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Grazing alters network architecture during interspecific mycelial interactions

T.D. ROTHERAY[a], T.H. JONES[a], M.D. FRICKER[b], Lynne BODDY[a,*]

[a]Cardiff School of Biosciences, Cardiff University, Biosciences Building, Museum Avenue, Cardiff CF10 3AX, UK
[b]Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK

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ABSTRACT

The changes that occur in mycelial architecture of *Phanerochaete velutina* interacting with *Hypholoma fasciculare* mycelium in soil microcosms in the presence and absence of the collembola *Folsomia candida* are investigated employing tools developed in graph theory and statistical mechanics. There was substantially greater overgrowth of *H. fasciculare* by *P. velutina* mycelium when grazed than when un-grazed. There was a marked disappearance of hyphal links in all un-grazed systems between 8 d and 34 d, predominantly in areas distant from the interaction, but this was much less evident in grazed systems. Further, new tangential cross-links connecting radial cords distant from the inoculum formed in grazed systems. The thickness of cords increased with time, and more so in grazed systems. There was no significant difference in transport efficiency between the grazed and un-grazed systems. The ability of the mycelial network to modify dynamically link strengths is crucial to achieving a balance between transport capacity/robustness to damage and overall cost of production.

Introduction

In the natural environment fungal mycelia have to compete with other fungi and microorganisms for space and resources, and suffer predation from grazing invertebrates. Interspecific interactions between basidiomycete mycelia trigger dramatic changes in mycelial morphology, both in the vicinity of the antagonist and often elsewhere in the mycelium (Dowson *et al.* 1988; Holmer & Stenlid 1997; Boddy 2000; Woodward & Boddy 2008). A range of responses are observed depending on individual species and species combinations, including production of stationary 'barrages' that are resistant to invasion by opposing mycelium, mycelial fans, linear organs and pigments. For example, non-destructive image analysis of *Stropharia caerulea* growing in soil revealed a reduction in extra-resource mycelial biomass and mass fractal dimension when grown in combination with *Phanerochaete velutina*, and reduction in number of major mycelial cords when grown in combination with *Phallus impudicus* (Donnelly & Boddy 2001).

Substantial morphological and physiological changes also occur in response to predation by invertebrates. For example, grazing of *P. velutina* by the collembola *Folsomia candida* resulted in changes to mycelial extension rate, hyphal coverage, fractal dimension and other qualitative aspects of morphology (Bretherton *et al.* 2006; Wood *et al.* 2006; Boddy & Jones 2008; Tordoff *et al.* 2008). Moreover, grazing altered the progression and outcome of interspecific mycelial interactions (TD Rotheray *et al.*, unpub.). It appears that different species have evolved different strategies to balance their ability to explore new territory, defend space already occupied

* *Corresponding author*. Tel.: +44 29 2087 4776.
E-mail address: boddyl@cardiff.ac.uk (L. Boddy).
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and maintain an effective transport network in the face of continuous attack. These are manifest in a range of network architectures and adaptive dynamics (Boddy 1999; Fricker *et al.* 2008a).

Recent work has begun to analyse mycelial network architecture (Bebber *et al.* 2007a, b; Fricker *et al.* 2007, 2008a, b; Lamour *et al.* 2007; Boddy *et al.*, in press) using tools developed in graph theory and statistical mechanics (Albert & Barabási 2001; Strogatz 2001; Dorogovtsev & Mendes 2002; Newman 2003; Amaral & Ottino 2004). This involves creating a digitised representation of the mycelial network as a graph, by plotting a series of connected nodes, representing branching points or anastomoses, and interconnecting links in the mycelium (Fricker *et al.* 2007). By mapping the network over time it is possible to examine changes in architecture, such as the formation of preferential transport routes through thickening of cords, development of cross linkages which increase resilience to attack, or local regression of mycelia as the network recycles redundant material. The network graph can be readily analysed to give theoretical predictions of the transport efficiency, resilience and cost of the network (Fricker *et al.* 2007). This analytical approach has demonstrated that mycelia of *P. velutina* develop a robust network that is resistant to damage, yet maintains a high transport efficiency (Bebber *et al.* 2007a). Moreover, this was achieved with a relative decrease in cost through preferential reinforcement of certain pathways and removal of intervening mycelium.

Here we investigate the changes that occur in mycelial network structure of *P. velutina* interacting with *Hypholoma fasciculare* in soil microcosms in the presence and absence of the collembola *F. candida*. In this three-way interaction, we hypothesise that: (1) *P. velutina* will reinforce the mycelial network in the vicinity of the interaction; (2) collembola grazing will reduce transport efficiency due to severance of cords; and (3) the biomass of the network in proportion to the area covered will be greater in un-grazed systems, as the collembola remove mycelia in grazed systems.

