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# Do proteins play a role in the development of soil hydrophobicity?

Elizabeth Bond (1), Alun Jones (1), Stefan Doerr (2), Ed Dudley (1), and Geertje van Keulen* (1)

(2) Geography Department, Swansea University, Swansea, UK, (1) Institute of Life Science, School of Medicine, Swansea University, Swansea, UK *(g.van.keulen@swansea.ac.uk)

Soil hydrophobicity is a common phenomenon affecting a wide range of soil and land use types in different climates. Despite the far-reaching environmental and (agro-) economic consequences, the fundamental biological causes of soil hydrophobicity and its transient behaviour remain poorly understood. Soil hydrophobicity is caused by organic compounds coating soil particle surfaces, though the exact nature of these compounds remains undetermined. Microbial as well as plant-derived substances have been implicated as sources of these organic materials, while some microbes have also been identified as degraders and/or emulsifiers of hydrophobic compounds. This is indicative of the uncertain role of soil micro-organisms in the development, reduction and temporal variability of soil hydrophobicity. Of particular interest are certain filamentous fungi and actinomycetes, which are able to render their hydrophilic cell surface hydrophobic by producing extracellular hydrophobic proteins. Previous in vitro studies showed that these extracellular proteins can form highly recalcitrant hydrophobic surfaces on the hydrophilic side of amphiphilic interfaces. The aims of our studies are to explore the relationship between the presence and/or absence of (hydrophobic) protein and soil hydrophobicity.

We sampled UK grassland and dune soils with varying temporal and severity of hydrophobicity on a weekly basis in the period May-Oct 2009. In preparation for protein extraction, a protease inhibitor cocktail was added to each sample, as well as the removal of humic acids from the grassland soil samples by a sodium pyrophosphate/hydroxide mixture. Soil moisture content and water drop penetration tests (WDPT), indicating the level of hydrophobicity, were determined for each soil sample. The combined results from moisture content and WDPT indicate that our grassland soil samples transiently developed strong hydrophobicity, whereas the dune samples displayed transient variation between strong and extreme hydrophobicity. Proteomic protocols have been optimised for extraction of total, extracellular and hydrophobic proteins from soil and dune samples. Proteins were then precipitated as a further purification step and separated via SDS-PAGE or HPLC. Proteins were then identified by mass spectrometry and database search peptide sequencing. Hydrophobic proteins were isolated using formic acid solubilisation after washing the soil first with detergent. Soil was also spiked with hydrophobic cell wall extracts of Streptomyces coelicolor grown in vitro as a positive control for hydrophobic protein extraction.

In summary, this study contributes to identifying soil proteomic dynamics, which may influence soil hydrology and structure, and ultimately the ability of soils to absorb water, support biomass growth, store carbon, and to capture and degrade pollutants.