Bohn and Magnasco, 2007). A different approach, taken by West and colleagues builds on the legacy of Cecil D. Murray, and the principle of minimum work (Murray, 1926; Sherman, 1981; West et al., 1997, 1999b, 2002; West and Brown, 2004; Savage et al., 2008). Fluid flow is essential to most biological transport networks, and so cost functions that reflect the energy required to overcome viscous drag are of particular biological significance. Murray observed that vascular networks represent an optimal or near optimal compromise between conflicting costs, as the work required to overcome viscous drag is much smaller in large vessels, but this benefit of thickening is offset by a cost, and as a simplifying assumption it is reasonable to assume that the metabolic cost of building and maintaining a vessel is proportional to its volume. The optimal arrangement is when the cube of the radius of the parent vessel equals the sum of the cubes of the radii of the daughters (Murray's law). Actual vascular systems approximately follow Murray's law (Sherman, 1981; Sherman et al., 1989; McCulloh et al., 2003; Kassab, 2006; Savage et al., 2008), but it is worth noting that significant deviations from Murray's law can result in only modest increase in the amount of energy required to overcome viscous drag (Sherman et al., 1989; Truskey et al., 2010).

Fluctuating demand, robustness and loops

Although vascular networks are frequently depicted as branching trees (Bernot et al., 2008; Pries et al., 2009), many natural and almost all man made networks contain loops. Street plans are full of loops, most fungal networks contain loops (Bebber et al., 2007a; Fricker et al., 2007, 2008a, 2009; Boddy et al., 2009, 2010), as do retinal vasculatures (Fruttiger, 2002), and the veins of many leaves contain recursively nested sets of loops (Roth-Nebelsick et al., 2001; Durand, 2006; Sack and Holbrook, 2006; Corson et al., 2009; Corson, 2010; Katifori et al., 2010). Recent theoretical analyses indicate that if a network is attacked, or subject to fluctuating loads, the optimal form is no longer a branching tree but will contain loops (Corson et al., 2009; Corson, 2010; Katifori et al., 2010; Katifori and Magnasco, 2011).

Optimisation under damage to links implies the formation of loops almost by definition, as if there were only one route connecting the source and a given sink, an infinite amount of power would be dissipated when that route is cut. Robustness to damage can be conferred by a (topologically minimum) ring joining the outermost nodes (Roth-Nebelsick et al., 2001), and more generally it is well known that redundant links can confer a degree of robustness to a transport network (Roth-Nebelsick et al., 2001; Rinaldo et al., 2004; Sack and Holbrook, 2006; Fricker et al., 2007, 2009; Barthelemy, 2010). Plants and fungi are under constant attack, from the elements as well as pathogens and a wide variety of grazing animals (Roth-Nebelsick et al., 2001; Sack and Holbrook, 2006; Rotheray et al., 2008; Boddy et al., 2010; Crowther et al., 2011a, b, c, in press). If fungal networks were branching trees, severing any branch would disconnect the network. Likewise, if the leaf vascular network was treelike, damage to any vein would result in the death of all the leaf sections downstream from that vein. The value of redundant links is therefore quite clear, but optimality under robustness to damage can produce hierarchical, recursively nested loops (Katifori et al., 2010; Katifori and Magnasco, 2011), as can be found in actual leaf venation networks (Roth-Nebelsick et al., 2001; Sack and Holbrook, 2006).

Katifori et al. (2010, 2011) and Corson et al. (2009, 2010) also show the importance of fluctuating demand for optimal transport networks. Many definitions of optimality yield treelike structures, with a single path connecting any two points

(Sherman, 1981; Banavar *et al.*, 1999, 2000a, b; Durand, 2006, 2007; Bohn and Magnasco, 2007; Bernot *et al.*, 2008). In this light, the many loops found in leaf venation networks, fungal networks or vascular networks could be interpreted as a compromise between transport efficiency and other requirements, such as tolerance to damage. However, Katifori *et al.* and Corson *et al.* show that the optimality of branching trees is contingent on the assumption of a stationary flow through the network. By contrast, they found that optimisation under a varying load leads can lead to the formation of dense, recursively looped structures (Corson *et al.*, 2009, 2010; Katifori *et al.*, 2010; Katifori and Magnasco, 2011). More specifically, when time variations or fluctuations are allowed for, the resulting optimal structures contain loops, although they do share the hierarchical organisation that is characteristic of treelike optimal networks.