Methods

Soil microcosm systems

Details of the experimental set up are given by TD Rotheray (2008). Briefly, beech wood blocks ($2 \times 2 \times 2$ cm) were pre-colonised with *P. velutina* and *H. fasciculare* for 3 months and then positioned 9 cm away from diagonally opposite corners of trays (24×24 cm) of compressed, non-sterile soil at different times, such that the mycelia met at the centre. Trays were incubated at 20 °C in the dark. Two days after mycelia had contacted each other, *F. candida* (20 at each corner of the tray) were added. Changes in the mycelial networks were recorded at 2–4 d intervals by digital photography (Nikon© Coolpix™ 5700) at a height of 47 cm with artificial illumination provided by two 1000 W spot flood lamps.

Network digitisation

Three replicates each of grazed and un-grazed systems were selected using randomly generated numbers from 10 available. Six images for each replicate were selected at $t = 0$ d, 4 d, 8 d, 12 d, 20 d and 34 d after collembola addition. Each image was cropped to remove background, resized to 1773×1773 pixels, and saved as an 8-bit greyscale .tif image. Image series were imported into a custom MatLab (The Mathworks Inc., Natick, USA) program (available from MDF on request) and aligned with respect to one another. Alignment was achieved by selecting consistent landmarks on successive images, and calculating a linear spatial transformation to correct for translation, rotation and scaling. The network was extracted as a series of N nodes, each representing a branch or anastomosis, joined by a set of K links representing the intervening cords. Node positions were stored as a list of their Cartesian (x,y) coordinates, whilst links were stored as a weighted $N \times N$ adjacency matrix, where each entry represents the diameter of the link between node i and node j (D_{ij}). As the structure of the network within the wood blocks cannot be characterised, the inoculum was represented as a single central node with multiple links leading to the cords emanating from the edge of the block (Figs 1 and 2). At each time point new growth was added as new nodes (with associated links), and complete regression was identified by disconnecting the relevant nodes. At this stage it is not possible to discriminate a genuine cord-fusion event from cords that are growing over each other. This will cause an over-estimate of anastomoses, particularly early in development before proper junctions have had time to become established. Nevertheless, manual dissection of fully networked systems shows that more established overlying cords are almost invariably linked in *P. velutina*. Nodes connected to only two other nodes (termed k2 nodes), representing a bend in a cord, were removed from the adjacency matrix during analysis and the weight of the resultant link between the junctions at either end adjusted to take into account the length and thickness of the intervening links.

Estimation of link weights

As cords differ in thickness, the links in the adjacency matrix were weighted by an estimate of the cord diameter between node i and node j (D_{ij}). The pixel resolution of the images was not sufficient to obtain direct measurements of diameter, so an average value of the local reflectance intensity was used as a proxy for cord thickness. Samples were taken 12 pixels away from each node along each cord to ensure that only intensities of the cord of interest were included. The local neighbourhood was averaged using a Gaussian smoothing filter with a radius of 5 pixels and the maximum intensity recorded. The values from both ends of each cord were averaged to give the intensity for that cord. Intensities were converted to diameter using a calibration based on the measured relationship between the reflected intensity of *P. velutina* cords with actual diameter (Bebber *et al.* 2007a). The cost of each link was estimated from its length (l_{ij}) times the cross-sectional area ($a_{ij} = \pi(D_{ij}/2)^2$), whilst the predicted resistance to transport was calculated as $l_{ij}a_{ij}^{-1}$, making the simplistic assumption that a cord comprises a circular bundle of equally sized hyphae. The link weight was colour-coded across the network according to a rainbow scale, with red representing thick cords. Development or regression of links was measured as

