Visualising the spatial organisation of soil microbial communities

K. Ritz, D. Crabb, K. Harris, N. Nunan, K. Wu, J.W. Crawford[1] & I.M. Young[1]

A very wide range of organisms inhabit soil. Larger creatures, such as rabbits, moles, earthworms, slugs and snails are familiar, but there is also a vast range of microbes and micro- and meso- fauna. A teaspoon of soil from under a pasture will typically contain 10^9 bacteria, several hundred metres of fungal hyphae, thousands of protozoa, hundreds of nematodes, dozens of mites, many other insects and spiders, and several metres of plant roots. The biodiversity belowground equals or exceeds that of a tropical rain forest canopy, and, just as there are many unrecorded species in the rainforest, we know very little in detail about the true range of species in the soil.

Soils function as a consequence of the myriad of interactions between these different organisms, but such interactions are strongly affected by the underlying physical structure of the soil. The architecture of the soil is fundamentally a labyrinth, comprising a complex network of blind and connected pores at spatial scales that span orders of magnitude, typically from micrometres to millimetres. It is these pore networks that govern the rate and limits of movement of gases, liquids, solutes, particles and organisms, and form the structural habitat for all life in the soil.

Techniques have been developed at SCRI that enable the visualisation of soil organisms in an intact state, and in their natural positions, in this complex soil matrix. The aim of these methods is to enable the production of 'underground maps' that allow the spatial distribution of microbes, pore networks and solid phases of the soil to be studied. The resultant maps can then be used to inform modelling frameworks that will allow the consequences of such spatial organisation to be understood. The approach is essentially a form of 'soil histology' akin to the sectioning and microscopic observation of plant and animal tissues. However, there are significant difficulties associated with producing thin sections of mineral soils in which biological material is to be preserved and observed. Soils are fragile and will collapse readily when disturbed; they contain extremely hard mineral components such as sand grains which preclude the simple cutting of sections. In contrast, most microbes are very soft – for example many protozoa are little more than naked masses of cytoplasm bound by a thin membrane – and sectioning such material in the proximity of quartz grains is technically demanding. Microbes are very small by definition and so it is difficult to visualise them in the context of much larger features such as pore networks or biological features such as roots. As discussed above, they are also very diverse in terms of the number of different forms and the size scales they encompass. Few microbes have any inherent contrast that would enable them to be visualised against the highly complex background of the

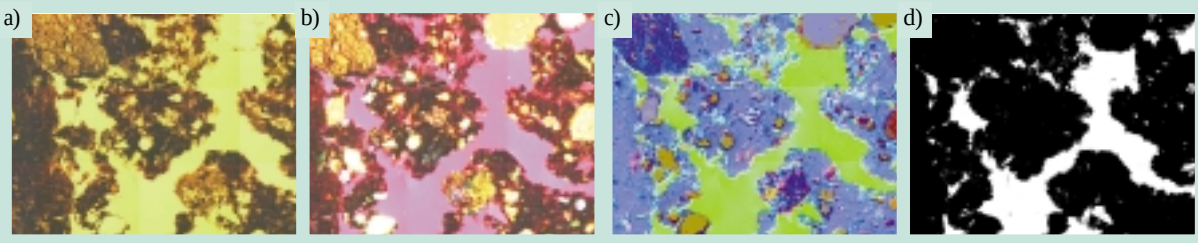


Figure 1 Segmentation of transmission pore networks from thin sections of undisturbed soil. Image width c.3 mm.
(a) Bright field transmitted illumination. The light penetrates both the pores in the soil (now full of resin) and sand grains and other clear mineral components, appearing yellow here. The brown material is organic matter and other minerals appear as various hues.
(b) Same image viewed with polarised light. Pores now appear pink and mineral grains various hues.
(c) False-colour image generated by computer-assisted image processing to extend the range of hues to differentiate the different components of the soil.
(d) Binary map of the pore network in the thin section.

[1] University of Abertay, Dundee.

