Effect of soil management on pore geometry and the implications for fungal invasion and interactions

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Abstract
Despite the importance of fungi in soil functioning they have received comparatively little attention and our understanding of fungal interactions and communities is lacking. This study aims to combine a physiologically based model of fungal growth and interactions with digitized images of undisturbed soil samples from contrasting management practices to determine the effect of physical structure on fungal growth and colonization. We quantified pore geometries of the undisturbed soil samples from long-term agricultural and native vegetation land uses, modelled invasion of a single fungal species and of two different fungal species within the soil samples; and evaluated the role of soil structure on fungal invasion and species interactions; in particular, we identified those characteristics of the pore volume that promote or exclude fungal invasion.

Key Words
Fungal growth model, 3D pore space, X-ray microtomography, land use.

Introduction
Fungi are a major player in soil functioning. They contribute to soil structure formation and shaping of plant communities through their role in nutrient cycling, pathogenesis and symbiosis. Surprisingly, fungi have received comparatively little attention; and theoretical approaches which have emerged over the years and improved considerably our understanding of above ground plant communities are still lacking below ground. A theoretical framework is needed, such that links soil physics, fungal biology, mathematical biology and statistics in order to understand fungal community dynamics and diversity in undisturbed soils. Such a framework is essential if we are to understand how environmental change or soil manipulation impacts biodiversity. Different land use and management practices significantly affect soil environmental characteristics crucial for fungal communities by contributing different quantities and qualities of biomass inputs, generating different levels of soil disturbance, influencing soil temperature and moisture regimes, and affecting structure and geometry of soil pore space. Differences in pore structures generated by long-term differences in land use and management are reflected in notable changes in soil physical and hydraulic properties, including soil porosity, hydraulic conductivity and water retention (Brye and Pirani 2005). Changes in numbers, shapes, and distributions of soil macropores have been often observed (e.g., Pachepsky *et al.* 1996; Giménez *et al.* 1997; Udawatta *et al.* 2008). However, specific implications of these differences in pore structure and geometries for ability of pathogenic as well as non-pathogenic fungi to colonize soil body have not been address yet. Recent advances in computed tomography and microscopy facilitate detailed examination of the inner pore structures of undisturbed soil samples as well as visualization of fungal mycelia. Such tools together with modelling generate a new level of understanding of the mechanisms governing fungal behaviour at microscopic scales, and for the first time allow us to examine species interactions in a 3D environment. The goal of this study is to assess how physical structure of the environment affects fungal growth and spread through the space? We hypothesize that analyses and comparisons of the pore structures and their colonization by fungi in soils of the same origin but subjected to long-term contrasting management, e.g., conventional agriculture with or without intensive tillage versus native vegetation, will provide insights for understanding functioning of soil fungal communities.

The specific objectives are (i) to quantify pore geometries of the undisturbed soil samples from long-term agricultural and native vegetation land uses based on the analysis of 3D images; (ii) to model invasion of a single fungal species and of two different fungal species within the soil samples; and (iii) to evaluate the role of soil structure on fungal invasion and species interactions; in particular to identify those characteristics of the pore volume that promote or exclude fungal invasion.

Methods

Sample collection

Soil samples were collected from Long Term Ecological Research (LTER) experiment located in Southwest Michigan, USA (42° 24' N, 85° 24' W) established in 1988. Soils are classified as well-drained, Typic Hapludalfs either fine-loamy, mixed mesic (Kalamazoo series) or coarse-loamy, mixed, mesic (Oshtemo series). The three of the LTER treatments used in this study are (i) conventionally tilled (chisel ploughed) (CT) and (ii) no-till (NT) corn-soybean-wheat rotation with conventional chemical inputs, and (iii) native succession vegetation established on the experimental plots abandoned from agricultural use in 1989 (NS). For each treatment we collected ~15 undisturbed soil samples from depth 2-7 cm in plastic cylinders 5 cm in diameter and 5 cm length.

