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## Modelling mycelial networks in structured environments

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### ABSTRACT

The growth habitat of most filamentous fungi is complex and displays a range of nutritional, structural, and temporal heterogeneities. There are inherent difficulties in obtaining and interpreting experimental data from such systems, and hence in this article a cellular automaton model is described to augment experimental investigation. The model, which explicitly includes nutrient uptake, translocation, and anastomosis, is calibrated for *Rhizoctonia solani* and is used to simulate growth in a range of three-dimensional domains, including those exhibiting soil-like characteristics. Results are compared with experimental data, and it is shown how the structure of the growth domain significantly influences key properties of the model mycelium. Thus, predictions are made of how environmental structure can influence the growth of fungal mycelia.

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### Introduction

Filamentous fungi grow in a diverse range of habitats. In the terrestrial environment their ecological roles, for example, in the cycling of nutrients, are well documented (Wainwright 1988), while the biotechnological applications of certain fungi continue to be investigated (Gadd 2001). The interaction between fungi and the built environment (e.g. *Serpula lacrmans*) and the role of mycelia in the medical sciences (e.g. certain *Candida* spp.) provides yet further instances of the diverse growth habitats of fungal mycelia. In certain cases, fungal growth is approximately planar, and, therefore, allows experimental investigation, either *in situ* or in an appropriate experimental system (e.g. in a Petri dish). However, in many cases, e.g. where the mycelium is expanding through a structurally heterogeneous environment, such as building materials and soils, fungal growth is not planar, and, therefore, it is difficult to obtain temporal data on the growth and function of fungal mycelia in such

circumstances. Although destructive techniques (e.g. extracting soil slices) provide the simplest way of physically observing a snap-shot of the mycelium, they are often complex to apply and have inherent difficulties in the interpretation of the data. Mathematical modelling provides a powerful and efficient tool to augment experimental investigation and allows the simulation and analysis of growth and function in fully three-dimensional environments exhibiting a diverse range of heterogeneities.

Historically two distinct approaches have been adopted for modelling fungal mycelia (see Davidson 2007, for a recent review). In many studies (e.g. Boswell *et al.* 2003 and references therein) the fungus has been represented as a continuous structure and differential equations describing the density of the mycelial network have been derived. Although this continuum approximation is ideal for investigating many of the properties associated with dense mycelia, it is incapable of generating an explicit hyphal network, such as those that form in highly heterogeneous conditions. Hence, a discrete

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approach is required for modelling mycelial growth in highly structured environments.

The first discrete model of mycelia networks was formulated by Cohen (1967), who simulated planar growth of generic branching networks, using fungi as an illustrative example. Cohen's approach, now often termed a 'vector-based' model, formed the basis of a number of subsequent models of fungal growth (Hutchinson *et al.* 1980; Kotov & Reshetnikov 1990; Yang *et al.* 1992; Meškauskas *et al.* 2004a,b). Of these models, only that of Meškauskas *et al.* (2004a,b) considered non-planar, that is fully three-dimensional, growth. The generated structures in all of these models can appear highly realistic but, because of computational difficulties and the necessary imposition of artificial rules, often neglect important characteristics of fungal networks. Anastomosis, in particular, was not considered in any of these vector-based models and neither was there a mechanistic relationship between the mycelium and its environment. Therefore, as a consequence of such omissions, simulated growth of mycelial networks in heterogeneous environments has typically been neglected.

An alternative approach to simulating the expanding mycelial network was adopted by Regalado *et al.* (1996), where the hyphal network was modelled by a continuous activator-inhibitor system. Although the model generated structures reminiscent of fungal networks and allowed for growth in nutritionally heterogeneous environments, there were difficulties in the identification of the activator and inhibitors for fungi. Moreover, this model again suffered from the absence of anastomosis.

Ermentrout & Edelstein-Keshet (1993) described a different method of simulating fungal growth by using cellular automata (CA). In such models space is divided into an array of 'cells' and the model variables take discrete values within each 'cell'. Boswell *et al.* (2007) adopted this approach by deriving a CA from a related continuum model and simulated fungal growth in a plane by restricting the hyphae to the edges of a triangular lattice. Although this approach did not generate networks that were as visually impressive as vector-based models, it did incorporate the important process of anastomosis (and so, unlike all the vector-based approaches, the modelled network was truly interconnected). Moreover, the interaction between the biomass network and the growth environment was explicitly modelled, allowing simulation of growth in nutritionally and structurally heterogeneous environments. Thus, the model was applied to simulate mycelial growth in soil slices.

In the current work, the planar model of Boswell *et al.* (2007) is reformulated to simulate growth in three spatial dimensions. The resultant model is, to my knowledge, the first capable of explicitly simulating the growth and function of fungal mycelia in soil-type conditions. The model is derived below and is focussed on the growth of the soil-borne fungal plant pathogen *Rhizoctonia solani*, but is sufficiently general to describe the growth characteristics of many mycelial fungi. The model is carefully calibrated and then applied to what represents uniform and soil-like growth conditions respectively. Model results are obtained in a manner that allows direct comparison with experimental results. Finally, the implications of the modelling results are discussed and compared with existing experimental data and predictions are made concerning fungal growth through complex media.

## Model description

The model described below was derived for the fungus *Rhizoctonia solani*, a soil-borne fungal plant pathogen capable of effective saprotrophic growth, to generate testable predictions. However, the model is sufficiently general to simulate the growth and function of other mycelial fungi by recalibrating and, if necessary, re-deriving certain expressions. For example, the model incorporates lateral branching, as exhibited by *R. solani*, whereas only a simple reformulation of the branching process would be required to model apical branching, as exhibited by, for example, *Neurospora crassa* (Riquelme & Bartnicki-Garcia 2004) and *Galactomyces geotrichum* (Webster & Weber 2007).

As with previous successful approaches (e.g. Boswell *et al.* 2003, 2007), the mycelium is modelled using three variables: active hyphae, inactive hyphae, and hyphal tips. Active hyphae, denoted by  $m$ , correspond to those hyphae involved in nutrient uptake and translocation. Inactive hyphae, denoted by  $m'$ , correspond to those hyphae still structurally present in the mycelium but no longer involved in growth. Hyphal tips, denoted by  $p$ , correspond to the end of active hyphae and provide the sole mechanism for biomass growth.

Although a combination of nutrients (e.g. carbon, nitrogen, oxygen, and trace elements) is essential for fungal growth, for simplicity and to enable the formation of testable predictions, it was assumed that a single generic substrate was responsible for biomass growth. The substrate was considered to be carbon because of its central role in fungal heterotrophy and data on the uptake and translocation of carbon by *R. solani* were readily available (e.g. Olsson 1995; Boswell *et al.* 2002), thereby facilitating the calibration of the model. To investigate the effects of various heterogeneities on fungal growth, it was assumed that the substrate existed in two forms, either external (i.e. free in the growth environment) or internal (i.e. acquired by the mycelium and held within the hyphae). The variables  $s_i$  and  $s_e$  denote the levels of internal and external substrate respectively.

