Emergent behavior of soil fungal dynamics: Influence of soil architecture and water distribution.

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13 Abstract

14 Macroscopic measurements and observations in two-dimensional soil thin sections indicate
15 that fungal hyphae invade preferentially the larger, air-filled pores in soils. This suggests that
16 the architecture of soils and the microscale distribution of water are likely to influence
17 significantly the dynamics of fungal growth. Unfortunately, techniques are lacking at present
18 to verify this hypothesis experimentally and, as a result, factors that control fungal growth in
19 soils remain poorly understood. Nevertheless, if only to design appropriate experiments later
20 on, it is useful to indirectly obtain estimates of the effects involved. Such estimates can be
21 obtained via simulation, based on detailed micron-scale X-ray computed tomography
22 information about the soil pore geometry. In this context, this article reports on a series of
23 simulations resulting from the combination of an individual-based fungal colony growth
24 model, describing in detail the physiological processes involved in fungal growth, and of a
25 Lattice Boltzmann model used to predict the distribution of air/liquid interfaces in soils. Three
26 soil samples with contrasting properties were used as test cases. Several quantitative
27 parameters, including Minkowski functionals, were used to characterize the geometry of
28 pores, air/water interfaces, and fungal hyphae. Simulation results show that the water
29 distribution in the soils is affected more by the pore size distribution than by the porosity of
30 the soils. The presence of water decreased the colonization efficiency of the fungi, as
31 evinced by a decline in the magnitude of all fungal biomass functional measures, in all three
32 samples. The architecture of the soils and water distribution had an effect on the general
33 morphology of the hyphal network, with a “looped” configuration in one soil, due to growing
34 around water droplets.. These morphological differences are satisfactorily discriminated by
35 the Minkowski functionals, applied to the fungal biomass.

36 Key words: fungal model, soil, Minkowski functionals, lattice Boltzmann model, X-Ray
37 computed tomography

38 According to available estimates, there may be as many as 1.5 million species of fungi in
39 terrestrial ecosystems (Hawksworth 1991). The activity of these fungi is crucial for the growth
40 of over 90 % of all vascular plants (Allen 1993), for which they constitute an essential life
41 support network (Bardgett et al., 2005, 2006). Fungi serve important functional roles as
42 nutrient recyclers and decomposers (Johnson et al., 2005). In exchange for carbon, they
43 provide soil-borne nutrients that are otherwise difficult for plants to access (White, 2003).
44 They protect plants against below-ground pathogens (Smith and Read, 1997), and fulfil a
45 range of other essential ecosystem services (Boumans, 2002).

46 In soils, fungal colonies grow as an interconnected network of hyphae, collectively
47 referred to as the mycelium, which propagates through the pore space. The hyphal tips
48 extend through the porous structure of soils, and, through these tips, the majority of nutrients
49 are acquired by the fungi (Ashford and Allaway, 2002). Resources are then distributed to the
50 more rigid fungal structures situated behind these tips, and which constitute the bulk of the
51 mycelium (Falconer et al., 2008). In part because of the rigidity of hyphae, contrasting with
52 the plasticity of their tips, and because of the average diameter of single hyphae, typically
53 much larger than that of bacteria, it has been conjectured for decades that 80 to 90% of
54 fungal hyphae may be restricted to the larger pores, in most soils. Also, since many fungi
55 appear to die off under conditions associated with full water saturation (Mitchell and
56 Alexander, 1962), it is reasonable to expect that fungal hyphae would predominantly occupy
57 the larger pores in soils, which are most likely to be air-filled under typical field conditions.

58 This restriction to larger pores has been observed experimentally by a number of
59 researchers using a variety of microscopic techniques (e.g., Hattori, 1988). Measurements of
60 fungal spread in light micrographs of soil thin sections, made by Otten et al. (1999) and
61 Harris et al. (2003), show preferential fungal exploration of the larger pores, and their virtual
62 absence in the finer ones. These observations are unfortunately mere 2-dimensional
63 snapshots of an evolving reality that unfolds in three dimensions. Nevertheless, they suggest
64 that the structure or “architecture” of soils, and more specifically, the geometrical features of
65 the pore space that this architecture harbors (Letey, 1991; Baveye, 2006), should profoundly

66 influence the propagation of fungal hyphae in soils, either directly or via the influence the soil
67 architecture has on the spatial distribution of water.

68 At present, it is not yet technically feasible to investigate, in real time, whether, and if so
69 how, fungal growth dynamics is affected by soil architecture. Part of the needed information
70 can be obtained, but unfortunately not all of it. Tremendous progress achieved in recent
71 years in the X-ray computed tomography (CT) of soils, carried out at synchrotron facilities or
72 using tabletop X-ray CT scanners, now allow researchers to obtain detailed data on the
73 geometry and topology of soil pores at sub-micron resolution (e.g., Sleutel et al., 2008). Even
74 taking into account operational problems that still affect the use of X-ray CT in terrestrial
75 systems, like those stemming from the thresholding/segmentation of CT grayscale images
76 (Baveye et al., 2010; Iassonov and Tuller, 2010), the resolution that can now be achieved is
77 in principle adequate to characterize the physical micro-environment of fungal hyphae in
78 soils. Unfortunately, visualization of the water distribution at that scale remains largely
79 unfeasible. Tippkötter et al. (2009) have recently been able to determine the distribution of
80 water in soil macropores using X-ray CT. However, measurements of the micron-scale
81 distribution of water in soil mesopores will have to await until thresholding issues be
82 resolved, suitable contrast-enhancing agents be identified, or procedures for dual-energy X-
83 ray CT scanning of soils be worked out, like those routinely used for gamma-ray scanning. A
84 similar situation pertains to fungal hyphae. The opacity of soils, as well as the virtually
85 identical X-ray absorption characteristics of hyphae and water, largely account for our
86 inability at this point in time to non-destructively monitor fungal dynamics in undisturbed soil
87 environments. If the technological advances of the recent past are any indication of how fast
88 CT applications in soils are likely to evolve in years to come, it may not take a decade for
89 researchers to be able to easily monitor fungal and water dynamics at micron scales in soils,
90 but one is not there yet.