X-ray scanning and image analyses

The 3D pore space of each of the soil samples has been visualised with an X-TEK HMX micro-tomography system at SIMBIOS (http://simbios.abertay.ac.uk/). The computed tomography data sets were reconstructed in 3D using filtered back-projection algorithm in 32-bit floating point format to enhance contrast between the phases in soil. From the centre of each soil sample image we selected a cube ~2x2x2 cm in size. Cube data were reconstructed at 110 micron resolution to lead to soil image data sets 180x180x180 pixels in size. Each voxel was classified either as a pore or a solid based on its gray-scale value using manual thresholding approach in ImageJ (http://rsbweb.nih.gov/ij/). After pore/solid thresholding, pore characteristics, including total porosity, pore connectivity and pore-size distribution were determined for each cube using an in-house developed SCAMP plugin for ImageJ.

Fungal growth modelling

Analyses of soil heterogeneity effects on fungal growth and interactions were built upon a modelling approach developed by Falconer *et al.* (2005). The approach explores fungal traits that allow for effective colonisation of heterogeneous environments. It describes the physiological processes of vegetative growth and development of fungal colonies in a 3D soil environment. The model formulation represents the pore space as a set of voxels and models an individual mycelial network growing in the pore space as comprising three fractions: immobilized insulated biomass, non-insulated biomass and mobilized biomass. The relative proportions of these components are dynamic and are determined by four physiological processes: uptake, inter-conversion rates between mobile and immobile biomass, redistribution of mobile biomass and growth. The model incorporates biomass recycling and production of extracellular enzymes and antibiotics through which interactions between fungal species are regulated.

Three inoculums placement densities were explored, i.e., 1, 5, and 20 inoculums were introduced in randomly selected pore voxels within the soil domain. Such range of inoculation densities allowed us to cover a variety of possibilities in terms of fungal colonization of the pore space. While 20 inoculums can be regarded as a representation of a scenario for a spread of a commonly present species after a dormancy period and encompasses almost the entire pore space, a single inoculum scenario may represent a pathogen spread from a single source and is likely to be influenced by the shape and connectivity patterns of the largest connected pores.

At each iteration step we recorded the percent of the total pore space that has been occupied by the fungus. Change in the percent of pores as a function of the model iteration step (time) was then fitted with a mathematical equation:

$$\%pore = \%final(1-\exp(-(iter/a)^b)) \quad (1)$$

where *%pore* is the percent occupied pore space, *iter* is the iteration number, *%final* is the plateau amount of pore space that has been accessed at the end of the simulation, and a and b are the parameters defining the shape of the curve. Equation (1) was fitted to the observed data from each model run of every sample using PROC NLIN procedure in SAS (SAS Inc. 2008). Statistical data analyses were performed using PROC MIXED in SAS (SAS Inc. 2008).

Results

Assessment of pore geometry characteristics

Porosity of NT was significantly lower than porosity of both CT and NS treatments. It was also much less variable, ranging only from 3 to 8%, as compared from 7 to 29 % for CT and 10 to 32% for NS. Analysis of the sizes of the largest connected pores indicated that the largest pores in CT and NS occupied much bigger

proportion of the total pore space than in NT (Table 1). On average the largest connected pore volume occupied 79 and 88% of pore space in CT and NS treatments, respectively, while it only occupied 46% of the pore space in NT. The second connected pore volume occupied around 11% of the pore space in NT, while became negligible (2-3%) in CT and NS treatments. Examples of the largest and the second largest connected pore volumes for samples from NS and NT treatments are shown in Figure 1.

Table 1. Percent of pore space occupied by five largest connected pores. Standard errors are shown in parentheses.

Treatments	Connected pore volume class				
	1	2	3	4	5
CT	79.2(9.2)a	2.8(1.4)a	1.8(1.0)ab	0.6(0.3)a	0.4(0.2)a
NS	88.5(6.7)a	1.5(0.9)a	0.5(0.4)a	0.4(0.3)a	0.3(0.2)a
NT	46.5(8.1)b	11.6(3.0)b	4.5(1.4)b	1.8(0.4)b	1.3(0.3)b

* means within the same column followed by the same letters are not significantly different from each other ($p<0.05$).