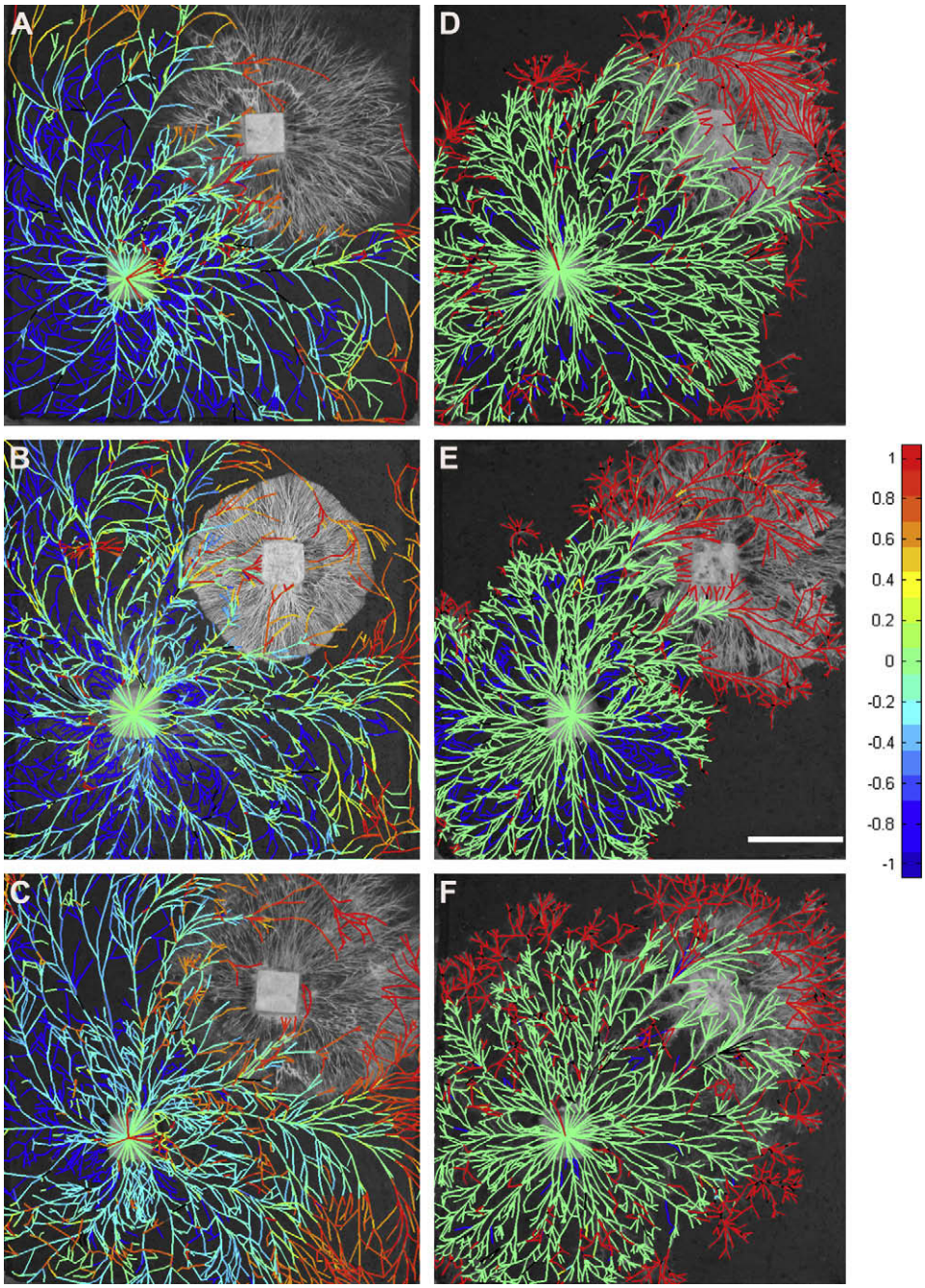

Fig 1 – Link evolution in all *Phanerochaete velutina* mycelial networks interacting with *Hypholoma fasciculare* when un-grazed (A–C) or grazed (D–F) between 8 d and 34 d. White scale bar, 5 cm. Rainbow scale bar values range from +1 for new growth, through 0 for no change, to −1 for complete disappearance of a given link.

the sum of the differences in cord diameter for successive time points, $\Delta D_{ij} = D_{ij(t+1)} - D_{ij(t)}$, over the interval 8–34 d, normalised to the maximum range of the difference, $\sum_{t=8}^{t=34} \Delta D_{ij} / (D_{ij,max} - D_{ij,min})$. This gives a value of 1 for consistently growing cords, a value of −1 for cords that shrink and 0 for cords that essentially remain constant through the time interval. Results for the link evolution were expressed as a rainbow scale, with red representing growth.

The weighted adjacency matrices, node positions and node identities were exported to R 1.9.0 (R Development Core Team 2006) to evaluate network structure and performance (Bebber *et al.* 2007a; Fricker *et al.* 2007). The area covered by the mycelium was determined from the convex hull encompassing all the outermost nodes. The total cost or biomass was estimated from the sum of the costs for each link, $\sum_{i=1}^{N} l_{ij} a_{ij}$. The predicted transport capability of the network was based on the path of least resistance between each node in the functional network, calculated using a shortest path algorithm (Gross & Yellen 2005; Long *et al.* 2008), with the assumption that low node-to-node resistance indicated better