91 In the mean time, however, if only to help in the design of future experiments, it would be
92 useful to try to estimate under what conditions the architecture and water regime of soils are
93 likely to exert a significant influence on fungal dynamics, based on the best information

currently available. In addition, one should also determine what quantitative characteristics of fungal hyphae, at different stages of their growth, are most sensitive to changes in soil architecture. Practically, such insight can be obtained at this stage only via simulation. Both for water dynamics in unsaturated soils and for the propagation of fungal hyphae on agar plates, sophisticated computer models have been developed in the last decade, and have been shown to provide a reasonably faithful, mechanistically plausible depiction of the behavior of real systems. In terms of water dynamics, theoretical approaches such as the Lattice Boltzmann (LB) models (e.g., Sukop and Thorne, 2006) can predict where liquid/vapor and liquid/soil interfaces are located at the pore (micro) scale. A key advantage of LBM over other approaches, e.g., mathematical network models, is their ability to envisage complex domain geometries, such as those obtained via X-ray CT. LBM has previously been used to demonstrate phase separation of fluids in a 2-D non-structured environment (Sukop and Thorne, 2006; Basit and Basit, 2010), a 2-D idealised porous medium (Sukop and Or, 2003) and a 3-D porous medium (Vogel et. al., 2005). Once the microscale water distribution is predicted by an LBM model, a computer program describing in detail the growth and metabolism of fungi in soil pores can be run to identify where one would expect fungal hyphae to propagate. A model of this process developed by Falconer et al. (2005, 2007) provides a very detailed account of the intricate network of diffusion processes and biochemical reactions that lead to hyphal elongation and propagation in various types of environments, from agar plates to soils. This model has been used, in particular, to model interactions among fungi, to link fungal individuals to community-scale patterns on plates and in soils (Falconer et al., 2008, 2010), and to analyze the effect of soil architecture on fungal growth dynamics. This model, however, has not yet been coupled with the output of LBM simulations to describe the combined influence of soil architecture and heterogeneous water distribution on fungal hyphae propagation. In order to quantify the impact of soil architecture and water on biomass distribution we can use the Minkowski functionals. The term “Minkowski functionals” is generally attributed to the collaboration of Georges Matheron and Jean Serra during their work that gave rise to the field of

122 *mathematical morphology.* The algorithm in its simplest form considers eight nearest
123 neighbour image elements in 3D space as forming a *cell* (i.e. a small but discernable
124 volume) for which measures are *estimated* based on a robust statistical treatment of the
125 available information. A prerequisite of the algorithms used is that each image element is
126 assigned membership of one of two classes; object or background, in other words the image
127 must first have been *segmented* using either a simple intensity threshold or some more
128 advanced technique. The first two measures belonging to the 3D Minkowski functionals are
129 the familiar *volume* and *surface area* about which little needs to be said other than to
130 reiterate that these are *estimated* measures based upon a particular scale of observation. In
131 other words, as more detail is included by employing greater resolution in the imaging
132 process, it is to be expected that many naturally occurring materials will exhibit significant
133 changes in measured properties (analogous with fractal problems such as measuring the
134 length of the British coastline). The remaining measures in 3D space are properly termed
135 *Integral Mean Curvature* and *Integral Total Curvature* neither of which is amenable to simple
136 and concise explanation; the interested reader is referred to Ohser & Mucklich (2000) for
137 formal mathematical definitions rooted in set theory and integral geometry. We will use the
138 Minkowski functionals to characterise the 3D geometry of the soil architecture, water
139 distribution and the fungal biomass distribution.
140
141 In this general context, the objectives of the research described in this article were
142 threefold. The first was to draw together a Lattice Boltzmann model of water distribution in
143 unsaturated soils and Falconer et al.'s (2005, 2007) model of fungal dynamics. A second
144 objective was to apply the combined model to a 3-D pore scale representation of soil
145 samples, obtained using X-ray CT. Finally, the last objective was to elucidate the role of soil
146 architecture and moisture distribution on fungal colonisation and to identify key geometric
147 parameters that most sensitively quantify this dependence of fungal dynamics on soil
148 architecture and moisture content (hereafter referred to as water content although the reader
149 should be aware of the density ratio limitation of the Lattice Boltzmann method used here

150 (Sukop & Or (2003))). Three different soil samples were selected as test cases, with
151 contrasting properties. To facilitate the analysis of the simulation results, several Minkowski
152 functionals are described and used to characterize not only the geometry of the pore space,
153 as has been done already by other researchers, but also the geometry of the air-water
154 interfaces and of the fungal biomass. Prospects for future research are discussed in detail.

