Combining X-ray CT and 3D printing technology to produce microcosms with replicable, complex pore geometries.

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Abstract:

Measurements in soils have been traditionally used to demonstrate that soil architecture is one of the key drivers of soil processes. Major advances in the use of X-ray Computed Tomography (CT) afford significant insight into the pore geometry of soils, but until recently no experimental techniques were available to reproduce this complexity in microcosms. This article describes a 3D additive manufacturing technology that can print physical structures with pore geometries reflecting those of soils. The process enables printing of replicated structures, and the printing materials are suitable to study fungal growth. This technology is argued to open up a wealth of opportunities for soil biological studies.

Microcosms have played a central role in the development of ecology, leading to model-driven insights into habitat fragmentation, competitive exclusion, resource allocation, and succession (Drake et al., 1996). These conceptual advances could be relevant to soils, whose complex geometry and heterogeneity is widely recognized as the key driver in many ecological processes. In soil science, the development of ecological theories is nevertheless in its infancy and the discipline still stands to benefit from more hypothesis-driven research (Prosser et al, 2007). This requires the level of experimental control afforded by model systems (Jessup et al. 2004). To date, introduction of heterogeneity in microcosms has been limited due to the difficulty in controlling and replicating the pore geometries of soil at scales relevant for microbial processes, and systematic study of the impact of soil structure on microbial invasions has been restricted to computer simulation studies (e.g., Falconer et al., 2005, 2012). There is therefore a pressing need to advance soil microcosms in ways that retain the control of laboratory-based studies, along with the heterogeneity encountered in the field (Baveye et al., 2011).

Engineers have been utilizing 3D printing or "additive manufacturing” for more than a decade. This technology is maturing and printing at small spatial scales is now possible even for complex stalactite like structures encountered in soil. Heterogeneous structures can be printed with a range of materials, including plastics, glass and ceramics. 3D printing technology is on the cusp of major exploitation in many areas (Marks, 2011). The latter tend to be at large spatial scales (> cm), but exploitation at the micron scale is an exciting opportunity, albeit with a few challenges. Here we demonstrate how micro X-ray CT imaging, which quantifies soil structure, can be combined with 3D printing to produce replicated static model microcosms that exhibit the physical heterogeneity found in soils. To produce microcosms, soil pore geometries can be quantified via X-ray CT or digitally designed to desired structures. The digital map is subsequently used in the 3D printing technique to produce replicated structures that can be used to explore for example the role of physical heterogeneity on fungal spread or transport processes. Soil samples including repacked sieved loam and undisturbed samples were scanned at a resolution of 29.3 um, with a Nikon HMX 225 X-ray micro-tomography system (Pajor et al., 2010). If required, these structures can subsequently be printed in different sizes to scale the porous medium (Fig 1). From the voxel data, the surface of pore network was extracted. The result of this process is a surface representation of the sample, with stereo lithography file format (STL). The polygonal mesh consists of up to 10.5 million triangles. This retains the key characteristics of the pore volume but does introduce some smoothening of the surface walls compared to real soil. An EOS P390 polymeric Laser Sintering machine (Additive Manufacturing Research Group, Loughborough University) was used to print the 3D structures in Nylon 12. The P390 has a heated chamber which is filled with a thin layer (0.1mm) of polymeric powdered materials (typically semi-crystalline polymers such as Nylon 12). A 50W CO_2 Laser is used to selectively melt (print) the polymeric powder according to the digital map. The powder offers a supporting surface during the printing process enabling so called stalactite-like structures. The powder is removed from the pore space after the printing process. Other printers use two plastics, one of which (representing the pore space) is dissolved after the printing process. Nylon 12, used in this study, is a resistant material enabling autoclaving and re-use of the samples, is resistant to most chemicals and has a low water adsorption. Up to two hundred replicated soil-like microcosms, as in Fig.1, can be printed overnight at very low cost covering the price of polymers only. The final stage is removal of the unprinted powder from internal cavities using a variety of methods such as vibration, ultrasonication, vacuuming, boiling, brushing and rinsing. This currently restricts the printing process to structures with well and fully connected pore volumes.

Replicate printed structures need be similar and tolerated by microorganisms. To ascertain that these conditions are met, ten Nylon-12 model systems were printed from the same structure. The Nylon 12 structures were then rescanned as above and data were converted to binarized images in ImageJ v 43 using Li's method. The surface area of the pore-solid interface and the pore volume for each replicate were determined. The average pore volume fraction, which is the total volume within which all microbial and physical processes occur, was 0.66 and highly reproducible with a small standard error (SE) of 0.0064. Similarly the volume of the solid phase (Nylon 12) was highly reproducible for each printed structure with an average of 10,985 mm^3 (SE = 223). The average solid-air interface of the structures was 38,320 mm^2 (SE = 1301). All standard errors were within 3% of the mean values showing a highly successful reproduction for complex geometries.