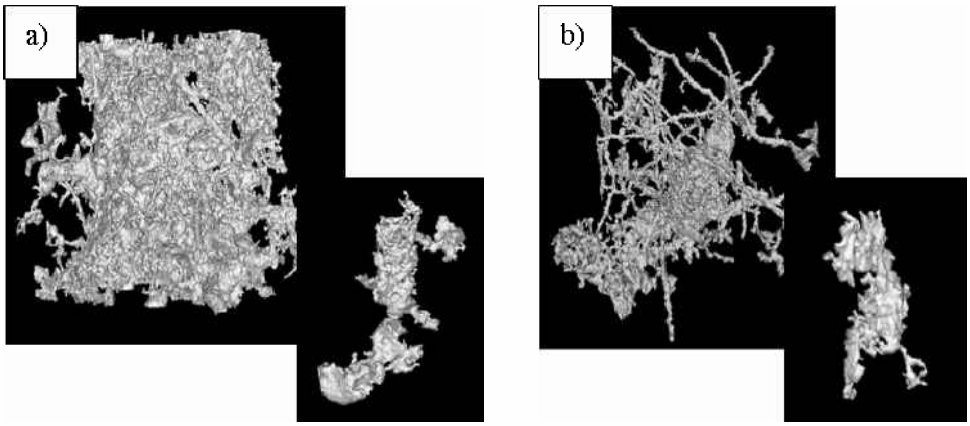

Figure 1. Examples of the largest and second largest connected pore volumes in a sample from NS (a) and NT(b) treatments.

Assessment of fungal growth

Percent of pore space occupied at the end of simulation with 5 starting inoculums in NT was only 53% while it was > 80% in NS and CT treatments (Table 2). With 20 inoculums the percentages of occupied pore space increased only slightly in CT and NS, and greatly increased in NT (to 65%). In CT and NS either with 5 or 20 inoculum points at least some seeds were placed within the largest connected pore volume which constituted on average 80-85% of the entire pore space and then were able to eventually colonize it. In NT, with 5 inoculum points the likelihood of initial seed placement to occur in more than 1-2 largest connected pore volumes is relatively low, thus only the largest and the second largest pores (combined ~58% of the pore space) were typically colonized.

Percent of pore space filled data fitted with Eq. (1) are shown on Figure 2a. There was no treatment or method differences in terms of parameter *a* at either 5 inoculum or 20 inoculum simulations (Table 2). For parameter *b* in 5 inoculum simulations, NT was lower than that of NS, but not significantly different from CT. This reflected a tendency for more S-shaped curve from NS, where possibly initial fungal spread was slow when the inoculum seeds ended up in tortuous components of the connected pore volumes. In all treatments pore space was occupied at a much slower rate with 1 and 5 inoculums points as compared to 20 inoculum points (Figure 2b).

Table 2. Parameters of Eq. (1) characterizing fungal colonization in 5 and 20 starting inoculum seeds simulations. Standard errors are shown in parentheses.

	%final		Parameter *a*		Parameter *b*	
Treatments	5	20	5	20	5	20
CT	81.5(5.5)a	83.8(5.2)a	14.1(1.4)a	6.6(0.6)a	1.9(0.2)ab	1.6(0.2)a
NS	86.5(4.3)a	88.2(4.5)a	12.4(1.2)a	7.0(0.5)a	2.1(0.2)b	1.6(0.2)a
NT	52.6(5.3)b	65.7(4.7)b	11.7(1.4)a	5.8(0.5)a	1.7(0.2)a	1.4(0.2)a

* means within the same column followed by the same letters are not significantly different from each other ($p<0.05$)