155 MATERIALS AND METHODS

156 **Soil sampling and characterization by X-ray computed tomography**

157 Soil samples were taken from experimental plots established at the Scottish Crop research
158 Institute (Invergowrie, Scotland), on a Dystic-Fluvic Cambisol (FAO) with a sandy loam
159 texture (Sun et al. 2011). From 2003 onwards, these experimental plots were tilled annually.
160 Two soil samples, labelled P1 and P2, were taken from the top 0-5 cm from fields ploughed
161 yearly to a depth of 40 cm and disked, whereas another sample (N) was obtained from a
162 field subjected to zero tillage treatment, and where seeds have been drilled directly. These
163 samples were selected on the premise that they would exhibit contrasting pore-size
164 distributions.

165 Characterisation of the micro-scale heterogeneity of the soils was achieved by scanning
166 samples in a Nikon Metrology/METRIS HMX micro-tomography system (Nikon Metrology,
167 Tring, Herts, UK) at 150 kV and 50 µA, with a 2mm Al filter, and 1200 angular projections.
168 The radiographs were reconstructed into a 3-D volume using CT-Pro (Nikon Metrology,
169 Tring, Herts, UK) at a resolution of 35 µm, imported into VGStudiomax (Volume Graphics
170 GmbH, Heidelberg, Germany) and converted into image stacks with voxel-thick slices. Image
171 stacks were imported into ImageJ (open source software, National Institute of Health,
172 Washington, D.C., USA). A median filter was applied prior to automated thresholding using
173 the ISO-data procedure available in ImageJ. Small cubes, of size 128 * 128 *128 voxels,
174 were selected from the thresholded volumes to serve as input for the water distribution and
175 fungal growth simulations.

176 Lattice Boltzmann modelling of liquid distribution in soils

177 The Single-Component, Multiple-Phase Lattice Boltzmann (SCMP-LB) model developed by
178 Shan and Chen (1993; see also Sukop and Thorne, 2006) was used to predict the water
179 distribution, and in particular the liquid/vapor and liquid/solid interfaces, in the 3 soil samples
180 P1, P2, N. Previous research has shown that the SCMP-LB model can be used to predict the
181 liquid-vapor behavior of a fluid in partially saturated porous media (Sukop and Thorne 2006).
182 The Lattice Boltzmann model is viewed from a particle perspective where collisions,
183 streaming, and particle-particle, particle-surface interactions constitute the conceptual
184 framework. It is considered a bottom up approach to fluid dynamics and requires a reduction
185 in spatial and temporal state space in order to be tractable. The number of possible particle
186 positions and microscopic momenta are defined by the lattice used, here we use the D3Q27
187 which defines a three dimensional lattice with a neighbourhood of 27 containing 27
188 velocities. The model implemented here uses the standard collision and steaming operators
189 as described in Sukop & Thorne (2006) p 35. The parameters which are well cited in the
190 literature and used to describe the particle-particle and particle-surface interactions are
191 described below. Interparticle interactions characterize the forces between fluid particles
192 where G is the interaction strength and Ψ0 and ρ0 are arbitrary constants. A $G < 0$ results in
193 attraction between particles and the force is stronger when the density is higher, therefore
194 dense regions (liquids) experience a stronger cohesive force than vapour which leads to
195 surface tension phenomena. In addition to interparticle interactions for porous media the
196 particle-surface interaction is also required, this can be considered the wettability of the
197 porous media. The method used is based on Martys and Chen (1996) and is similar to
198 interparticle force calculation but the number of solid nodes in the neighbourhood is also
199 considered. By assigning a virtual density (vd) to the solid nodes we can alter the wettability
200 of the porous media. The higher the vd the stronger the fluid-surface interaction and
201 therefore the more wettable the surface. Values selected for G, ψ_0, ρ_0 and vd (Table 1) were
202 found in Sukop and Thorne (2006). They were also adopted by Basit and Basit (2010).

203 The resulting version of the SCMP-LB model, incorporating solid phase wettability, was
204 implemented using the open source PALABOS program (available at
205 http://www.lbmethod.org/palabos/), first in 2-D to reproduce the liquid-vapour phase
206 separation dynamics described by Sukop and Thorne (2006). The simulations were
207 performed on a mesh of 128 by 128 pixels. The entire domain was initialised with an average
208 density of 200 mu.lu^{-2} (where "mu" denotes a non-dimensional mass and "lu" a lattice unit),
209 and was perturbed with a random number in the interval [0, 1] at each node. The simulations
210 were then extended to three dimensions on a volume of 128 x 128 x128 voxels. The SCMP-
211 LB model was run on each soil structure (P1, P2, N), with two levels of wetness determined
212 by the initial average density of the fluid.

213 Concretely, as in the 2D simulations, the entire domain was initialised with an average
214 density (ρ_o) of 150 or 200 mu.lu^{-2} and was perturbed with a random number in the interval [0,
215 1] at each pore node, achieving two levels of partial water saturation, associated respectively
216 with the vapor and liquid phases. The associated volumetric liquid water content in each
217 case was determined by selecting a threshold water density above which a voxel is
218 considered to contain liquid water, and below which it is filled with vapor. This threshold
219 water density was determined by analysing the functional measures of the water. They
220 showed a very sudden transition at a certain density, which was used as the threshold value.
221 The initial average density of ρ_o = 150 was determined as the lowest value that was possible
222 while maintaining stability of the SCMP-LB model across the three soil samples, whereas the
223 use of the value ρ_o = 200 seemed justified in view of the fact that it has been previously used
224 in the literature (Sukop and Or, 2003; Sukop and Thorne, 2006; Basit and Basit, 2010). The
225 surface area and volume, in the form of Minkowski functionals, of the liquid phase is
226 calculated every 10,000 model iterations to monitor the dynamics of the LB model and to
227 ensure it is consistent with the 2D case. A periodic boundary was assumed for the 2D and
228 simulation runs were pursued until the systems reached equilibrium.