To assess whether the printed microcosms could host fungi, we introduced 3 poppy seeds that were previously colonised by *Rhizoctonia solani* into the 3D printed soil and incubated it at 23 ^{0}C for 3 days. The colonisation by this fungus was similar to that previously observed in bulk-soil (Harris et al., 2003) and in cracks (Otten et al., 2004), with preferential spread within larger pores and fungal hyphae bridging air gaps (Fig 2). This indicates that the soil-derived model systems are suited to study the effect of physical heterogeneity on fungal growth and species interactions (Fig 2).

In conclusion, 3D printing makes it possible to produce replicable static model systems possessing some of the physical complexity of soils. The current example is focused on relatively large pores, with the original structure scaled up three times to ensure all powder could be removed from the intricate pore network, and to produce pores with diameters in which we can study fungal invasion. Future work will address the limits of 3D printing technology in accurately replicating soil samples with more complicated geometries (lower porosities, high tortuosity) from which powder removal is a key challenge and to test the microscopic characteristics of the surface. Nevertheless, advancements can be made to comprehend interactions whilst explicitly considering structural heterogeneity, something hitherto not possible with alternative methods. Finally alternative polymers can be used to alter hydrophobicity of surface properties and determine its effect on hydrological properties of the structure. Many printers are available at prices of a few thousand pounds. Although the cheaper versions may not be able to cope with the complexity of soil structures, it is likely that rapid advances will make this an accessible technology in the near future.

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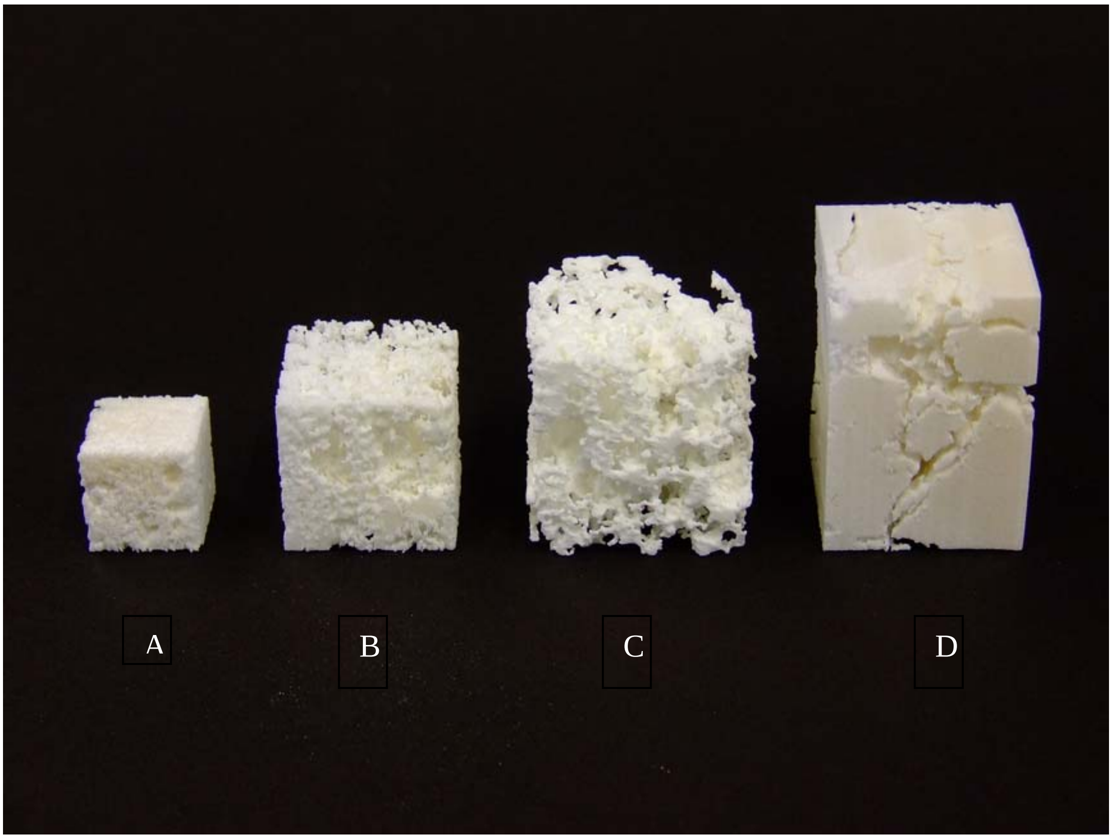

Fig 1 Soil-like structures demonstrating the range of microcosms that can be reproduced with 3D printing from a digital map. Printed structures from repacked sieved loamy sand (scaled to (A) 1.8 and (B) 2.7 cm wide), (C) the same sample but now with the pore space printed, and (D) an example of printed undisturbed soil sample with macro-pores.

