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Developing 3D polymer nanostructured fabric as a soil-like model for studying interactions between microorganisms and soil structure - The case of bacterial biofilm development

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Soil structure is the organisation of soil particles in aggregates with increasing hierarchical levels, from nano- to macro-architectures. Several processes and the functioning of the entire soil ecosystem fundamentally depends on soil structure. Soil is also a very heterogeneous and complex matrix to study because of several components with different nature (mineral, organic and biological), physics and chemistry comprise it. Its study often involves techniques that profoundly alter its natural composition or destroy its original 3D arrangement, including the pore distribution and organisation, which is crucial in preserving a suitable habitat for soil ecology and functioning.

Microbial life has been discovered in the last decades to exist in biofilms, 3D spatial organisations of microbial communities adhering to solid surfaces. In these well-organised assemblages of one or more different microbial species, extracellular polymeric substances (EPS) play remarkable functions for microorganisms in biofilms and facilitate aggregation of soil particles.

As a model system, a self-standing polymer biodegradable nanostructured scaffolds (NS) composed of a mixture of nano- to microfibrils and microbeads mimicking the fibrous materials and particles comprising the main morphological types of soil (organic matter and mineral particles) and the relative spatial architecture at the micro- and nanoscale were created by electrospinning. Electrospinning is a nanotechnology producing 2D and 3D nano- and microfibrillar scaffolds under an electric field. The resulting NS were characterised by considerable porosity and extensive surface area. A PGPR species was employed as a model microbial type to test the capacity of similar NS of supporting the biofilm development. Incubation was performed under stirring to stimulate only stable interactions between microorganisms and the various morphological types of the NS, and also to assess the stability of the NS mimicking the soil aggregates. To shed some light into the nexus between microorganisms and soil structure and the reciprocal influence, combination of imaging techniques such as optical, SEM and TEM microscopy were used to observe "in situ" associations of microbes with mineral and organic materials at

nano- and microscale and the consequent effects on porosity usually destroyed under investigations.

The typical phases of conditioning film release, initial and stable adhesion mediated by appendages and EPS release, micro- and macrocolony formation until a mature biofilm development were observed. Morphological modifications of bacteria and the involvement of other components in the mentioned stages were also detected. The bacteria growth rate, the overall respiratory activity and its spatial distribution throughout the NS were recorded.

Hence, the tools here proposed can have high potentials in reproducing the spatial and temporal dynamics of microbial hotspots of activity typically present in the rhizosphere, the sphere of soil surrounding roots, which is of central importance for the entire soil ecosystem functioning.

Similar 3D NS can also provide the opportunity of zooming in microbial lifestyle observing microbes at work, from the dynamics of interactions with organic matter and particle surfaces to their spatial distribution and colony formation, then linking biological processes to specific physical and chemical features of soil at different scales (from nm to mm).