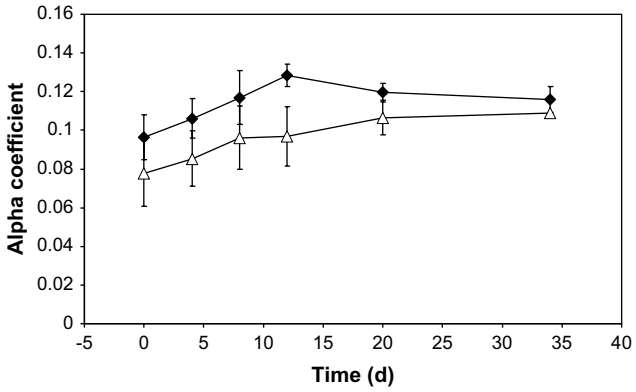

Fig 2 – Changes in the degree of cross-linking, measured by the α-index, in mycelial systems of *Phanerochaete velutina* interacting with *Hypholoma fasciculare* when un-grazed (◆) or grazed (△). Values represent mean ± s.e.m. ($n = 3$).

connection and more efficient nutrient distribution. The transport performance of the network was summarised as the root or local network efficiency (E_{loc}), defined as the mean of the reciprocal of shortest path lengths from the inoculum to all other nodes (Latora & Marchiori 2001, 2003). To examine the effectiveness of the way in which the fungus allocates biomass by differentially weighting cords, these measures were also calculated for networks with the same topology, but with uniform allocation of biomass to all areas of the network creating cords of equal weight (Bebber *et al.* 2007a; Fricker *et al.* 2008b).

Statistical analysis

Network development in grazed and un-grazed systems was compared using analysis of covariance. The analysis was performed for convex hull against root efficiency and convex hull against cost. For the root efficiency against convex hull data, comparisons were made between actual data and between model data assuming uniform distribution of biomass across all links. In all cases the model contained convex hull (area), treatment status (grazed or un-grazed) and an interaction between convex hull and treatment: the latter was of primary interest, testing for a difference in root efficiency–area or cost–area relationship in the presence of grazing. Because of pseudo-replication (inherent in time series on the same replicate), the linear regressions were fitted using generalised estimating equations (GEE), which adjust the regression model to allow for the potential correlations within trays, to permit valid statistical inference (Liang & Zeger 1986). As observations were taken over time, the correlation was modelled with a first order autoregressive structure (Liang & Zeger 1986). Models were fitted using the GEE generalised linear model (GLM) function in the geepack library in R. This form of analysis has been proposed as a suitable method for analysing datasets containing individual data points which are not statistically independent of one another, a phenomenon that is common in ecological research (Vaughan *et al.* 2007).

Results

Network development: link evolution

In all replicate experiments *P. velutina* formed a relatively sparse network in comparison to the more compact, dense mycelium formed by *H. fasciculare*. In the absence of collembola, *P. velutina* spread throughout most of the microcosm (Fig 1A–C). There was a marked regression of links in the *P. velutina* network for all un-grazed systems between 8 d and 34 d, predominantly in areas distant from the interaction, leading to a sparser network (Fig 1A–C). In contrast, although the total area covered by *P. velutina* was lower in grazed systems, the network was denser and there was much greater new network formation (Fig 1D–F). Furthermore, there was substantially greater overgrowth of *H. fasciculare*, with cords from *P. velutina* turning in from both sides and enveloping the *H. fasciculare* inoculum (Fig 1D–F). The grazed systems were characterised by much denser networks with many short branches, arising from continuous trimming of the fine hyphae and local stimulation of new growth. Nevertheless, fewer major links disappeared in the grazed systems, although there was variability between replicates with one colony showing more regression than the other two (Fig 1E), particularly in areas away from the interaction zone. Despite continuous grazing, more new cross-links were formed in grazed systems that connected radial cords distant from the inoculum, being especially pronounced in the two replicates that showed little loss of links (Fig 1D, F). These are highlighted in the graphs of network evolution as tangential red links appearing within the existing network. Thus, although there is an increase in terminal branches in grazed systems, part of the response appears to be an increase in new cross-links to maintain the integrity of the network.

While there were cross-links in un-grazed systems, these were fewer and many of them already existed prior to 8 d (Fig 1A–C). Thus, the link evolution graphs show considerable regression of links, coded blue, around the original inoculum, leaving behind a much sparser network. There was also correspondingly little new growth within the established mycelium and no evidence for increased tangential link formation.

The overall degree of cross-linking was calculated from the alpha (α) index or meshedness coefficient which normalises the number of cross-links present to the maximum number possible for a planar network with the same topology. A branching tree with no cross-links would have an α-index of zero, whilst a fully connected Delaunay triangulation of all nodes would have an α-index of 1. At the start of the grazing treatment, both grazed and un-grazed networks had an α-index of 0.08–0.1 which increased marginally to ~0.12, 5 weeks later. There was some evidence that the grazed systems had a lower α-index, particularly after 12 d. This may result from the initial effects of grazing damage, breaking links and stimulating proliferation of new growth that would both contribute to lowering the α-index, followed by an increase in tangential cross-links to restore the topology. However, as the α-index was slightly lower at the start for these systems it is not clear that this represents a predictable

hallmark of the response to grazing. Overall it appears that, despite the substantial differences in network density and the effect of grazing, networks evolved to have remarkably similar values for the α-index (Fig 2).