The model is of a type most commonly-known as a CA. In such models, space is treated as a regular array and the elements in the array contain the local status of the model variables. Typically these variables take discrete values, but in the model constructed below, the substrates were, as was most appropriate, modelled as continuous variables. Hence, the model was of a hybrid discrete-continuum type. Time was also divided into a series of discrete steps, and at the end of each time step the status of each element was updated according to a set of prescribed (transition) rules depending, typically in a stochastic manner, on the status of the surrounding elements. There were several fundamental problems to consider when forming the CA, including choosing the most appropriate spatial and temporal scales, how to formulate the transition rules and how to calibrate the model. In the work described below, a simple and powerful technique was applied to create the CA from a previously calibrated continuum model. Thus, many of the above complications disappeared.

The growth environment was modelled using a face-centred cubic (FCC) lattice, which comprises a volume packed

as densely as possible with layers of balls of equal radii, and where each layer has the same basic hexagonal formation, but subsequent layers have a different alignment that repeats every third layer. Except at the boundaries, each ball is in contact with 12 neighbours; six from its own layer, three from the layer directly above, and three from the layer directly below. The centres of these balls (hereafter referred to as 'elements' in the FCC lattice) were used to denote the possible locations of hyphal tips, while the straight lines connecting the centres of adjacent elements were used to denote the possible locations of hyphae. It was assumed that internal and external substrates were equally accessible to all the hyphae and hyphal tips within that element.

For computational purposes, the FCC lattice was mapped to a standard three-dimensional cubic array. Each entry in the cubic array contained information about the corresponding element in the FCC lattice including the presence/absence of a hyphal tip, the presence/absence of hyphae in all of the 12 possible directions and the quantity of both internal and external substrate.

An established method (e.g. Anderson & Chaplain 1998) was applied to generate the transition rules in which a continuous partial differential equation (PDE) model was discretized. This powerful approach allowed the rules governing the change of the state variables to be formed in a simple and straightforward manner from the previously calibrated model of Boswell et al. (2003). Central to this process was the introduction of a spatial and a temporal discretization parameter,  $\Delta x$  and  $\Delta t$  respectively. The spatial parameter  $\Delta x$  defined the distance between the centres of adjacent elements in the FCC lattice and represented the maximum distance moved by a hyphal tip during a time interval of  $\Delta t$ . The value chosen for  $\Delta x$  is stated below, while the value of  $\Delta t$  was chosen such that the probabilities described below summed to less than unity (see Boswell et al. 2007, and comments below for details).

The hyphal tips of many mycelial fungi, including *R. solani*, move predominately in a straight line but exhibit small changes in direction. It has long been postulated that the cause of such a growth habit is a consequence of the delivery of tip vesicles from the Spitzenkörper and their subsequent incorporation into the hyphal wall (Reynaga-Pena et al. 1997). Any variation in the supply of tip vesicles to the hyphal walls may cause the small variations in the direction of growth alluded to above. By adopting this approach, a hyphal tip was, therefore, regarded as the end of a hypha and moved on average in a straight line (i.e. advection) but with some variation (i.e. diffusion), and the hypha itself was regarded as a trail left behind a hyphal tip as it moved. The straight line growth habit was hence considered to be a consequence of advective and diffusive growth, whereas the changes in direction were regarded as being due entirely to a diffusive process. Because of the shape of the hyphal tip and the manner in which the vesicles are supplied to the hyphal walls (Carlile et al. 2001), these changes in direction were assumed to be small and certainly at acute angles to the previous direction of growth. Moreover, since tip extension only occurs because of the supply of tip vesicles, and the tip vesicles are created only under a sufficient supply of nutrients (Carlile et al. 2001), it was assumed that tip movement in the model depended on local levels of internal substrate.

The probability of tip movement during a time interval of duration  $\Delta t$  was (see Boswell et al. 2007 for a detailed explanation of these probabilities)

$$P(\text{move in same direction}) = vs_i \frac{\Delta t}{\Delta x} + D_p s_i \frac{\Delta t}{\Delta x^2},$$

$$P(\text{move at acute angle}) = D_p s_i \frac{\Delta t}{\Delta x^2},$$

$$P(\text{do not move}) = 1 - P(\text{move in same direction}) - P(\text{move at acute angle}),$$

where  $D_p$  and  $v$  were non-negative parameters describing the strength of the diffusive and advective movement terms, respectively. That the probabilities depended on  $s_i$  reflect the fact that tip growth was a consequence of the supply of tip vesicles, which in turn arose because of the supply of internalized nutrients. Hence, the movement of hyphal tips increased with respect to internal substrate. The above modelling technique tacitly implied that hyphal tips exhibit a move/do-not-move characteristic, which has been recognised in mycelial fungi (Lopez-Franco et al. 1994). There is, of course, a metabolic cost associated with the movement of each hyphal tip, which was assumed to be proportional to  $\Delta x$ , the distance moved. Consequently, when a hyphal tip moved, the internal substrate in the corresponding element was reduced by an amount  $c_2 \Delta x$ .

*R. solani* exhibits lateral branching, whereby hyphal tips emerge from hyphal walls, and it has long been established that the build-up of vesicles and turgor pressure play important roles in such branching characteristics. Hence in the model system it was assumed that branching was related to internal substrate concentration and that the probability of an active hyphae branching during a short time interval  $\Delta t$  was

$$P(\text{branching}) = bs_i \Delta t,$$

where  $b$  was a non-negative constant describing the branching rate. The direction of branching was uniformly distributed from all the possible directions that were at an acute angle to the existing direction of hyphal growth.

The regular structure of the FCC lattice allowed anastomosis to be simply incorporated by assuming that it occurred when a hyphal tip moved into a FCC element that already contained a hypha. The hyphal tip was then removed from the lattice and a new network connection established.