229 ***Fungal growth modelling***

230 The model of Falconer et al. (2005) is individual-based and incorporates the essential
231 physiological processes of nutrient absorption, within colony biomass transport and
232 recycling, inhibitor production and growth, and these occur differentially within a single
233 mycelium as a consequence of local and non-local contexts. This differential behaviour
234 permits different parts of the mycelium to expand and senesce concurrently as observed in
235 nature. The assumption is that all species of fungi carry out these processes to varying
236 degrees and this can be characterized by a trait set. This framework was developed to
237 capture the minimal set of physiological processes required to reproduce the observed range
238 in phenotypic response in the growth and development of single and interacting colonies.
239 These processes are known to be important for vegetative growth of fungi but have not
240 collectively been incorporated into a single framework. The model formulation represents
241 individual mycelial network growing in the environment as comprising three fractions:
242 insulated biomass (b_i), non-insulated biomass (b_{ni}) and mobile biomass (n). These
243 essentially relate to but are not limited to older inactive biomass, active hyphal tips and
244 internal resource respectively. The relative proportion of these components is dynamic and
245 determined by the physiological processes. Further, a fungal individual is characterised by a
246 trait set (genotype) which regulates the physiological processes and its interaction with the
247 environment. The model is based on a set of Partial Differential Equations (Box 1) which
248 represent the interdependencies amongst the types of biomass - non insulated (b_n),
249 insulated (b_i) and mobile biomass (n) and external resource (s) - and how these change
250 over space and time.

251 The set of modelled processes describe uptake of resource from the environment, the
252 conversion of this resource into mobile biomass, which can be translocated within the
253 structural fungal network. A key advancement of this modelling framework is the ability to
254 interconvert the mobile biomass into structural biomass and vice versa. This process allows
255 the fungal colony to recycle and reallocate its biomass depending on local environmental
256 context. The colony spreads through space by a diffusion process. The fungal colony can

257 also exude an inhibitor field which is proportional to the local mobile biomass concentration.
258 The presence of a non-self inhibitor field stops local spread of the colony.

259 This model has been used to show that single (Falconer et al. 2005) and interacting
260 colony Falconer et al. (2008) morphologies on agar plates, as observed in the laboratory, are
261 sensitive to the trait set controlling the physiological processes and the environmental
262 context. Also, simulation results indicate that specific physiological processes (biomass
263 recycling) are required for survival in resource-limited and heterogeneous environments
264 (Falconer et al. 2007). We also demonstrated the use of a physiologically-based model to
265 explore the factors that influence the nature of fungal community diversity, as well as the link
266 between individual behaviour and the structure and function of fungal communities (Falconer
267 et al. 2010).

268 ***Coupling of fungal growth and SCMP LBM***

269 The Lattice Boltzmann model described above is used to explore the effect of liquid/vapour
270 and liquid/solid interfaces on fungal colonisation using a completely air filled (dry) sample
271 and 2 levels of unsaturation (different water contents (wc)). New extensions to the fungal
272 model include response to the presence of water. Consistent with experimental work by
273 Otten et al (1999) we reduce the colony spread, in areas of high fluid density (liquid). The
274 distribution of fluid density derived from the LBM encapsulates how much vapour and liquid
275 is present in a given voxel. This density is mapped using linear interpolation to the diffusion
276 coefficient (D_b) governing colony spread (see Box 1) resulting in areas of the pore space with
277 dense fluid voxels having less colony spread. Similarly regions of the pore space that are
278 less dense will have more colony spread allowing more speedy spread. The spread of fungi
279 is now a function of water content (wc) and structure (v) i.e. (D_b function of (wc, v)) A
280 linear mapping of D_b to fluid density was used as this is the simplest continuous mapping
281 function.

$$\frac{\partial b_i}{\partial t} = \left(\frac{\zeta}{1-\zeta}\right) b_n + \gamma\left(\alpha_i \pi^{\theta} - \beta_i \pi\right) b_i,$$

$$\frac{\partial b_n}{\partial t} = (1-\zeta)\left[\nabla . D_b(wc, v) \nabla b_n + \gamma\left(\alpha_n \pi^{\theta} - \beta_n \pi\right) b_n\right],$$

$$\frac{\partial n}{\partial t} = \nabla . D_n(n) \nabla n - \left(\alpha_n \pi^{\theta} - \beta_n \pi\right) b_n - \left(\alpha_i \pi^{\theta} - \beta_i \pi\right) b_n + \left(\lambda_1 b_n + \lambda_2 b_i\right) s,$$

$$\frac{\partial s}{\partial t} = \omega(s_m) - \left(\lambda_1 b_n + \lambda_2 b_i\right) s$$

where:

$$\pi = \frac{n}{b_i + b_n}$$

$$D_b(wc, v) = \begin{cases} 0 & \text{voxel(v) = solid} \\ D_b = & \text{voxel(v) = pore,} \\ & D_{\max} \times wc + D_{\min} \times (1 - wc) \end{cases}$$

$$D_n(n, b_n, b_i) = \begin{cases} 10^{-7} D_b & n > n_0, b_n + b_i > 0 \\ D_b & n < n_0, b_n + b_i > 0 \end{cases}$$

282

283 Box 1 The mathematical model of fungal colony growth as in Falconer et al 2005. The
284 dependency of D_b to water content (wc) via a linear mapping is the main extension of this
285 work in order to couple with water dynamics. Dmax and Dmin correspond to the minimum
286 and maximum diffusion coefficients,

287 Fungal growth was initiated from a single plane (first z-y plane) of the 3D sample. The
288 growth of the colony is affected by colony traits and here we aim to assess the effect of
289 structural heterogeneity and distribution of water on fungal colonisation and not the intrinsic
290 properties of the colony (i.e. other colony traits) therefore we use the same colony traits in
291 the three samples (as in Falconer et al 2005). The fungi colonise the 3D structure until it
292 reaches the opposing plane from the inoculation (termed crossing time). The colonisation
293 ability of the fungi is described using the Minkowski functionals at the crossing time, as
294 explained above.