Link weight development within networks

In un-grazed interacting systems of *P. velutina*, about 12–15 major cords radiated away from the inoculum at roughly equal angular spacing and were interconnected through a network of narrower cords (Fig 3A). Following contact with *H. fasciculare*, link weight became progressively polarised, with thicker links developing towards the opposing *H. fasciculare* mycelium after 8 d (Fig 3C) and a substantial thinning out of the network, particularly in more distal regions after about 20 d (Fig 3E, F). There has to be some caution in interpretation of the estimated cord thickness in regions overtopping the *H. fasciculare* mycelium, as the local intensity used in the calibration may be biased upwards by reflectance from the underlying *H. fasciculare* mycelium. Nevertheless, preferential cord thickening was also apparent in cords *en route* to the interaction zone where such potential errors are not present (Fig 3B–F).

Initial development in grazed systems was similar, but polarised growth in the direction of the interaction started earlier and was more pronounced (Fig 3G–L). In general, the grazed networks covered a smaller area but had more, thicker cords compared to un-grazed systems. There was an even stronger allocation response towards the *H. fasciculare* interaction zone, with the *P. velutina* network invading the territory previously occupied by *H. fasciculare* and preferentially strengthening the cords contacting the opposition wood block after around 12 d (Fig 3J).

As might be expected, there was a significant positive correlation between mycelial area, measured from the convex hull, and network biomass in both grazed and un-grazed systems (Table 1; Fig 4). For any given area, the biomass of the grazed network was greater than in the un-grazed systems (Fig 4), reflecting the increase in density of these networks (Fig 1). Furthermore, the rate of biomass increase matched the rate of increase in area in the grazed systems, suggesting a constant biomass density as the network developed. The lower slope in the un-grazed networks reflects thinning out of the network as the area covered expanded, giving a decrease in the biomass density over time.

Network transport efficiency

The predicted transport efficiency of the weighted networks based on the shortest path from the inoculum to all other nodes (root efficiency, E_{loc}) declined slightly as the networks increased in area (Fig 5). The rate of decline, however, was not as great as might be expected from the simple increase in surface area covered, as preferential cord thickening effectively compensated for the increase in Euclidean separation between nodes and the physical path length. Thus, there was only a ~30 % decline in root efficiency with a 3 fold increase in area covered, equivalent to a 1.8 fold increase in effective colony radius. The root efficiency was ~1.5–2 fold greater for the weighted networks in comparison to the model networks with uniformly weighted links (Fig 5; Table 1). However, there was no significant difference in the relationship between convex hull and root efficiency, based on the comparison of regression lines, between the grazed and un-grazed actual systems or between grazed and un-grazed model networks (Table 1).

Discussion

Changes in biomass allocation during fungal interactions have been previously documented, based on total pixel counts and fractal geometry (Donnelly & Boddy 2001). However, the network analysis approach used in the present study followed the evolution of individual links and allowed, for the first time, visualisation of exactly where and when links were added and removed from the mycelial systems. The considerably greater proliferation of *P. velutina* mycelium over the opposing *H. fasciculare* in grazed compared with un-grazed systems was completely unexpected. Over-compensation and stimulation of growth following collembola grazing of *P. velutina*, in the absence of other basidiomycete mycelia, have been reported previously (Bretherton *et al.* 2006), but these changes followed cessation of grazing or were observed at low grazing pressure. The response differs further in the present study as the mycelium growing directly on soil did not increase the area covered generally, but did preferentially invade the *H. fasciculare* territory. The cause of proliferation on the opponent's mycelium is unclear. One effect of grazing damage to *H. fasciculare* might be to release nutrients that stimulate local growth of *P. velutina* (Tlalka *et al.* 2008) and act as cues for the presence of a more substantial organic resource nearby. Replacement of the opponent would allow access of *P. velutina* to those resources, which would in turn provide a location 'safe' from grazing.

Thinning out of mycelia distal to the interaction, as seen in the present study, has previously been detected (Donnelly & Boddy 2001). The reduced regression in the grazed systems, evidenced by link evolution and higher biomass density, was again unexpected, and our hypothesis that the biomass of the network in proportion to the area covered would be greater in un-grazed systems was rejected. A simple explanation would be that continuous grazing maintains the network in a developmentally juvenile state characterised by proliferation of fine hyphae rather than selective reinforcement of only a subset of routes and thinning out of redundant mycelium. Nevertheless, an advantage of maintaining extra-resource biomass might be a sacrificial strategy whereby collembola would graze on hyphae and fine cords, which would normally regress, leaving other parts of the mycelium intact and allow cords to grow wider and hence be less prone to destruction. There was certainly a dramatic increase in cord diameter (i.e. increasing link weight) of mycelium close to and over-growing the opponent in grazed systems in support of our first hypothesis. If the remaining mycelium were to regress as in the un-grazed scenario, the interconnecting cords might be placed at greater risk of disconnection. It is interesting to note that, despite the different mycelial densities between grazed and un-grazed systems, the local degree of cross-connection, measured by the α-index, converged at a similar value.