Carbon translocation in *R. solani* is known to have a diffusive component and a metabolically-driven component (Olsson 1995), often termed *active translocation*, and therefore these components were modelled separately. Following Boswell et al. (2007), the amount of internal substrate moving from element  $k$  to element  $j$  due to diffusion over a time interval  $\Delta t$  was obtained using a finite-difference approximation

$$A_{\text{passive}} = \begin{cases} D_i \Delta t \frac{s_i(k) - s_i(j)}{\Delta x^2}, & \text{if } k \text{ and } j \text{ were connected by an active} \\ \text{hyphae,} \\ 0, & \text{otherwise,} \end{cases}$$

where  $D_i$  was a non-negative coefficient of diffusion. Active translocation is the process where internally-located material is moved to regions of the mycelium that require nutrients. Thus, throughout the mycelium it was assumed that there

was a local demand for nutrients and active translocation occurred in response to that demand. The demand was modelled by the function  $\Omega$ . Assuming that active translocation moved internal substrate to regions of greatest demand by using the gradients of  $\Omega$ , the flux of internal substrate from element  $k$  to element  $j$  due to active translocation over a time interval  $\Delta t$  was given by

$$\Delta_{\text{active}} = \begin{cases} -D_a \Delta t \frac{\Omega(k) - \Omega(j)}{\Delta x^2}, & \text{if } k \text{ and } j \text{ were connected by an active} \\ & \text{hyphae,} \\ 0, & \text{otherwise,} \end{cases}$$

where  $D_a$  was a non-negative constant. Since hyphal tips denoted the largest energy sinks in the mycelium, it was therefore reasonable to assume that they were the regions of the mycelium with the greatest demand for internal substrate. Consequently the function  $\Omega$  was set to be a decreasing function depending on the distance through the network to the nearest hyphal tip. For simplicity it was assumed that  $\Omega$  was a linear function and its value in an element was taken to be the negative value of the network distance between the element and the nearest hyphal tip, with the constant of proportionality absorbed into the parameter  $D_a$ . As with hyphal tip movement, there is a metabolic cost associated with active translocation. It was assumed that the internal substrate quantity in the element from which the substrate was translocated was reduced by an amount proportional to the amount of material moved during the time interval, and that  $c_4$  denoted that constant of proportionality.

To insulate hyphae and prevent internalized nutrients from leeching out of the mycelium, internally-located nutrients are used to maintain the structure of the hyphal walls (Carlile *et al.* 2001). Hence, it was reasonably assumed that each active hyphae of length  $\Delta x$  depleted the internal substrate from the appropriate element by an amount  $c_5 \Delta x \Delta t$  over a time interval  $\Delta t$ , for the purpose of hyphal wall maintenance. If after that subtraction the internal substrate was negative, there was insufficient energy available to maintain the hypha and it was assumed to become inactive (e.g. lysis) and had no further role in uptake, translocation or branching. Hyphae that became inactivated still formed part of the mycelial structure and were assumed to degrade at a constant rate  $d_i$ . Therefore the probability that a length  $\Delta x$  of inactive hypha degraded in a time interval  $\Delta t$  was given by  $d_i \Delta t$ .

*R. solani* acquires nutrients through the active transport of externally-located material across the plasma membrane. This process is auto-catalytic and depends upon the status of internally-located material to drive the process, the hyphal surface area over which the uptake occurs, and the amount of external nutrients available for uptake. Consequently the uptake process was modelled as depending on the amount of internal substrate in a hypha  $s_i/m$ , the amount of hyphae  $m$  and the amount of external substrate  $s_e$ . Typically such uptake processes are modelled using Michaelis–Menten dynamics, which involve the approximation of two parameters relating to uptake under low nutrient conditions and saturating effects. However, since in the current application the substrate concentration was assumed to be significantly less than that encountered in saturating conditions (see [Supplementary Material Appendix A](#)), a linearization of the Michaelis–Menten form was used (i.e., it was assumed that

the uptake depended in a linear fashion on the amount of substrate in a hypha, the amount of hyphae, and the amount of external substrate available to be taken up). Therefore the amount of substrate taken up during a time interval  $\Delta t$  was

$$\Delta_{\text{uptake}} = c \frac{s_i}{m} m s_e \Delta t = c s_i s_e \Delta t,$$

which depended on a single non-negative parameter  $c$ . As energy is lost when it moves between tropic levels, it was assumed that there was an imperfect conversion of external to internal substrate and the parameter  $c$  above was, therefore, less in the acquisition of internal substrate than in the loss of external substrate. That is, the quantity of external substrate was reduced by an amount  $c_3 s_i s_e \Delta t$  while the internal substrate was increased by an amount  $c_1 s_i s_e \Delta t$  where  $c_1 < c_3$ .

In the absence of ground water flow or other such advective processes, externally-located nutrients diffuse in a standard Fickian manner. The inclusion of advective processes will also influence the growth characteristics of hyphal tips and so as a first approximation and in the absence of any detailed experimental evidence, such advective flows were ignored. Hence, the amount of external substrate over a time interval  $\Delta t$  moving from element  $k$  to element  $j$  was modelled by a finite difference approximation

$$\Delta_{\text{diffusion}} = D_e \Delta t \frac{s_e(k) - s_e(j)}{\Delta x^2},$$

where  $D_e$  was a non-negative diffusion coefficient. There were no source terms of external substrate within the growth domain and hence the only external substrate available to the biomass was that present when the simulation was started.

The CA was written using the C programming language and, to ensure portability, was compiled and tested on machines running Windows and Linux. The results presented below were obtained on a 2.6 GHz Pentium 4 machine with 1 GB of RAM running Windows XP. In all that follows, unless otherwise specified, the model was solved in what represented a growth domain of approximately  $1 \text{ cm}^3$ , where zero-flux boundary conditions were applied at the edges of the domain. Therefore, there was no flux of external substrate across the boundary of the domain and similarly no movement of hyphal tips across the boundary. The initial data were representative of an inoculum positioned in the centre of the domain whereby the central element had 12 hyphae radiating outwards to the adjacent elements, each of which contained a hyphal tip. The initial internal substrate was confined to these 13 elements while the initial external substrate was uniformly distributed throughout the domain.

The model was calibrated for the fungus *R. solani* anastomosis group 4 (R3) (IMI 385768) growing at  $30^\circ \text{C}$  in a mineral salts media (see [Supplementary Material Appendix A](#) for a detailed description of the calibration procedure). The parameter values used are given in [Table 1](#). The spatial parameter  $\Delta x$  was chosen to be  $100 \mu\text{m}$  and was evaluated by comparing experimental data to the radial expansions obtained by the model (see [Boswell \*et al.\* 2007](#), for a complete description of the method). As explained above, the temporal discretization parameter  $\Delta t$  was chosen to be sufficiently small so as to ensure that the tip movement probabilities summed to less than unity and that stability of the numerical integration of