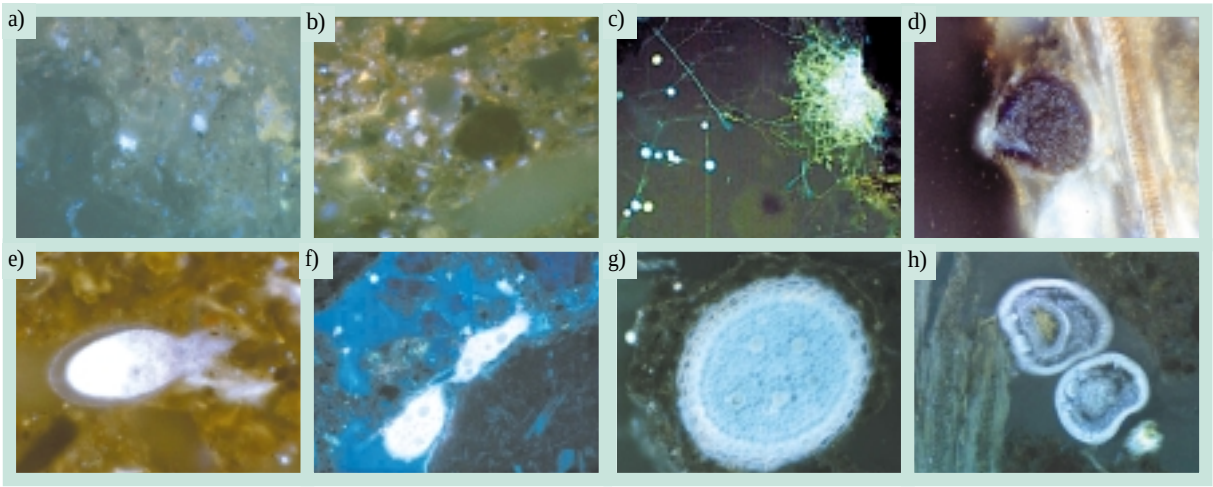
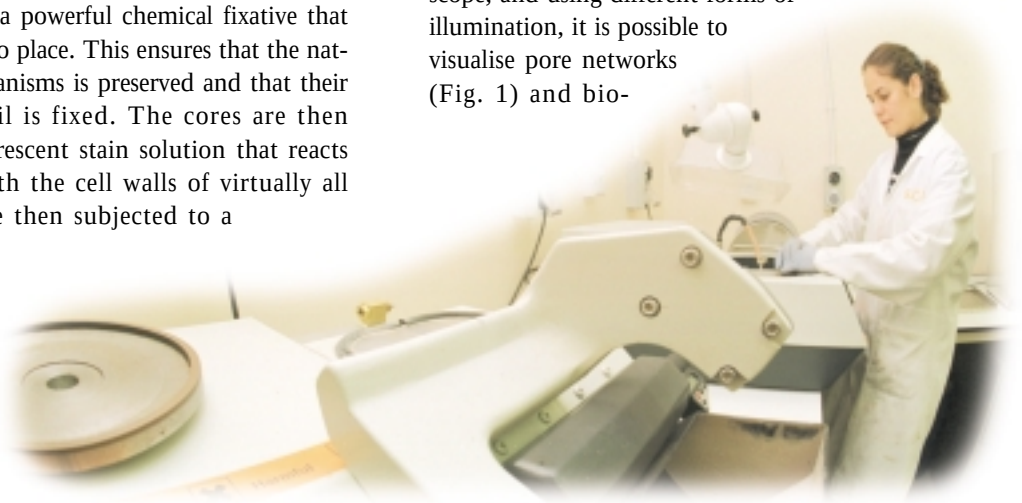


Figure 2 *In situ* visualisation of soil organisms in undisturbed soils as revealed by the biological thin-section technique.
(a) Bacteria (fluorescing blue under UV epifluorescence illumination) in a bulk soil sample from an arable field. Image width c.120µm.
(b) Bacteria in soil from an earthworm cast. Note considerably greater biological activity compared to (a). Image width c.120µm.
(c) Fungal hyphal network growing in pore space. Fluorescent spheres are spore clusters. Image width c.500µm.
(d) Fungal perithecium in decomposing root. Note root vascular tissue on right of image; the perithecium is embedded in the cortex, and the orifice through which spores are ejected opens into a soil pore on the left of the image. Image width c.120µm.
(e) Testate amoeba. Note the cytoplasm extruded from the silicaceous test; this indicates the organism was mobile and actively foraging for bacterial prey before being preserved by the biological thin-section procedure. Image width c.80µm
(f) Naked amoeba penetrating narrow neck between two adjacent pores. Note this is essentially a 2-D thin section, and above the plane of section the pore neck may widen; the organism is a 3-D mass of cytoplasm. Image width c.60µm
(g) Transverse section of root. Note the vascular cylinder is intact and the majority of cortex has disappeared, and that the root is surrounded by a closely-associated soil matrix. Image width c.500µm
(h) Transverse section of enchytreid worm. There are two sections due to the worm looping through the 2-D plane of the section. Note the soil deposited in the lumen of the gut in the upper section. Image width c.200µm.

