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Quantification of spatial distribution and spread of bacteria in soil at microscale

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Soil bacteria play an essential role in functioning of ecosystems and maintaining of biogeochemical cycles. Soil is a complex heterogeneous environment comprising of highly variable and dynamic micro-habitats that have significant impacts on the growth and activity of resident microbiota including bacteria and fungi. Bacteria occupy a very small portion of available pore space in soil which demonstrates that their spatial arrangement in soil has a huge impact on the contact to their target and on the way they interact to carry out their functions. Due to limitation of techniques, there is scant information on spatial distribution of indigenous or introduced bacteria at microhabitat scale. There is a need to understand the interaction between soil structure and microorganisms including fungi for ecosystem-level processes such as carbon sequestration and improving the predictive models for soil management. In this work, a combination of techniques was used including X-ray CT to characterize the soil structure and in-situ detection via fluorescence microscopy to visualize and quantify bacteria in soil thin sections.

Pseudomonas fluorescens bacteria were introduced in sterilized soil of aggregate size 1-2 mm and packed at bulk-densities 1.3 g cm^{-3} and 1.5 g cm^{-3}. A subset of samples was fixed with paraformaldehyde and subsequently impregnated with resin. DAPI and fluorescence in situ hybridization (FISH) were used to visualize bacteria in thin sections of soil cores by epifluorescence microscopy to enumerate spatial distribution of bacteria in soil. The pore geometry of soil was quantified after X-ray microtomography scanning.

The distribution of bacteria introduced locally reduced significantly ($P<0.05$) with increasing bulk-density, consistent with a significant difference in the pore geometry. The results indicate that soil structure affects spread and colonization of bacteria in soil. We showed how the technique of resin impregnation and thin sectioning can be combined with 3D X-ray CT to visualize bacterial colonization of a 3D pore network. This combination of research methods is a first step towards a full mechanistic understanding of microbial dynamics in structured soils.