**Table 1 – Initial data and calibrated parameter values**

Initial variables/ parameter	Physical description	Calibrated value
$s_{i0}$	Initial internal substrate	1e-06 mol
$s_{e0}$	Initial external substrate	1e-06 mol
$V$	Directed tip velocity	1e-02 cm day <sup>-1</sup> mol <sup>-1</sup>
$D_p$	Diffusive tip velocity	1e-03 cm <sup>2</sup> day <sup>-1</sup> mol <sup>-1</sup>
$D_a$	Directed translocation	3.456 cm day <sup>-1</sup> mol <sup>-1</sup>
$D_i$	Diffusive translocation	0.3456 cm <sup>2</sup> day <sup>-1</sup>
$D_e$	Substrate diffusion	0.3456 cm <sup>2</sup> day <sup>-1</sup>
$B$	Branching rate	1e+06 cm <sup>-1</sup> day <sup>-1</sup> mol <sup>-1</sup>
$d_i$	Degradation rate	1e-02 day <sup>-1</sup>
$c_1$	Net uptake rate	9e+07 mol day <sup>-1</sup>
$c_2$	Cost of growth	1e-07 mol cm <sup>-1</sup>
$c_3$	Gross uptake rate	1e+08 mol day <sup>-1</sup>
$c_4$	Translocation cost	1e-11 mol cm <sup>-1</sup> day <sup>-1</sup>
$c_5$	Maintenance cost	1e-11 mol cm <sup>-1</sup> day <sup>-1</sup>

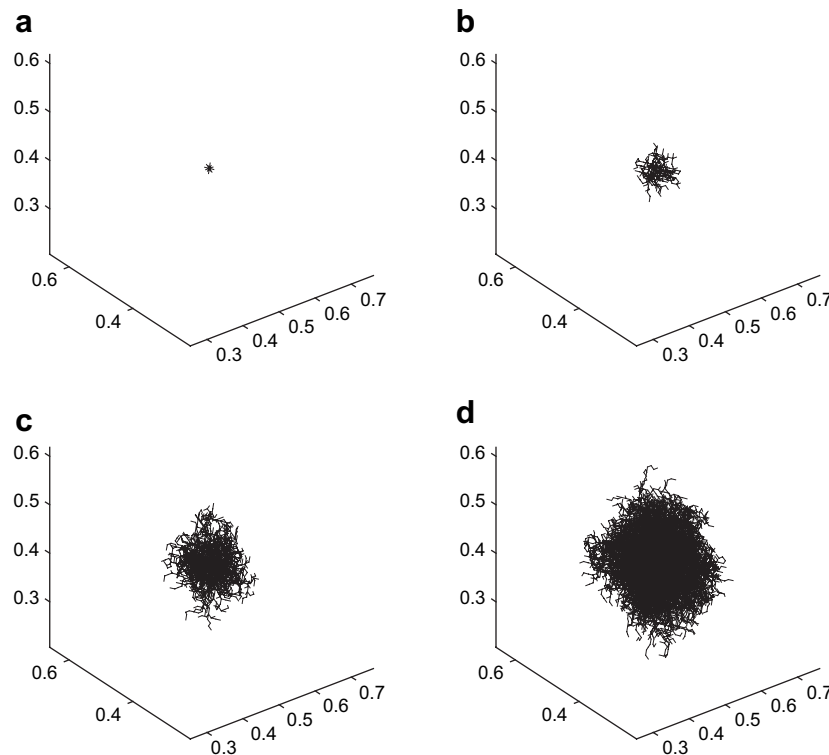
Details of the calibration experiment are given in [Supplementary Material Appendix A](#).

the substrate fluxes was preserved. If either of these conditions were violated, the simulation was aborted and restarted with a reduced value of  $\Delta t$  automatically selected to prevent further violations.

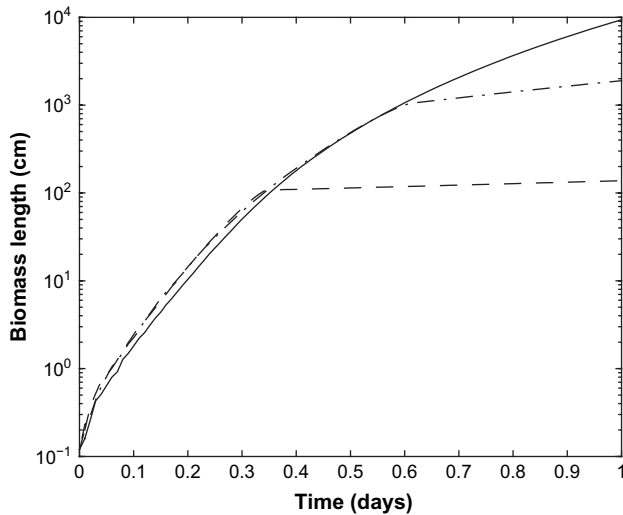
## Simulations under uniform conditions

The model was first considered in what represented initially uniform conditions corresponding to fungal growth through a homogeneous substance exhibiting no structural heterogeneity. To investigate the effects of nutritional conditions, a number of simulations were performed with different amounts of initial external substrate. The simulation was started with parameter values and initial data given in [Table 1](#). The model biomass expanded in essentially a radially-symmetric fashion, with the biomass density greatest at the colony centre but with a number of ‘fingers’ preceding the bulk of the biomass growth ([Fig 1](#)).

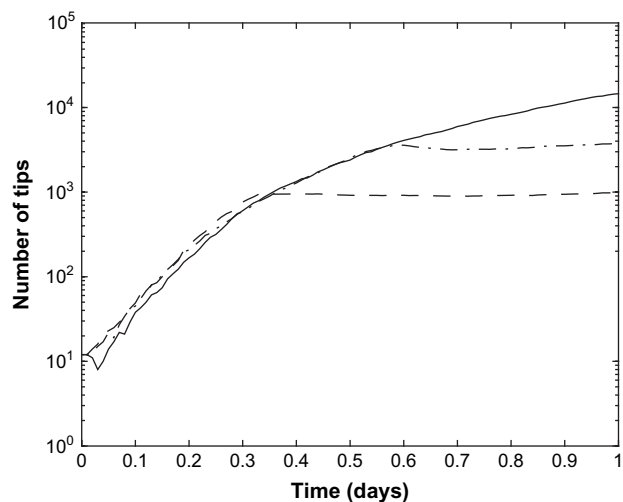
Total biomass length (*cfr* mycelial length in [Trinci 1974](#)), calculated by multiplying the total number of model hyphae by  $\Delta x$ , the length of an individual hypha, was determined at regular time intervals in each of the different growth domains. It was seen that after an initial transient phase, biomass length increased exponentially at a rate dependent on the external amount of substrate with the greatest growth rates corresponding to the greatest quantity of substrate ([Fig 2](#)). Such behaviour has been seen experimentally for numerous fungal mycelia ([Trinci 1974](#)). The initial increase in biomass length was independent of the external amount of substrate indicating that early biomass expansion was determined principally by internal substrate.



**Fig 1 – Biomass expansion from the centre of an initially uniform CA environment of volume approximately 1 cm<sup>3</sup>.** (a) The initial biomass distribution was chosen to represent an ‘inoculum’ at time  $t = 0$ . The biomass network expanded approximately spherically over times (b)  $t = 0.125$  day, (c)  $t = 0.25$  day, and (d)  $t = 0.375$  day, but exhibited a number of ‘explorer hyphae’ preceding the bulk of biomass. [A movie of this simulation is available as [Supplementary Material \(movie1.mpg\)](#)].