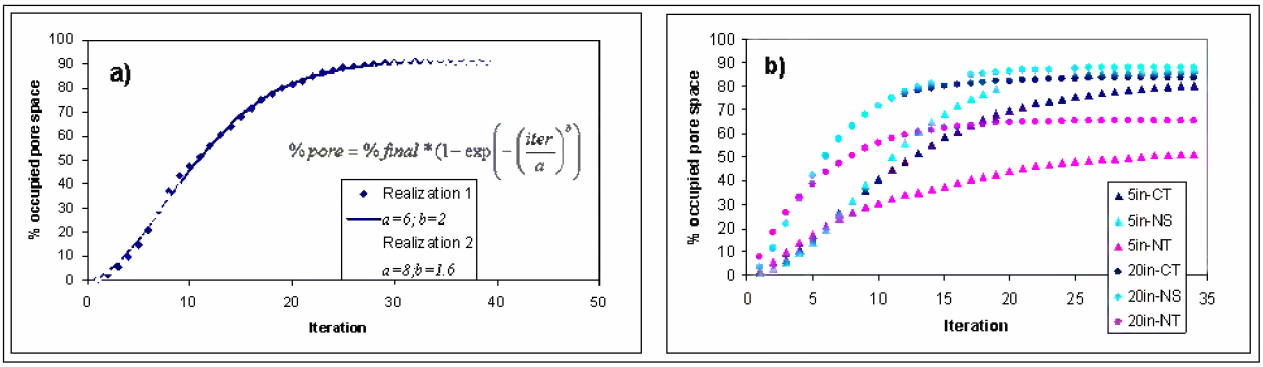

Figure 2. Example of percent of pore space occupied by fungus in a course of growth simulation fitted with mathematical function (Eq. (1)) (a) and an average % pore space occupied in simulations with 5 and 20 inoculums.

Modelling of two species interactions is currently in progress. An example of the model outcomes for two species interaction within a 3D soil pore space is shown on Figure 3. Preliminary results clearly indicate that two fungal species occupy different volumes of the soil and that the pore space heterogeneity will be a key factor in their interaction.

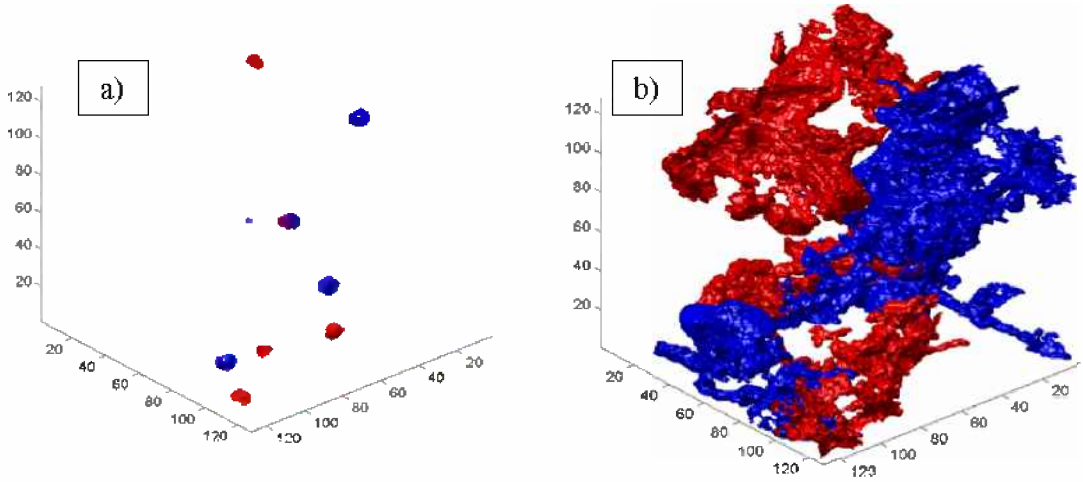

Figure 3. Example of colonization of the 3D soil pore space by two fungal species at the initial stage (a), at and final iteration (b) steps. X-, y-, and z- axes represent dimensions of the sample in voxel units (one voxel is equal to 100 μm). Two fungal species are shown in red and blue, respectively.

Conclusions

No-till had substantially lower porosity (pores >100 μm) and connectivity of pores >100 μm than CT and NS soils. These characteristics significantly reduced possibility of fungal pathogen invasions from a single inoculum, while still enabled almost complete colonization of pore space in case of multiple (20) inoculums.

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