295 ***Geometrical characterization of pores, moisture distribution, and biomass***

296 The development of non-invasive techniques and the interpretation of the output of
297 modelling studies that predict spatial dynamics and interactions in 3-D, requires the
298 development of novel spatial descriptors. Simple characteristics such as volume and
299 connectivity of pores were previously used to analyse the impact on fungal growth (Pajor et
300 al. 2010; Kravchenko et al.,2011). However, more advanced descriptors can be used for the
301 3D structures of pore space, water and biomass volumes using the fundamental set of
302 Minkowski functional measures. In the case of 3-dimensional space, the Minkowski
303 functionals are a four-tuple of linear measures that describe an object within the space
304 (Hadwiger 1957).The measures relate to volume, surface area, curvature and the topological
305 measure Chi (the Euler-Poincare characteristic). The significance of the first two measures
306 stems from the fact that the volume and surface area of pores within a soil sample strictly
307 limit the biomass that can be accommodated. The Integral Mean Curvature (IMC) measure
308 describes the manner in which the surface of an object fills space; a large positive value
309 implies a surface that is predominantly convex whereas a large negative value implies
310 concavity. The topological measure, also referred to as the Integral of Total Curvature (ITC)
311 describes the overall form of an object; a large positive value implies a disjoint object
312 consisting of many isolated fragments, a large negative value implies an object that is
313 punctured by many holes. Figure 1 shows the relationship between binary structures and
314 Minkowski measures for simple geometries. Vogel et al. (2010) apply Minkowski functionals
315 to (static) soil structures imaged at different resolutions. In this case, each functional
316 measure is calculated as a distribution over pore size classes (the volume measure can
317 therefore be interpreted as a dimensionally scaled pore size distribution). This approach
318 allows information obtained over a range of spatial scales to be combined, thus revealing a
319 broader picture of structural properties.

320 The thresholded 3-D tomography images, the water distribution predicted by the LBM model,
321 and the predicted fungal networks in the three soils, at different water contents, are analysed

using algorithms described by Ohser and Mucklich (2000) (implemented in bespoke software) to obtain estimates of the four Minkowski functional measures, i.e., the volume fraction (VF), surface area (SA), integral mean curvature (IMC), and integral total curvature (ITC). In fact these measures can provide more intuitive information when expressed as ratios e.g. the measure-wise ratio of one object relative to another. This cancels out the units of each measure and makes for numerical values that may be easier to handle and interpret. Also, the ratio of two distinct measures may be taken for a single object (in which case a derived physical unit will result) in order to summarise some more abstract property.

These measures are hereafter standardised to the canonical [0, 1] interval, i.e., each volume image is treated as being a cube of unit sidelength, and standardised Minkowski functional measures are computed on this basis. In particular, the volume functional measure is simply the pore (object) volume fraction. When considering water or biomass, the functional measures are standardised relative to those of the relevant binary (pore-versus-solid) image, i.e., relative to structural features of the soils. In addition to the Minkowski measures for tomographic images the pore size distribution is calculated. For each pore voxel, one determines the sphere of maximum diameter that fits at least partly within the local pore space with the proviso that pore space having been previously covered by another sphere is excluded from the calculation. Diameters (exceeding a small threshold) are recorded and used to compute the pore size distribution (Figure 2).

Due to the number of methods and models used in this paper we present a schematic which provides an overview of how these are used in the context of this research (Figure3).

RESULTS AND DISCUSSION

Physical pore space properties and functionals of pore space

The soil structure metrics show that N has the lowest porosity and connected pore fraction (Table 2). P1 and P2 are similar in terms of porosity and connectivity but the surface area functional is much less for P1. Surprisingly sample N has a larger surface area than P1

suggesting a more twisty and tortuous pore space. The IMC and ITC measures are much larger, by an order of magnitude, for N than P1 and P2 samples. IMC is positive for all three samples implying that the space that describes the pore surface is convex. IMC for N sample is large and positive implying a disjoint object consisting of many isolated fragments and this is consistent with a connected pore fraction of 62.84%. Samples P1 and P2 contain mostly large pores, with sample P1 containing a higher proportion of larger pores than P2. The largest pore diameter was 45 voxels in P1 and 30 voxels in P2. The pore size distribution of N is quite different with all pore diameters smaller than 15 voxels.

Evolution of functionals of liquid phase from SCMP-LB

Using PALABOS and the parameter values in Table 1 we extended the previously published phase transition dynamics in 2D (Sukop and Thorne 2006, Basit and Basit 2010) to 3D porous media. Figure 4 a) – c) shows the evolution of phase transition for a 2D, non-structured environment. Figure 4 (d-f) shows the segregation of the vapour/liquid phases within the pore space for sample.