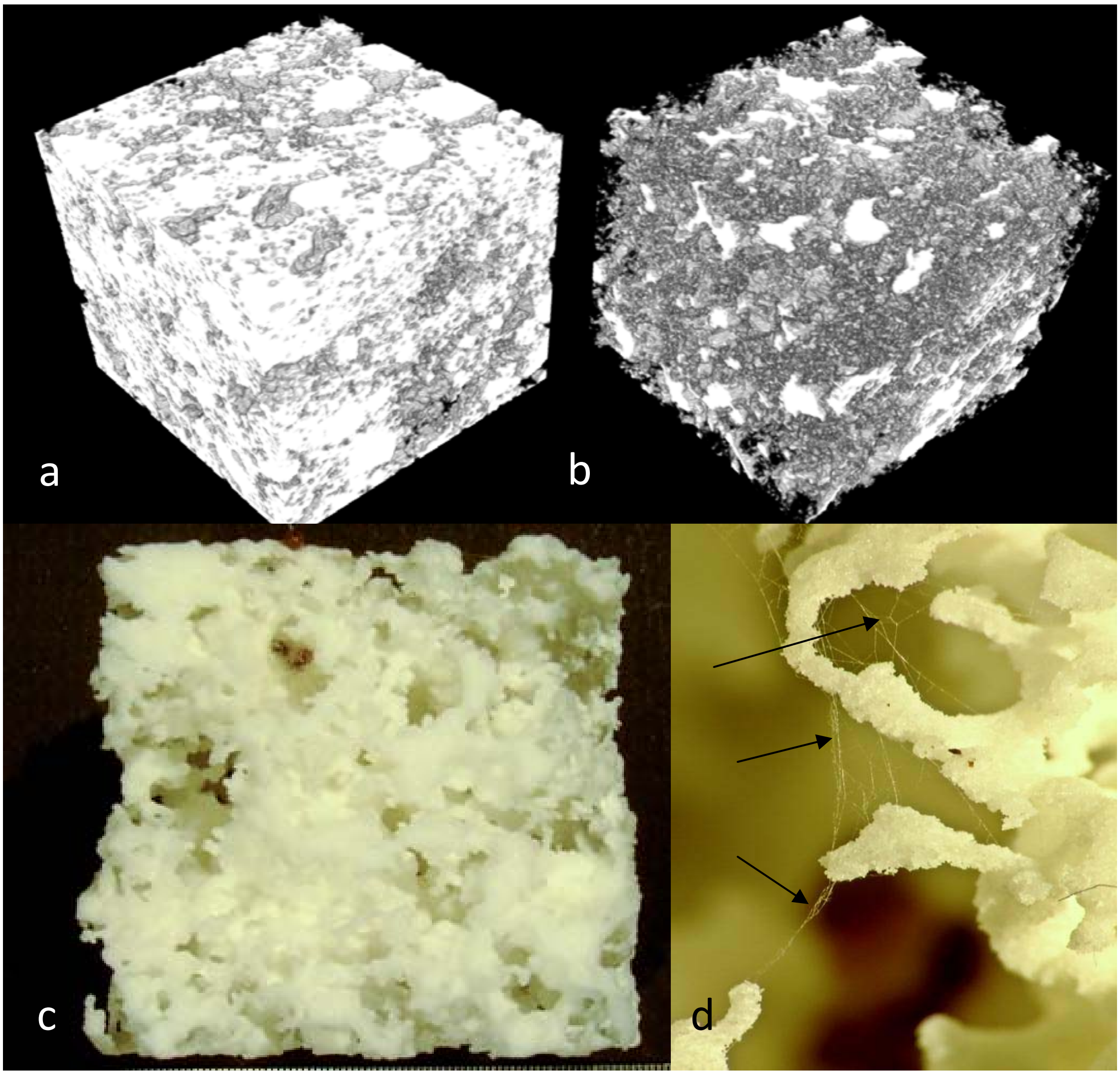

Figure 2. X-ray CT permits the visualization of the solid volume (a) and pore volume (b) at a spatial resolution of 30 µm. In the 3D printed Nylon 12 replica of the soil structure (c), fungal hyphae are easily visible in a close-up view (d; hypha indicated by an arrow).