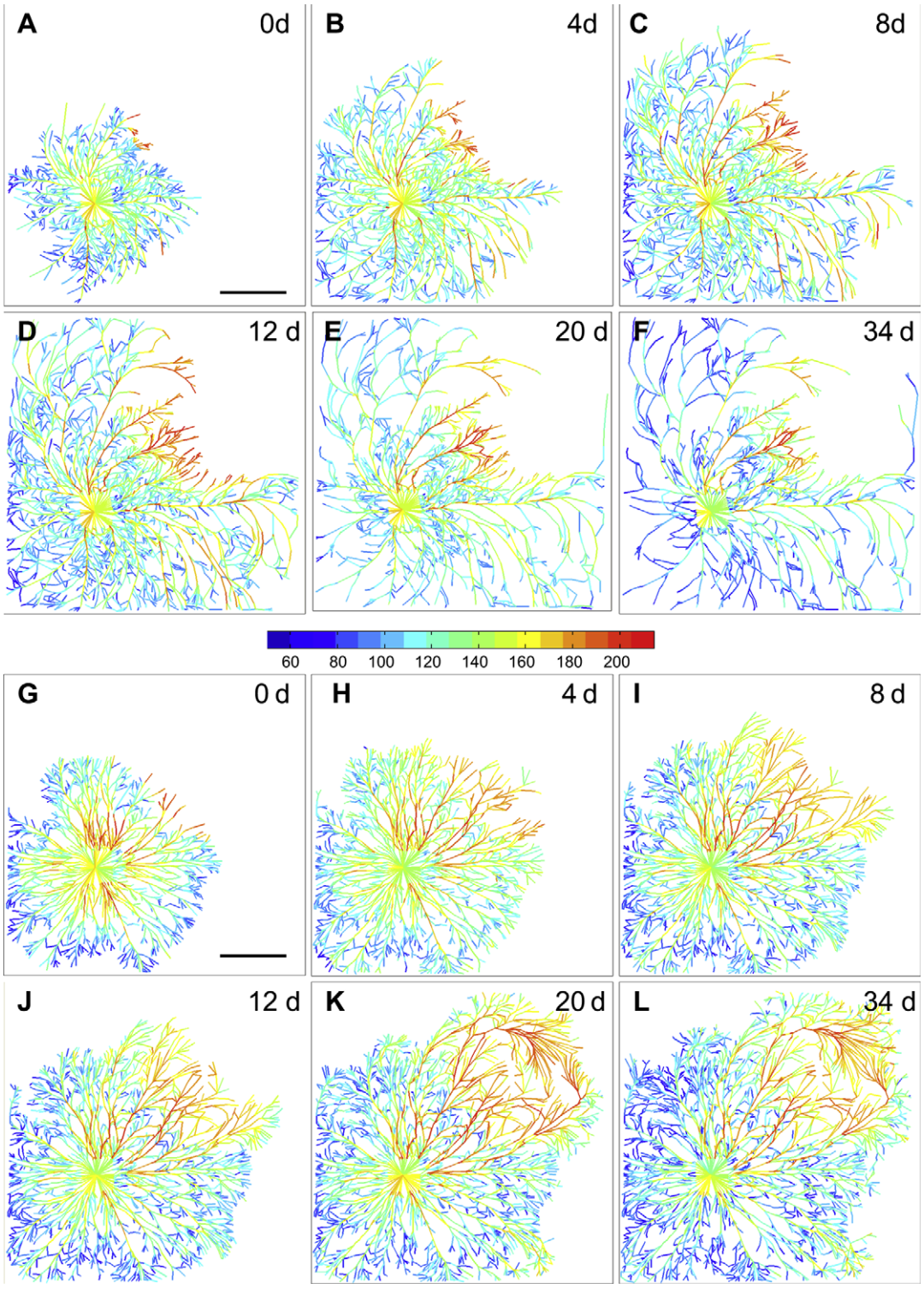

Fig 3 – Development of link weight in a representative *Phanerochaete velutina* mycelial network interacting with *Hypholoma fasciculare* when un-grazed (A–F) or grazed (G–L) at time intervals after addition of collembola. Rainbow scale bar indicates relative cord thickness based on reflected intensity, red being thickest cords. Scale bar is 5 cm.