If we consider all four Minkowski functional measures simultaneously, the liquid volume is characterised as a point in 4-dimensional Minkowski functional space; a full comparison of the geometry of the liquid volume therefore tends to become a 5-dimensional problem. In either case, the high dimensionality requires that the information to be presented either in tabular form or as a set of graphs. This can be a relatively "indigestable" presentation format and so there is an incentive to consider only a subset of the functional measures. For example, the relationship between volume and surface area can be quite informative; for any given volume the minimum surface area is achieved by a sphere, as the surface area increases from this minimum then the object becomes less sphere-like and this has important implications for the relationship between points interior to the object. Hence a scatter plot of surface area against volume permits an immediate visual comparison of many different data sets (simulation time points for example) (Figure 5). Here we present the evolution of surface area and volume functional ($\rho 0 = 200$) for the three structures during the

course of the SCMP LB simulation. The reduction of both surface area and volume fraction, as seen for the 3 structures, indicates that aggregation of the higher density phase (liquid) is occurring due to the interaction forces in the SCMP LB model and the Minkowski functionals, SA and VF, can be used to determine when the system is in equilibrium. This is consistent with the 2D droplet formation in Figure4. We can see from Figure 4 a large reduction in the SA and VF at the second time step and this is due to the interface minimization that occurs as a result of the interparticle and particle-surface interactions. Essentially the 1st and 2nd time points on Figure 5 relate to Figure 4a and Figure 4b where we start off with a noisy density distribution (large SA and VF) and this is subsequently reduced. The surface area and volume fraction of the liquid phase are less than the corresponding surface area and volume fraction of the pore space, as liquid phase is constrained by pore phase. There are also distinct differences among 3 structures with respect to volume fraction occupied and surface area and by plotting the volume fraction and surface fraction we can clearly see the functionals can separate the structures in surface/volume parameter space. The last time point for SCMP LB model is then used to provide the air/liquid interface configuration and input into the fungal growth model. There is less change over time for N, compared with P1 and P2. N has a much lower volume fraction but a relatively high surface area, indicating tortuous distribution. The water content associated with each sample at the two initial densities was determined providing an indication of the water content of the sample and is provided in Table 3. The table demonstrates that water content increases with an increase in average initial density (ρ0) over the three structures, and the water content appears to increase, not with porosity, but with the increased occurrence of large pore diameters.

To illustrate how water distribution will impact on fungal colonisation the linearly mapped diffusion coefficients, from fluid density, for fungal spread are shown in Figure 6 for sample P2 where a) is completely air filled, b) has a water content of 2.96% and c) has a water content of 4.75%. We can see that large sections of the pore space is liquid filled (dark blue pixels) as predicted by SCMP Lattice Boltzmann method, and these areas are less likely to

be invaded by fungal colony. This fundamentally alters the connectivity of the pore volume and may have consequence for fungal colonisation and interactions.

Fungal Invasion

Inspecting table 4 and Figure 7we conclude that the presence of liquid (water level) has decreased the magnitude (i.e. ignoring the sign of numbers) of all fungal biomass functional measures in all three samples. Such a consistent decrease in magnitude strongly indicates a progressive restriction on growth i.e. fungal colonisation is impeded due to pore space being made inaccessible by the presence of water. Further evidence supporting this conclusion is provided by the trend of increased crossing time in relation to water content. We can see that as the water content is reduced the Minkowski measures for the fungal colony tend towards that of the pore space, however these are not exactly the same due to areas of the pore space being disconnected.

Looking in more detail at the functional measures it is possible to deduce some interesting characteristics of the fungal biomass spatial distributions. In the case of the N sample, the IMC measure is far greater than that of P1 and P2 while the converse is true in terms of ITC. These factors, in conjuction with a surface area that is large in relation to the volume occupied, strongly indicate that N has a more spatially convoluted or tortuous fungal network. Additionally, the strongly negative ITC measure of N biomass indicates that it is punctured by many holes i.e. the fungus has grown around many obstacles (solid structure and water distribution) giving a significantly "looped" morphology. All of these same general conclusions may, to a lesser degree, be reached for the P1 structure. Contrastingly, in the case of P2, a large positive value for the ITC measure indicates a significantly disjoint morphology i.e. the biomass exists as numerous distinct "clumps".

By plotting the surface area vs volume functions we can clearly see that there is clustering based on structure/sample and some separation within clusters due to water content. Figure 7 illustrates most variation within P2 cluster, intuitively one might assume the sample with the largest water content would most strongly inhibit colonisation but it is the location of the water that is most important. This may be due to the location of the water

blocking off the growth channels completely. The effect of water is least in the case of the N structure.

DISCUSSION AND CONCLUSIONS

We showed that both structure and water distribution impacted on fungal colonization as characterized by the Minkowski functionals. Water content decreased both surface area and volume available for biomass for the three samples reflecting reduced ability to colonize. This decrease is consistent with reduced colonisation due to inaccessibility of pore space due to presence of liquid. This is the first attempt, as far as the authors are aware, that such measures, which are a valuable tool describing the geometric structure of an object, have been applied to fungal networks and fluid distribution. The set of functional measures: surface area (SA), volume fraction (VF), integral mean curvature (IMC) and integral total curvature (ITC) forms a multi-dimensional space in which each point summarises key structural properties. Transformation of structure can thus be understood as motion through functional space. Although only sub-spaces can be graphically presented (surface vs volume and ITC vs IMC) these can still provide useful insight into patterns of temporal change and for classifying fungi response, in terms of colonisation capacity, to structure and moisture. For both the Surface vs Volume and IMC vs ITC plots we can see clear clustering by structure, and within these clusters there is variance relating to the effect of water content. The effect of water content is more apparent in the IMC vs ITC functional space. It seems however that some structures (N) are less sensitive to the presence of moisture and this can possibly be explained by the structural characteristics of the soil (small pores and low porosity therefore low water content).