This result is biologically very significant, as in general biological transport networks operate in the face of considerable spatiotemporal irregularities. As fungal networks grow and the supporting mycelium matures, different parts of the growing margin will need to be supplied with resources at different times. If fungal networks have indeed evolved to maximise transport efficiency, they should be optimal in relation to a history of transport demands, and we should not necessarily expect that at any instant the network is the best way of linking the sites of resource uptake with the sites of resource demand. Nevertheless, we note that fungal transport networks have unusual features compared to the preceding models of networks for the delivery of nutrients. The fungal network is both a transport system *and* the organism itself. Fungal networks are vastly more dynamic than vascular networks in plants and humans and it seems that fungi use their networked state to process their environment, possibly using their fluid based nature to rapidly couple behaviour even in disparate parts of themselves. This type of analysis has strong parallels with the conceptual ideas set out by Rayner two decades ago (Rayner, 1991; Rayner *et al.*, 1994, 1999).

10. Future developments

Characterisation of mycelial networks is still in its infancy. However, the network approach provides a way of quantifying and analysing complex fungal systems in detail, and also makes it possible to link measurements in laboratory microcosms to observations of networks in the field. The simplest models predicting transport through such networks are based on shortest path considerations through the weighted network and are relatively straightforward to calculate, but only capture part of the experimentally determined transport behaviour. Models that include parallel-flow pathways coupled to the empirically-measured growth of the network improve the match between simulation and experiment significantly and explain several previously challenging empirical observations. The next conceptual advance will be to identify the rules that allow the network iteratively to refine its structure and transport behaviour to yield the network architectures observed. It is conceivable that the identification of such rules will allow development of generic "fungal colony optimisation" (Hanson *et al.*, 2006; Xu *et al.*, 2009) algorithms similar to those that have evolved from the study of ant colony foraging patterns (Dorigo *et al.*, 1999) or based on *Physarum* (Tero *et al.*, 2006, 2007, 2008; Nakagaki *et al.*, 2007; Watanabe *et al.*, 2011).

Even at this stage, some common features of biological network formation seem to emerge. Fungal networks are constructed by local iterative developmental processes rather than predetermined blueprints or centralised control, with growth involving over-production of links and nodes, followed by selective pruning of some links and reinforcement of others. Such a process mimics the process of Darwinian evolution in which natural selection removes less fit offspring. This 'Darwinian network model' may be applicable to other biological systems, including foraging ant trails, *Physarum*, axon development and angiogenesis, and may represent a generalised model for growth of physical biological networks. Based on the ant colony and *Physarum* models, we might expect the generic ingredients in such a model will involve a non-linear positive reinforcement term related to the local flux and a linear decay term. Notably this model differs from other models of weighted network evolution that only incorporate differential strengthening of links, i.e., 'the busiest get busier', rather than additional differential weakening and loss that is the hallmark of evolution by natural selection. However, the model has parallels with the selective link removal model proposed for unweighted networks (Salathe *et al.*, 2005). In infrastructure networks where costs are associated with creation and maintenance of links, where links differ in some measure of fitness, and where material can be recycled, such a Darwinian model may be applicable. In practical terms such a process may also be witnessed in the evolution of real infrastructure networks, such as British railways following the Beeching reviews in the early 60's (British Transport Commission, 1963; British Railways Board, 1965). In these reviews, the flux along various routes was measured and routes with too low a level of traffic, mainly branch lines, were targeted for closure. At the same time, major routes were strengthened to cope with the expected source–sink relationships for both passenger and freight traffic. Interestingly, the reports focussed on efficiency rather than any explicit consideration of resilience, which may explain the sensitivity of the current network to disruption.

A second feature of interest emerging, particularly through consideration of the *Physarum* and fungal networks, is the extent that coupled flows may contain global information. Networks involving physical flows obey continuity equations and are therefore intrinsically coupled across the network. This automatically means that increasing the flow in one part of the network will lead to reductions elsewhere, even though the local conditions in the distal region remain the same. Thus each part of the network is influenced by and can influence the whole network, but without any global assessment of behaviour. Useful properties of the network may emerge from the interaction between the local update rules governing topology and flows without the need for long-distance communication or calculation of aggregate properties of the network. It is this coupling in the *Physarum* model that allows the network to resolve from a fine mesh into a quasi-optimal solution (Tero *et al.*, 2006, 2007, 2008;

Nakagaki et al., 2007). Furthermore, the computational overhead for such self-organised networks scales well with the number of additional nodes.

Set against this progress are some equally difficult challenges. The transition to three dimensions, particularly in realistic soil or wood microcosms, is immensely problematic for the vast majority of imaging approaches. There is potential to record the micro-structure of a porous soil system using high-resolution X-ray tomography and then predict the behaviour of different fungi using modelling approaches (Blair et al., 2007; Pajor et al., 2010), but there is currently not sufficient contrast to resolve the actual fungal distribution within the soil. Likewise, the observation that fungi can take up and translocate quantum dots (Whiteside et al., 2009), and the increasing number of species that can now express fluorescent proteins (Lorang et al., 2001; Czymmek et al., 2004; Leroch et al., 2011) coupled with confocal or multi-photon imaging facilitate 3-D or 4-D data collection from living systems (Czymmek, 2005), but only in relatively translucent media to a depth of around 100 μm.

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