soil matrix by conventional microscopy. Finally, any resultant images must be amenable to quantification, and to ensure such measurements are representative, a high degree of replication is necessary. The production of such maps therefore requires automated image processing, analysis and quantification procedures.

The techniques devised achieve most of these aims. The production sequence involves removing undisturbed cores of soil from their natural position and infusing them with a powerful chemical fixative that locks all proteins into place. This ensures that the natural state of the organisms is preserved and that their position in the soil is fixed. The cores are then immersed in a fluorescent stain solution that reacts non-specifically with the cell walls of virtually all microbes. They are then subjected to a controlled chemical dehydration in solvent until all water has been removed, followed by impregnation with a low-viscosity polyester resin which subsequently polymerises to produce a solid block of resin-embedded soil. Thick slices (about 5 mm) are cut from these blocks using diamond-coated saws, and adhered to glass microscope slides; thin sections are then produced by a protracted series of cutting and grinding steps with lubricated diamond pastes until the slices are approximately 25 µm thin. The sections are then viewed with a compound microscope, and using different forms of illumination, it is possible to visualise pore networks (Fig. 1) and bio-



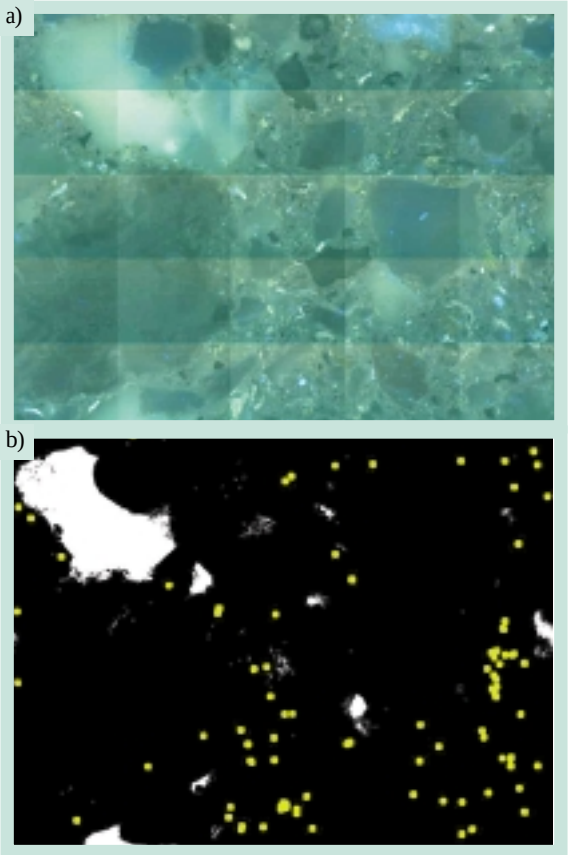


Figure 3 Large-scale imaging of the *in situ* distribution of bacterial cells in undisturbed fallow soil. Image width ≈ 600 µm
(a) Tessellated montage image (total 25 fields-of-view) under UV epifluorescence illumination. Digital file size is 32 Mb. Individual cells are not visible by eye due to their small size.
(b) Overlay map of locations of bacterial cells and transmission pore network produced by automated image processing techniques.

logical material (Fig. 2). The degree of preservation of biological material is very high, as demonstrated by the intact nature of such delicate structures as fungal mycelia and naked amoebae.