While the grazed mycelium was more expensive to produce per unit area convex hull (i.e. a greater biomass per unit area), the occupied area at any particular time was less than in un-grazed systems. The cost of mycelial production could in future be determined by estimating decay rate of the wood inoculum, and comparisons made between different systems. The strategy of accelerated growth in *P. velutina* may be a risky strategy, as high expenditure on mycelium would consume the initial wood inoculum more rapidly, necessitating the rapid discovery of new resources.

Table 1 – Summary statistics for GEE GLM regression analyses of data in Figs 4 and 5

Descriptor		Coefficient	Standard error	Critical value (df = 1,32)	*P*-value
a	Y intercept (α)	0.0025	0.0002	112.2588	**<0.0001**
	X and Y correlation (β_1)	<0.0001	<0.0001	7.8056	**0.0052**
	Y intercept difference (β_2)	0.0007	0.0006	1.3886	0.2386
	Regression line gradient comparison (β_3)	<0.0001	<0.0001	1.0843	0.2977
b	Y intercept (α)	0.0017	0.0002	73.8352	**<0.0001**
	X and Y correlation (β_1)	<0.0001	<0.0001	31.4800	**<0.0001**
	Y intercept difference (β_2)	0.0001	0.0004	0.0835	0.7727
	Regression line gradient comparison (β_3)	<0.0001	<0.0001	0.0699	0.7914
c	Y intercept (α)	0.0018	0.0004	21.6787	**<0.0001**
	X and Y correlation (β_1)	<0.0001	<0.0001	14.3782	**<0.0001**
	Y intercept difference (β_2)	0.0014	0.0007	4.6354	**0.0313**
	Regression line gradient comparison (β_3)	<0.0001	<0.0001	0.8708	0.3507
d	Y intercept (α)	0.0017	0.0002	69.2373	**<0.0001**
	X and Y correlation (β_1)	<0.0001	<0.0001	29.6107	**<0.0001**
	Y intercept difference (β_2)	0.0008	0.0003	7.2344	**0.0072**
	Regression line gradient comparison (β_3)	<0.0001	<0.0001	0.0980	0.7543
e	Y intercept (α)	86.4358	13.9927	38.1579	**<0.0001**
	X and Y correlation (β_1)	0.0137	0.0011	143.5756	**<0.0001**
	Y intercept difference (β_2)	−58.4274	67.1661	0.7567	0.3844
	Regression line gradient comparison (β_3)	0.0111	0.0043	6.5733	**0.0104**

Comparisons between regression lines examining the correlation between: (a) convex hull (mycelia area) and root efficiency of actual networks in grazed and un-grazed systems, (b) as (a) but for model networks, (c) convex hull and root efficiency of actual and model networks in grazed systems, (d) as (c) but un-grazed systems and (e) convex hull and network cost of actual networks in grazed and un-grazed systems. The regression equation $Y = \alpha + (\beta_1 \times \text{area}) + (\beta_2 \times \text{treatment}) + (\beta_3 \times \text{area} \times \text{treatment})$ is employed where the treatment value is 0 for un-grazed (or model in actual *vs*. model comparisons) and 1 for grazed or actual systems. β-values are tabulated coefficients. For each GEE GLM: Y intercept is the estimate and standard error for the grazed (a,b,e) or actual (c,d) system and significance indicates a difference from 0; X and Y correlation shows whether both regression lines are correlated; Y intercept difference indicates any significant difference between the Y intercepts of the two regression lines; and the gradient comparison shows whether the two regression lines have a different gradient. Values in bold indicate a significant difference at <0.05.

The increase in cross-links within the established network of two of the grazed systems might again represent a defence against grazing. The higher the grazing pressure the greater the chance of parts of the mycelial network becoming disconnected from the inoculum resource. The greater the mycelial biomass, the less chance there will be of disconnection, following the arguments above. Likewise, the greater the networking, i.e. the more cross-links/tangential connections there are, the lower the chance of major regions becoming disconnected from the resource.

The lack of significant difference in network transport efficiency (measured as root efficiency) between the grazed

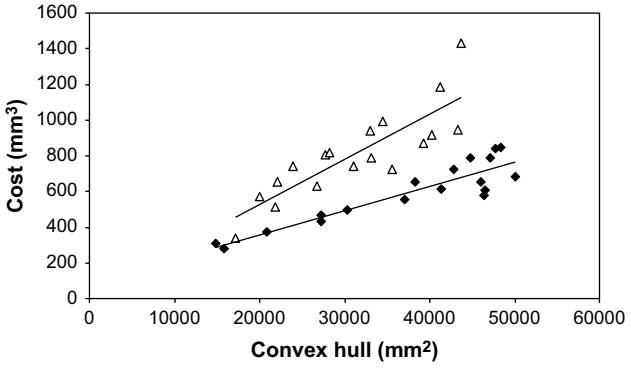

Fig 4 – Scatter plot (derived from 3 replicates) of convex hull (mycelial area) against cost of a *Phanerochaete velutina* mycelial network interacting with *Hypholoma fasciculare* when un-grazed (◆) or grazed (Δ). Grazed and un-grazed were significantly different ($P \leq 0.05$; Table 1).