We have also shown for the first time that a model of fungal growth and dynamics can be coupled to a model predicting the micro-pore distribution of fluid. The structural heterogeneity and in particular the pore size distribution appears to effect the distribution of moisture and this requires further investigation. Future work can investigate effect of water

distribution on soil samples with similar porosities but different pore size distributions, although identifying or generating soil samples with specific structural characteristics is not trivial. Secondly, this work is an essential step towards extending the model to include carbon dynamics as it enables to incorporate both particulate and soluble carbon sources. Here we restricted ourselves to investigating a single set of parameters for fungal growth, as at this stage we focus on the effect of structure and moisture on fungal colonisation, however different trait sets may be more or less affected by soil structure and moisture. This coupled model is an important first step towards developing a framework that can functionally classify fungi in terms of their essential traits and provide a tool that can predict shifts in colonisation ability associated with soil management strategies or climate change (changes in rainfall pattern).

REFERENCES

Allen, M. 1993. The Ecology of Mycorrhizae, Cambridge University Press, Cambridge, UK.

Ashford, A., W. Allaway. 2002. The role of the motile tubular vacuole system in mycorrhizal fungi. Plant Soil 244: 177–187.

Bardgett, R., W.D. Bowman, R. Kaufmann, and S. Schmidt. 2005. A temporal approach to linking aboveground and belowground ecology. Trends Ecol. Evol. 20: 634–641.

Bardgett, R., R.S. Smith, R.S. Shiel, S. Peacock, J.M. Simkin, and H. Quirk. 2006. Parasitic plants indirectly regulate below-ground properties in grassland ecosystems. Nature 493: 969–972.

Basit, R., and M. A. Basit. 2010. Simulation of phase separation process using lattice Boltzmann method. Canadian Journal on Computing in Mathematics, Natural Sciences, Engineering and Medicine 1(3): 71-76.

Baveye, P. 2006. Comment on “Soil structure and management: A review” by C.J. Bronick and R. Lal. Geoderma 134 (1-2): 231-232.

Baveye, P.C. 2010. Comment on “The role of scaling laws in upscaling” by B.D. Wood, Advances in Water Resources 33: 123–124.

485 Baveye, P.C., M. Laba, W. Otten, L. Bouckaert, P. Dello Sterpaio, R.R. Goswami, D. Grinev,
486 A. Houston, Y. Hu, J. Liu, S. Mooney, R. Pajor, S. Sleutel, A. Tarquis, W. Wang, Q. Wei,
487 and M. Sezgin. 2010. Observer-dependent variability of the thresholding step in the
488 quantitative analysis of soil images and X-ray microtomography data. Geoderma 157: 51-
489 63.
490 Boumans, R. 2002. Modelling the dynamics of the integrated earth system and the value of
491 global ecosystem services using the GUMBO model. Ecological Economics 41: 529–560.
492 .
493 Falconer, R.E., J.L. Bown N.A. White, and J.W. Crawford. 2005. Biomass Recycling and the
494 origin of phenotype in fungal mycelia. Proc. Roy. Soc B. Lond. 272: 1727-1734.
495 Falconer, R.E., J.L. Bown N.A. White, and J.W. Crawford. 2007. Biomass recycling: a key to
496 efficient foraging. Oikos 116: 1558-1568.
497 Falconer, R.E., J.L. Bown N.A. White, and J.W. Crawford. 2008. Modelling interactions in
498 fungi. Royal Society Interface 5: 603-615.
499 Falconer, R.E., J.L. Bown N.A. White, and J.W. Crawford. 2010. Linking fungal individuals to
500 community scale patterns; Fungal Ecology: In Press.
501 Gras, A., M. Ginovart, X. Portell, X., and P.C. Baveye. 2010. Individual-based modelling of
502 carbon and nitrogen dynamics in soils: Parameterization and sensitivity analysis of abiotic
503 components. Soil Sci. 175: 363-374.
504 Hadwiger, H. 1957 Vorlesungen uber Inhalt, Oberflache und Isoperimetrie. Springer, Berlin,
505 Germany.
506 Harris, K., I.M. Young, C.A. Gilligan, W. Otten, and K. Ritz. 2003. Effect of bulk density on
507 the spatial organisation of the fungus Rhizoctonia solani in soil. FEMS Microbiol. Ecol. 44:
508 45–56.
509 Hattori, K. 1988. Soil aggregates as microhabitats of microorganisms. Rep. Inst. Agric. Res.
510 37: 23–36.
511 Hawksworth, D.L. 1991. The fungal dimension of biodiversity: magnitude, significance, and
512 conservation. Mycological Research 95: 641–655.