The majority of soil bacteria are approximately 1 µm in diameter . In order to visualise them, high-power microscope objectives are needed which provide a concomitantly small field-of view, of the order of 0.01 mm^2. To increase the scale of visualisation, a computer-controlled microscope stage is used to capture a series of precisely aligned adjacent fields-of-view which are then tessellated to form a contiguous image. The limit to the total area which can be visualised is set by available computer memory; an example of such a tessellated image is shown in Fig. 3a. Although

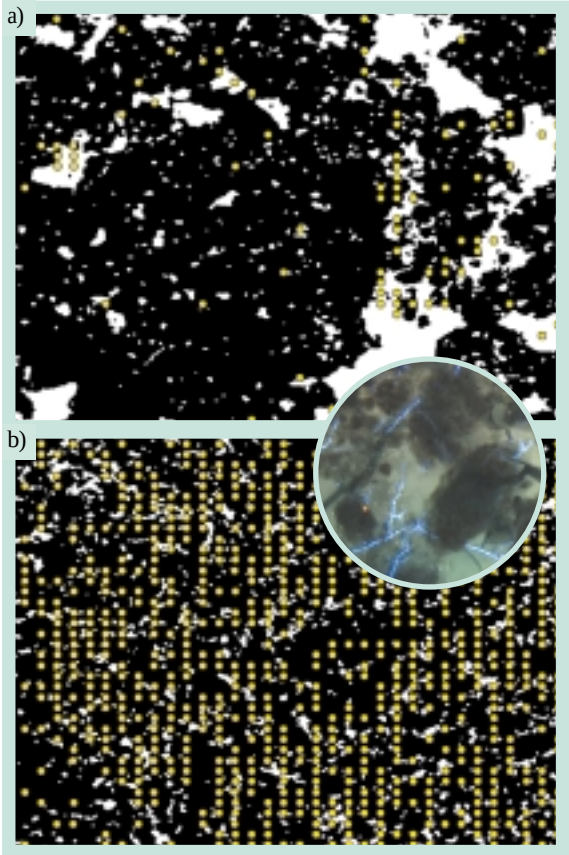


Figure 4 *In situ* spatial distribution of *Rhizoctonia solani* growing in soil at different bulk densities but at the same air-filled porosity of 17 %. Maps produced from analysis of 1400 individual and contiguous images for each section. Inset shows example image where fungus is present. Image width = 25mm.

individual bacterial cells in such an image cannot be resolved by eye, the digital information denoting them is present. By use of sophisticated image processing procedures, which involve some 23 individual steps applied to the red green and blue channels of the digital images, the bacterial cells are automatically segmented and their positions mapped (Fig. 3b). By combining such information with the pore images, we have produced the first ever maps of *in situ* bacterial distribution at these sorts of spatial scales. These maps of a fallow soil, produced for a DTI LINK project with Aventis plc, reveal that a very small proportion of the soil volume contains bacterial cells, even though the numbers of cells per gram are huge, and that in such fallow soils a relatively small proportion of bacterial colonies consist of more than a few cells. These maps make it strikingly clear that if, for example, a pesticide were being transported in a heterogeneous manner through such a spatial pattern of cells (which

is almost certain due to the tortuosity of the pore networks), the extent to which the degradation rate may be reduced by the lack of contact of the compound with potential degrader bacteria.

The first large-scale spatial distribution maps of soil fungi have also been produced using these techniques. Fungi can be visualised at lower magnification than bacteria, hence much larger areas of section can be simultaneously visualised using the tessellation procedure, in this case the entire area of 25 x 23 mm sections. In an experiment in collaboration with the University of Cambridge, the effect of soil bulk-density upon the growth of the plant pathogenic fungus *Rhizoctonia solani* was studied. It was shown that the biomass and spatial distribution of fungus were strongly reduced in low bulk density soil compared to higher densities (Fig. 4). There was relatively little proliferation of mycelia in the larger soil pores that prevailed in the low bulk density systems. These spatial patterns of mycelia have important implications for whether the fungus may locate a host root and thus initiate a point of primary infection.

Thin sections provide an essentially 2-dimensional slice taken from a 3-dimensional volume of soil. Extrapolating from 2-D to 3-D can only be achieved practically by mathematical modelling, and this represents the next key step in this unique insight into the organisation of soils that these techniques are enabling.