**Fig 2 – Biomass length plotted against time for model expansion in uniform conditions. The solid line denotes the biomass length obtained when the initial external substrate was  $s_{e0}$ , the dot-dash line denotes the biomass length when the initial external substrate was  $s_{e0}/2$ , while the dashed line denotes the biomass length obtained when the initial external substrate was  $s_{e0}/10$ . After a transient phase, the biomass length increased exponentially. In all cases the internal substrate levels were the same (see Table 1).**

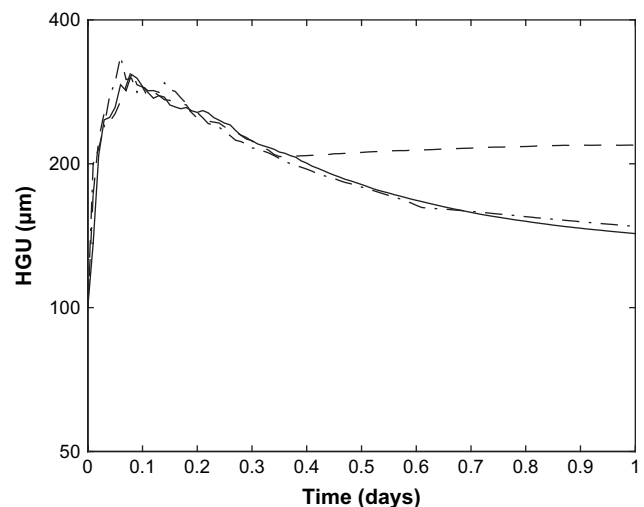


**Fig 3 – The number of model tips plotted against time for biomass expansion in uniform conditions. The solid line denotes the number of tips when the initial external substrate was  $s_{e0}$ , the dot-dash line denotes the number of tips when the initial external substrate was  $s_{e0}/2$ , while the dashed line denotes the number of tips when the initial external substrate was  $s_{e0}/10$ . After a transient phase, tip numbers increased exponentially. In all cases the amount of internal substrate was the same (see Table 1).**

The total number of hyphal tips was similarly recorded at various stages of biomass growth in the different domains. After an initial transient phase there was a period of ‘continuous tip production’ (cfr Trinci 1974) during which the number of tips increased exponentially (Fig 3). As above, the increase in hyphal tip numbers depended upon the status of external substrate.

A statistic of much interest in the investigation of mycelial networks is the hyphal growth unit (HGU), which is usually defined as the total mycelial length divided by the number of branches in the mycelium. The HGU, therefore, denotes the length of hyphae associated with each tip over the lifetime of the mycelium. When a fungus grows in a planar uniform environment under constant conditions, the HGU is a constant depending upon the fungus and the growth environment (Trinci 1974; Carlile et al. 2001). In the CA, the model HGU was calculated from the data and after an initial and brief increase, the model HGU decayed to a constant value (Fig 4). Such qualitative behaviour has often been observed in mycelial fungi such as *Geotrichum candidum*, *Aspergillus nidulans* and *Neurospora crassa* growing in planar conditions (Trinci 1974). Similar to the other data obtained from the simulations, the model HGU depended on the status of external substrate and obtained a greater value for domains having lower external substrate (Fig 4).

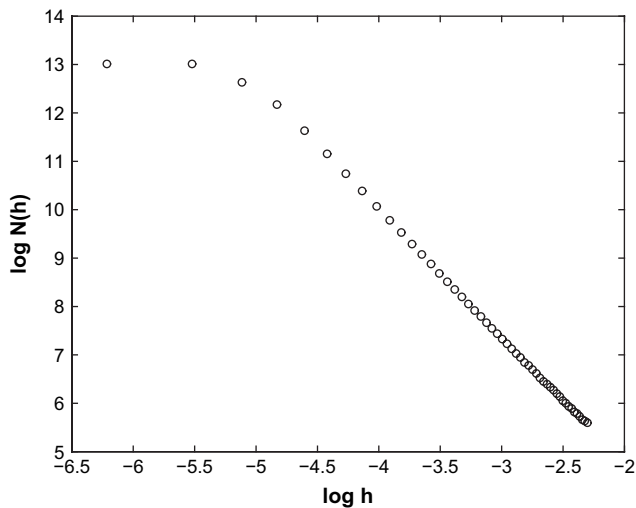
The simulated biomass network (such as that in Fig 1) was an approximate fractal structure (but not strictly fractal since it had a regular geometry) and hence its fractal dimension was approximated accordingly. The most common



**Fig 4 – The model HGU, calculated by dividing the total biomass length (see Fig 2) by the total number of branches in the biomass structure) plotted over time for biomass expansion in uniform conditions. The solid line denotes the HGU when the initial external substrate was  $s_{e0}$ , the dot-dash line denotes the HGU when the initial external substrate was  $s_{e0}/2$ , while the dashed line denotes the HGU when the initial external substrate was  $s_{e0}/10$ . The logarithmic scale allows direct comparison with related experimental data (Trinci 1974). In all cases the initial amounts of internal substrate were the same (see Table 1).**

method of calculating the fractal dimension of fungal mycelia uses the box-counting method (e.g. Boddy *et al.* 1999) where  $N(h)$ , denoting the minimum number of boxes (or cubes in three spatial dimensions) of side  $h$  required to completely contain the structure is determined for various values of  $h$ . As the fractal dimension  $D$  is related to  $N$  and  $h$  by the expression  $N = h^{-D}$  the negative value of the gradient of the regression line of  $\log N$  plotted against  $\log h$  gives the fractal dimension. This measurement of the fractal dimension is, therefore, only valid over those values of  $h$  such that the graph of  $\log N$  against  $\log h$  is linear. To determine the range over which the fractal approximation was valid in the model, a typical simulated network was examined and the number of cubes of side  $h$  required completely to contain the network was calculated for values of  $h$  between  $0.1\Delta x$  and  $5\Delta x$ . There was a linear relationship, and hence a valid approximation of the fractal dimension, for box sizes  $h$  between  $0.4\Delta x$  and  $5\Delta x$  (Fig 5) and therefore these were the ranges used in all calculations of the fractal dimension.

The fractal dimensions of the biomass networks for three typical simulation runs with the different initial amounts of external substrate described above were obtained (Fig 6). The fractal dimension initially increased at a rate independent of the external substrate. This was an unsurprising artefact given that internal substrate drove the initial expansion process and that the internal substrate concentrations were the same in the different simulations. However, after this initial transient phase, the biomass network continued to expand and its fractal dimension approached a limiting value proportionate to the quantity of external substrate.