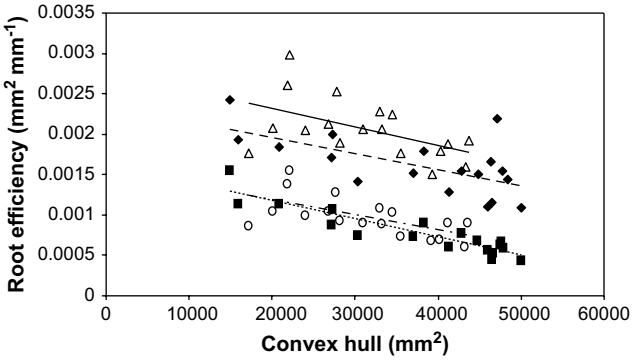

Fig 5 – Scatter plot (derived from 3 replicates) of convex hull (mycelial area) against root efficiency (transport efficiency) of a *Phanerochaete velutina* mycelial network interacting with *Hypholoma fasciculare* when grazed (Δ, · —) and un-grazed (◆, - - -). Data are also presented based on a uniform link weight for grazed (○, · · ·) and un-grazed (■, - · -) systems. Regression statistics are given in Table 1. Grazed and un-grazed systems were not significantly different ($P > 0.05$), but the actual and model systems were ($P \leq 0.05$).

and un-grazed systems emphasises mycelial robustness to grazing. Previous studies of *P. velutina* growing alone have suggested that *F. candida* grazing activity is concentrated on the fine mycelium and that substantial cords are largely unaffected (Tordoff *et al.* 2008). That *P. velutina* exhibits the same robustness during interactions is particularly important due to the likely need for nutrient transport to the interaction site as it is an area of heightened metabolic activity (Baldrian 2004). The decline in network transport efficiency with increasing area (convex hull) was expected as the distance over which nutrients are transported increases with increasing mycelial extent. Lack of difference in the rate of efficiency change with increasing area between grazed and un-grazed systems contrasts with the effect of grazing on mycelial biomass (discussed above), and further highlights the robustness of the network to grazing as mycelial coverage increased.

Robustness to grazing can be examined by artificial grazing ('bombing') digitised networks *in silico*, by random removal of links and determination of associated changes in network connectivity (Bebber *et al.* 2007a; Boddy *et al.*, in press). Saprotrophic cord-forming basidiomycetes on soil are extremely robust to *in silico* grazing, but robustness varies between species depending on network configuration. Thus *P. impudicus* with many cross-links is more robust than *P. velutina*, which in turn is more robust than *Resinicium bicolor* with few cross-links (MD Fricker & L Boddy, unpub.). There is a trade-off between the cost of producing highly interconnected networks that confer robustness and sparser networks that can extend further, increasing the chance of finding new resources but also increasing the chance of losing transport pathways if part of the network is damaged. The ability of a fungus to change its strategy by constructing a more highly interconnected network under conditions where damage is more likely is obviously highly advantageous. Natural fungal networks weighted by link area were more robust than mathematical 'toy' networks, containing the same amount of material, connected by a minimum spanning tree or Delaunay triangulation (Bebber *et al.* 2007a; Boddy *et al.*, in press). Evolution has thus resulted in a mechanism for achieving robustness while reducing overall cost.

In conclusion, analysis of network architecture has provided highly informative qualitative and quantitative information on mycelial development during grazed and un-grazed interspecific interactions in soil. *P. velutina* is robust to grazing and responded to it. Our hypothesis that *P. velutina* will reinforce the mycelial network in the vicinity of the interaction was accepted, but the other two hypotheses that collembola grazing will reduce transport efficiency due to severance of cords and that the biomass of the network in proportion to the area covered will be greater in un-grazed systems were rejected. As in the previous study on *P. velutina* growing alone (Bebber *et al.* 2007a), the ability of the mycelial network to modify dynamically link strengths is crucial to achieve adaptive polarisation of the network whilst maintaining a balance between transport capacity, robustness to damage and overall cost. At present, the network analysis tends to focus on global measures of the entire system, such as transport efficiency. However, fungal development is often polarised, particularly when another variable such as a resource (Tlalka *et al.* 2008), opposing fungus (Donnelly & Boddy 2001) or grazer (Kampichler *et al.* 2004) is present. Given that the network analysis includes detail of the behaviour of every node and link over time, it will be informative in future to develop new methods to analyse the fine-grained behaviour of the network in various regions to capture the different behavioural responses.

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