513 Iassonov, P., and M. Tuller. 2010. Application of segmentation for correction of intensity bias
514 in X-ray computed tomography images. Vadose Zone Journal 9 (1): 187-191.
515 Johnson, D., M. Kresk, E. Wellington, A. Stott, L. Cole, R. Bardgett, D. Read, and J. Leake.
516 2005. Soil invertebrates disrupt carbon flow through fungal networks. Science 309: 1047.
517 Killham, K. 1994. Soil Ecology. Cambridge University Press, Cambridge, UK.
518 Kravchenko, A.R., R.E. Falconer, D. Grinev, and W. Otten. 2010. Fungal colonization in soils
519 of contrasting managements: modelling fungal growth in 3D pore volumes of undisturbed
520 soil samples. Ecological Applications: in press.
521 Letey, J. 1991. The study of soil structure - Science or art. Australian Journal of Soil
522 Research 29(6): 699-707.
523 Martys, N. S., and H. Chen. 1996. Simulation of multicomponent fluids in complex three-
524 dimensional geometries by the lattice Boltzmann method. Phys. Rev. E. 53: 743–750.
525 Mitchell, R., and M. Alexander. 1962. Microbiological changes in flooded soils. Soil Science
526 93(6): 413-419.
527 Ohser, J., and F. Mücklich. 2000. Statistical Analysis of Microstructures in Material Science.
528 Wiley, New York.
529 Otten, W., C.A. Gilligan, C. Watts, A.R. Dexter, and D. Hall. 1999. Continuity of air-filled
530 pores and invasion thresholds for a soil-borne fungal pathogen, Rhizoctonia solani. Soil
531 Biol. Biochem. 31: 1803–1810.
532 Pajor, R., R. Falconer, S. Hapca, and W. Otten. 2010. Modelling and quantifying the effect of
533 heterogeneity in soil physical conditions on fungal growth. Biogeosciences Discussion, 7:
534 3477-3501
535 Shan, X.W., and H.D. Chen. 1993. Lattice Boltzmann model for simulating flows with multiple
536 phases and components. Physical Review E 47: 1815-1819.
537 Sleutel, S., V. Cnudde, B. Masschaele, J. Vlassenbroek, M. Dierick, L. Van Hoorebeke, P.
538 Jacobs, S. De Neve. 2008. Comparison of different nano- and micro-focus X-ray
539 computed tomography set-ups for the visualization of the soil microstructure and soil
540 organic matter. Computers and Geosciences 34: 931-938.

541 Smith , S. E., and D.J. Read. 1997. Mycorrhizal symbiosis. Academic Press, San Diego, CA.
542 Sukop, M.C., and D. Or. 2003. Invasion percolation of single component, multiphase fluids
543 with lattice Boltzmann models. Physica B 338: 298-303.
544 Sukop, M.C., D.T. Thorne, Jr. 2006. Lattice Boltzmann Modeling: An Introduction for
545 Geoscientists and Engineers, 1st Edition, Springer Verlag Berlin Heidelberg, Germany.
546 Sun, B., P. Hallett, S. Caul, T. Daniell, and D. Hopkins, D. 2011. Distribution of soil carbon
547 and microbial biomass in arable soils under different tillage regimes. Plant and Soil 338: 17-
548 25.

549 Tippkötter, R., T. Eickhorst, H. Taubner, B. Gredner, and G. Rademaker. 2009. Detection of
550 soil water in macropores of undisturbed soil using microfocus X-ray tube computerized
551 tomography (ìCT). Soil and Tillage Research 105 (1): 12-20.
552 Vogel, H.-J., U. Weller, and S. Schluter. 2010. Quantification of soil structure based on
553 Minkowski functions. Computers and Geosciences 36(10): 1236-1245.
554 Vogel, H.-J., J. Tolke, V.P. Schulz, M. Krafczyk, and K. Roth. 2005. Comparison of a Lattice-
555 Boltzmann model, a full-morphology model, and a pore network model for determining
556 capillary pressure–saturation relationships. Vadose Zone Journal 4: 380–388.
557 White, N. A. 2003. The importance of wood-decay fungi in forest ecosystems. In Fungal
558 biotechnology in agriculture, food and environmental applications (ed. D. K. Arora), pp.
559 375–392. Marcel Dekker, New York, NY.

560

561 FIGURE CAPTIONS

562 **Figure 1.** Minkowski measures for simple 3D geometries, middle 2D slice is shown.

563 **Figure 2.** Schematic showing the interaction between the various models and quantification
564 measures used. The middle slice of the 3D soil structure, water distribution and
565 biomass distribution is shown. Input into the Lattice Botzmann Model (LBM) are the
566 three structures with varying properties (black = solid, white = pore) - samples from top
567 to bottom are N, P2 & P1..Output from the LBM is the water distribution represented as
568 grayscale within pore space. This water distribution together with the connectivity of
569 pore space effects fungal biomass distribution (white corresponds to presence of
570 fungal biomass). The Minkowski measures are applied to pore, water and biomass
571 volumes to characterise their 3D geometry.

572 **Figure 3.**Histogram of log transformed pore diameters for the 3 soil samples P1, P2 and N.

573 **Figure 4.** Phase separation with parameters specified in Table 1 in a 2-D, non-structured
574 context at (a) t=0, (b) t=300 and (c) t=1000 iterations, and within a porous environment
575 at d) t=0, (e) t = 300 and (f) t =50000

576 **Figure 45** Surface Area (SA) against Volume Fraction (VF) for the water phase of the 3 soil
577 samples P1, P2, and N. The arrows indicate increasing time.

578 **Figure 6.** Distribution of diffusion coefficients affecting colony spread for (a) dry soil sample,
579 i.e., with no moisture, b) moisture content of 2.96% and c) water content of 4.75% (Table
580 3). Black and white voxels correspond to solid (Diffusion coefficient = 0) and pore
581 voxels, respectively (Diffusion coefficient = 250). b) and c) show that the diffusion
582 coefficients are no longer binary but are a distribution derived from a linear mapping to
583 water distribution (as predicted by SCMP LB model.)

584 **Figure 7.** Plots of the surface area versus Volume fraction (a) and IMC versus ITC
585 measures for the biomass (b) at the 3 moisture contents. In the graph (a), P1_Pores,

586 P2_Pores, and N_Pores represent the functional measures of pore space. The other
587 samples are labelled as in Table 3.
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