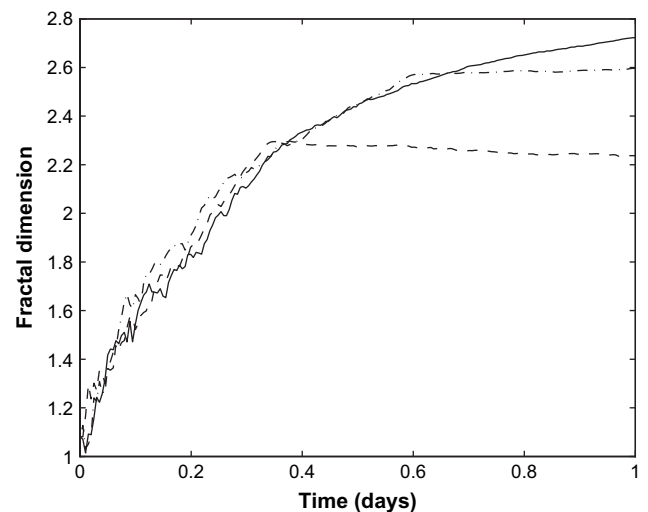


**Fig 5** – Plot of number of cubes required to contain a simulated mycelium against cube size. A simulated network was covered by a range of cubes of sizes  $h$  incrementing by units of  $0.1\Delta x$  from  $0.1\Delta x$  up to  $5\Delta x$  and the number of cubes required to completely contain the network were counted. The graph of  $\log N$  against  $\log h$  is approximately linear for  $h$  in the interval between  $0.4\Delta x$  and  $5\Delta x$  and hence the fractal dimension of the network was given by the negative value of the gradient of the regression line obtained by considering  $h$  over that interval.

## Simulations in a ‘soil environment’

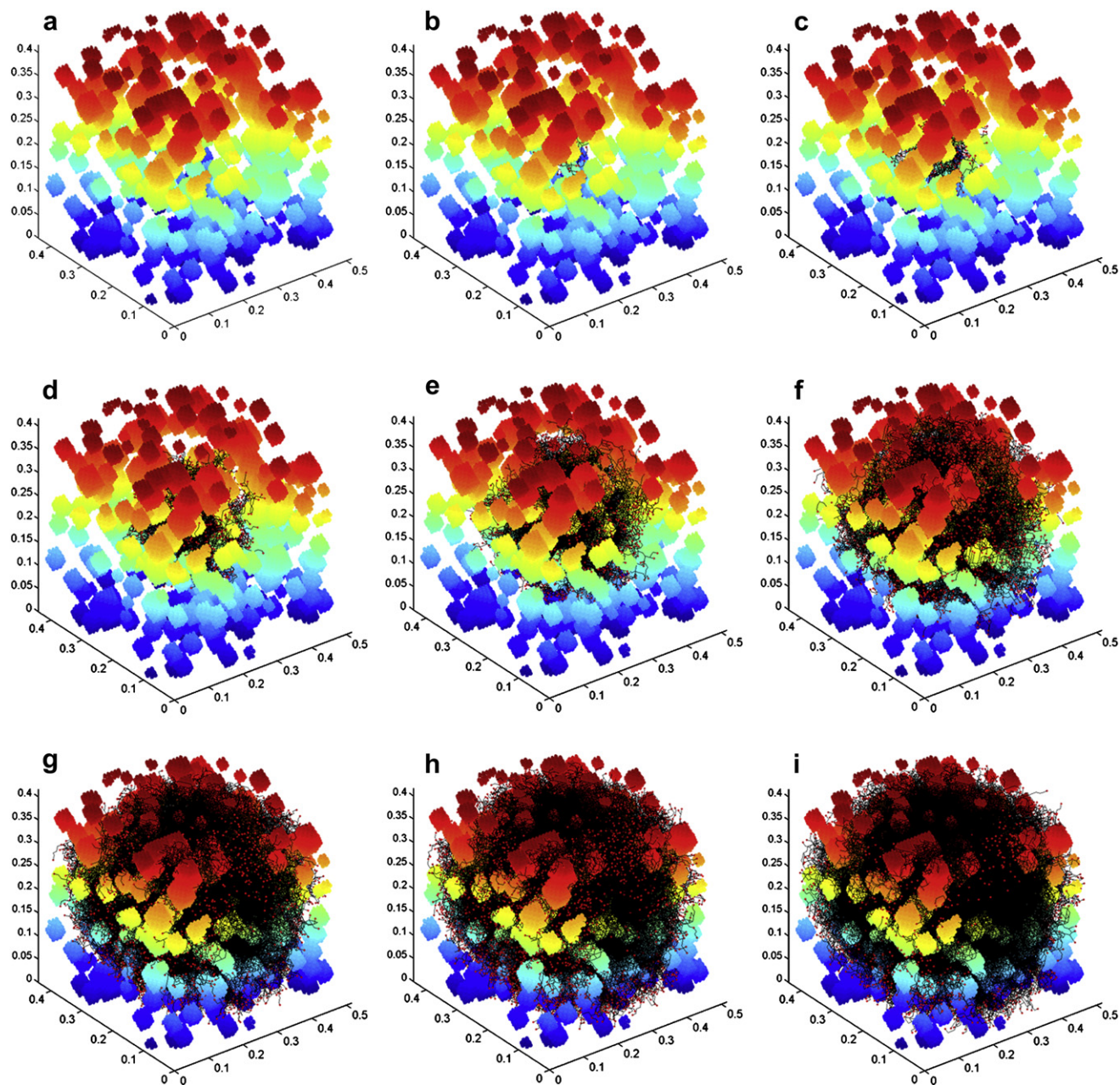
Typically fungal mycelia grow in conditions exhibiting both nutritional and structural heterogeneities, for example, soils. The model system can simulate a soil-like structure if elements are ‘removed’ from the FCC lattice (i.e. set to be uninhabitable) and so confining the expansion of the biomass network to the remaining elements. To this end, collections of elements (which can be thought of as soil particles, see Fig 7A) were ‘removed’ from the FCC lattice in a random fashion, and therefore, the remaining elements corresponded to the soil pore space in which the biomass could expand. The external substrate was initially set to be uniformly distributed throughout the representation of the soil pore space and was assumed to diffuse only among those elements forming part of the soil pore space, i.e. zero-flux boundary conditions were applied on the edges of the ‘soil particles’. Although this did not model soils in a strictly mechanistic sense, it incorporated the generic structures associated with soils systems in a simple manner that allowed investigation of the effects of various structural heterogeneities on mycelial growth.

As before, an ‘inoculum’ of biomass was introduced into the centre of the growth domain and allowed to expand using the rules described above. If any of the movement directions corresponded to relocation to a ‘removed’ element then the corresponding probability of that movement was set to zero and the excess probability was uniformly distributed among the remaining possible movement directions. Hyphal tip speed was, therefore, invariant under that rescaling. If, after that rescaling, no directions for tip movement were available



**Fig 6** – The fractal dimension of the expanding biomass network plotted over time. The solid line denotes the fractal dimension of the biomass structure when the initial external substrate was  $s_{e0}$ , the dot-dash line denotes the fractal dimension of the biomass when the initial external substrate was  $s_{e0}/2$ , while the dashed line denotes the fractal dimension of the biomass when the initial external substrate was  $s_{e0}/10$ . In all cases the internal amounts of substrate were the same (see Table 1).





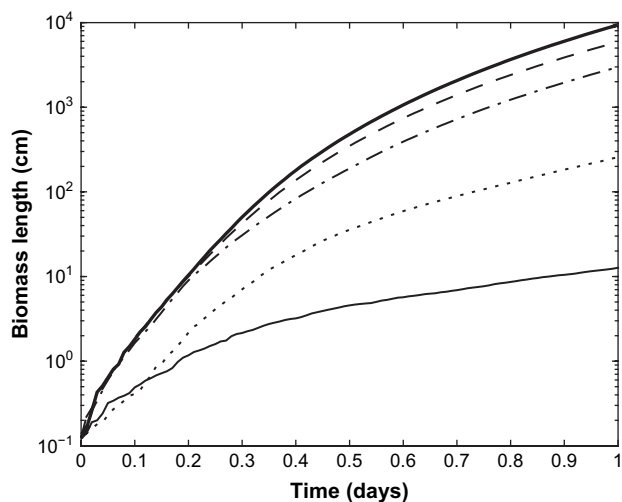
**Fig 7 – A simulated soil environment.** The FCC lattice had 16 % of the elements randomly removed while the remaining elements represent the soil pore space. For exposition, the ‘soil particles’ were coloured according to their z-coordinate (height), ranging from blue ( $z = 0$ ) to red ( $z = 0.5$ ), and the domain represented a cube of soil having side 0.5 cm. (a) The initial biomass distribution represented an inoculum placed in the centre of the domain. The biomass network expanded from the initial ‘inoculum’ and is shown at times (b)  $t = 0.05$ , (c)  $t = 0.1$ , (d)  $t = 0.15$ , (e)  $t = 0.2$ , (f)  $t = 0.25$ , (g)  $t = 0.3$ , (h)  $t = 0.35$ , and (i)  $t = 0.4$  day. [A movie of this simulation is available as [Supplementary Material \(movie2.mpg\)](#)].

then the hyphal tip was assumed to ‘die’ and was removed from the simulation.

Simulations were performed in several distinct growth domains having different proportions of elements ‘removed’ to investigate the effects of structural heterogeneities that corresponded to mycelia growing in soils of different densities. A typical biomass network is shown in [Fig 7](#) expanding over time from an initial ‘inoculum’ into an interconnected network intertwining the ‘soil particles’.

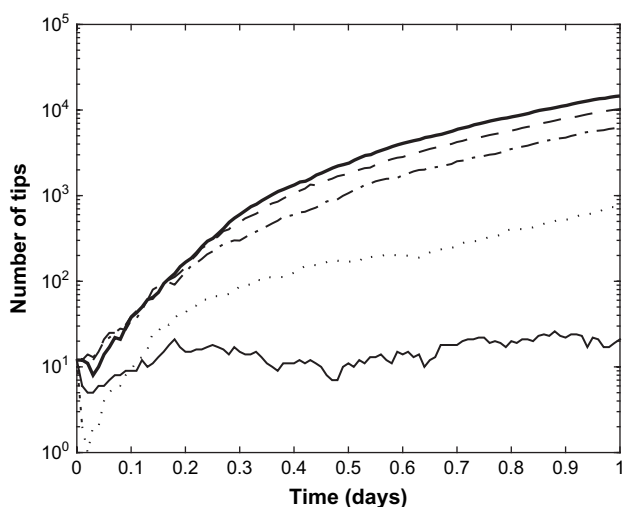
The total biomass length, the number of hyphal tips, and the model HGU were calculated throughout the simulations. The biomass length and the total number of hyphal tips were inversely proportional to the ‘soil particle’ density ([Figs 8–9](#)), which followed because of the restrictions in the volume available for biomass growth. Features of the biomass HGU in the soil-like simulations were qualitatively similar to that obtained under simulated growth in uniform conditions in that there was an initial transient period of exponential



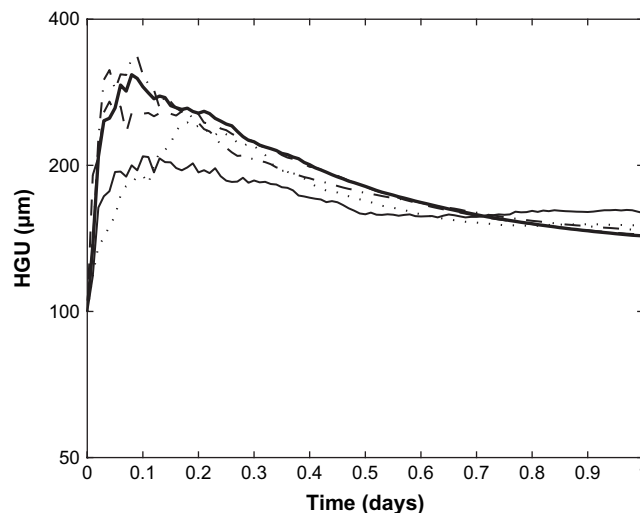


**Fig 8** – The total biomass length plotted over time for growth in soil-like conditions. The thick solid line denotes expansion in uniform conditions (i.e. the soil particle density was 0 %); the dashed line denotes expansion when the soil particle density was 16 %; the dot-dash line denotes expansion when the soil particle density was 32 %; the dotted line denotes expansion when the soil particle density was 64 % and the thin solid line denotes expansion when the soil particle density was 80 %. The initial data are given in Table 1. After an initial phase, biomass length increased exponentially except when the soil particle density was 80 %, where the length increased approximately linearly.

increase before tending toward a constant value (Fig 10). In the simulated soil systems, this constant value depended on the density of the ‘soil particles’ in the growth domain; the densest ‘soils’ gave the greatest HGU. Such a result may have



**Fig 9** – The total number of model tips plotted over time for expansion in soil-like conditions. Symbols as in Fig 8. The initial data are given in Table 1. The number of hyphal tips increased exponentially except when the soil particle density was 80 % and the number of tips was approximately a constant.



**Fig 10** – The model HGU plotted over time for biomass expansion in soil-like conditions. Symbols as in Fig 8. The initial data is given in Table 1. The logarithmic scale allowed direct comparison with existing experimental data (Trinci 1974).

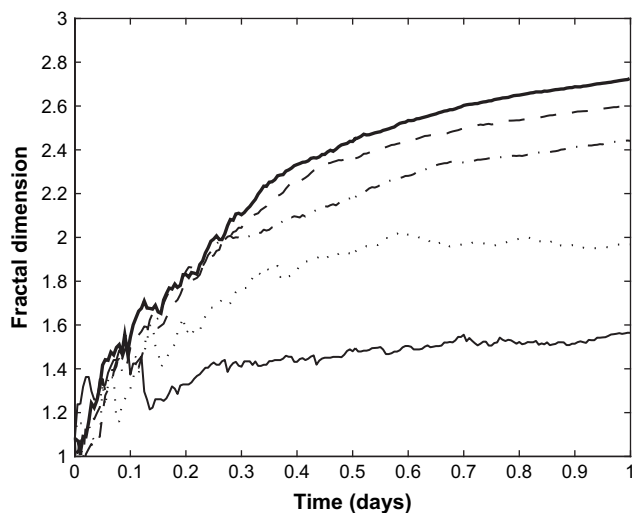
arisen because the restriction of the soil pore space resulted in the biomass having less opportunity to branch, there therefore being more hyphae associated with each hyphal tip, and consequently a greater HGU.

Not only was the biomass network an approximate fractal structure (Fig 6), but the representation of the soil pore space was also an approximate fractal structure. To this end, the box counting fractal dimensions were calculated for the model biomasses and the respective growth domains, as above (Fig 11). By randomly ‘removing’ elements of the growth lattice, the fractal dimension of the resultant growth domain was reduced, and this impacted on the fractal dimension of the expanding biomass network. As above, this impact was most prominent for what corresponded to densely-packed soils.

## Discussion

A mathematical model of fungal growth that incorporated the branching and anastomosing nature of mycelial networks has been constructed and has investigated the effects of various nutritional and structural heterogeneities found in soil systems. The model, a CA, was derived using the FCC lattice to form a structure containing the biomass network and a growth-promoting substrate. The state variables and probabilistic rules governing the expansion of the biomass network and its interaction with the growth environment were obtained by discretizing a previously calibrated continuum (i.e. differential equation) model. This powerful and efficient approach also naturally linked the microscale (i.e. the hyphal level) and the macroscale (i.e. the mycelium level).

The model was applied to both uniform and structurally-heterogeneous environments where the external nutrients were initially homogeneously distributed. Qualitative results



**Fig 11 – The fractal dimension of the model biomass structure plotted over time for growth in soil-like conditions. The thick solid line denotes expansion in uniform conditions (i.e. the soil particle density was 0 %) and the fractal dimension of the soil pore space  $D_{\text{pore}} = 3$ ; the dashed line denotes expansion when the soil particle density was 16 % and  $D_{\text{pore}} = 2.92$ ; the dot-dash line denotes expansion when the soil particle density was 32 % and  $D_{\text{pore}} = 2.85$ ; the dotted line denotes expansion when the soil particle density was 64 % and  $D_{\text{pore}} = 2.55$ ; and the thin solid line denotes expansion when the soil particle density was 80 % and  $D_{\text{pore}} = 2.19$ . The initial data are given in Table 1.**

obtained in uniform conditions were consistent with existing experimental data on total mycelial length, hyphal tip numbers and, surprisingly, on the HGU (Trinci 1974). Indeed, there was no concept of a HGU incorporated into the model and instead it arose solely as a result of local branching and hyphal tip growth. Moreover, the HGU was dependent on the growth conditions and obtained greater values in less favourable growth conditions. This observation is consistent with the widely accepted belief that fungi growing in low-nutrient conditions adopt an ‘exploratory’ phase where the HGU is greater than would be observed by the same fungi growing in nutrient-rich conditions. These results, therefore, strongly suggest that the HGU is a consequence, rather than a cause, of network growth in mycelia and, while being a useful statistic upon which to quantify mycelial networks, is not a requirement to generate such branched and anastomosed structures.

When growth was simulated in domains that exhibited soil-like properties, there were differences in the biomass length, number of hyphal tips, HGU and fractal dimension of the biomass network depending on the density of the ‘soil particles’ (Figs 8–11). These differences arose because an increase in the soil particle density corresponded to a restriction in the region available for biomass growth, as demonstrated by the corresponding reduction in the fractal dimension of the soil pore space (Fig 11). A notable feature common to all these data was that the differences were most apparent in the densest soils. For example, the differences between the biomass that expanded in 0 % soil particles (i.e. the uniform conditions)

and 16 % soil particles was much less than the differences between the biomass that expanded in 64 % and 80 % soil particles. Percolation theory provides the simplest explanation for such behaviour and has been successfully applied to problems in fungal growth where heterogeneities arose through nutritional differences in two-dimensional agar droplet tessellations (Bailey *et al.* 2000). However, the application of percolation theory is more suited to situations where the growth domain is divided into regions in which growth is possible or impossible, such as in the current investigation. If a small proportion of elements are randomly removed from the FCC lattice, the remaining structure (the soil pore space) is still connected, i.e. there exists a continuous route through the lattice connecting any two points. However, as more and more elements are removed from the lattice, the route connecting any two points becomes increasingly tortuous and eventually, when a critical proportion of elements are removed, the probability of being able to travel between any two points becomes less than unity. This proportion is called the critical percolation threshold and depends upon the geometry of the lattice. The notation  $p_c$  is used to denote the critical percolation threshold and is usually given in terms of the proportion of elements present rather than removed from the lattice. For an infinitely large FCC lattice, when elements are individually and randomly removed,  $p_c = 0.198$  (Stauffer & Aharony 1992). In the simulations considered above, the elements were not removed individually, but instead as collections of elements. The critical percolation threshold for such situations will, therefore, be close to, but less than, 0.198. The precise value of  $p_c$  depends on the shapes and relative frequencies of the collections of elements removed, i.e. the soil particles, and can only be determined by extensive computer simulation. Thus, when the soil particle density was close to the critical percolation threshold (i.e., 80 % soil particle density in the above simulations), the soil pore space was highly tortuous, as evidenced by its low fractal dimension (Fig 11), and biomass growth was therefore severely restricted.

The modelling techniques described above allowed mycelial growth to be considered in spatially-complex environments exhibiting certain characteristics typically found in soils. In particular, the CA model allowed a branched and anastomosed network to be formed that depended on the structure of the growth medium. It was seen that important aspects of fungal growth, e.g. total biomass and HGU, could be determined by specifying the structure of the growth medium and its nutritional quality. Clearly the potential for the control of fungal mycelia in this manner has numerous interesting implications and requires experimental investigation.

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## Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.mycres.2008.02.006](https://doi.org/10.1016/j.mycres.2008.02.006)

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