

# DEVELOPING 3D POLYMER NANOSTRUCTURED FABRIC AS A SOIL-LIKE MODEL FOR STUDYING INTERACTIONS BETWEEN MICROORGANISMS AND SOIL STRUCTURE

*Fabrizio De Cesare<sup>1,3</sup>, Elena Di Mattia<sup>2</sup>, and Antonella Macagnano<sup>1,3</sup>*

<sup>1</sup>University of Tuscia, Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), Viterbo, Italy (decesare@unitus.it)

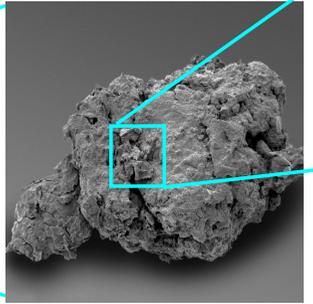
<sup>2</sup>University of Tuscia, Department of Agriculture and Forest Sciences (DAFNE), Viterbo, Italy

<sup>3</sup>National Research Council (CNR), Institute of Atmospheric Pollution Research IIA, Monterotondo (RM), Italy

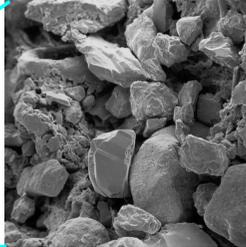
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# 1. THE CONTEXT: Impact of soil structure and microhabitat on soil biota

## MINERAL AND ORGANIC COMPONENTS

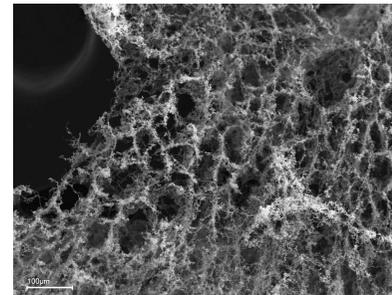


Macroaggregates

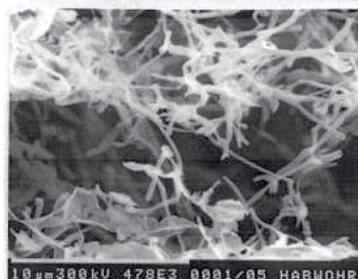
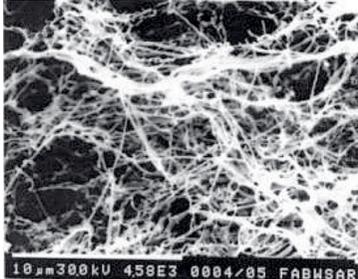


Soil particles

Source: T. Eickhorst & R. Tippkoetter. *Micropedology—The hidden world of soils.* University of Bremen, Germany (<http://www.microped.uni-bremen.de>). All rights reserved



Fulvic and humic acids showing tubular and membrane structures and honeycomb-like aggregations



Source: Kim H. Tan (2014) *Humic Matter in Soil and Environment.* CRC Press. All rights reserved

- 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 comprising 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.

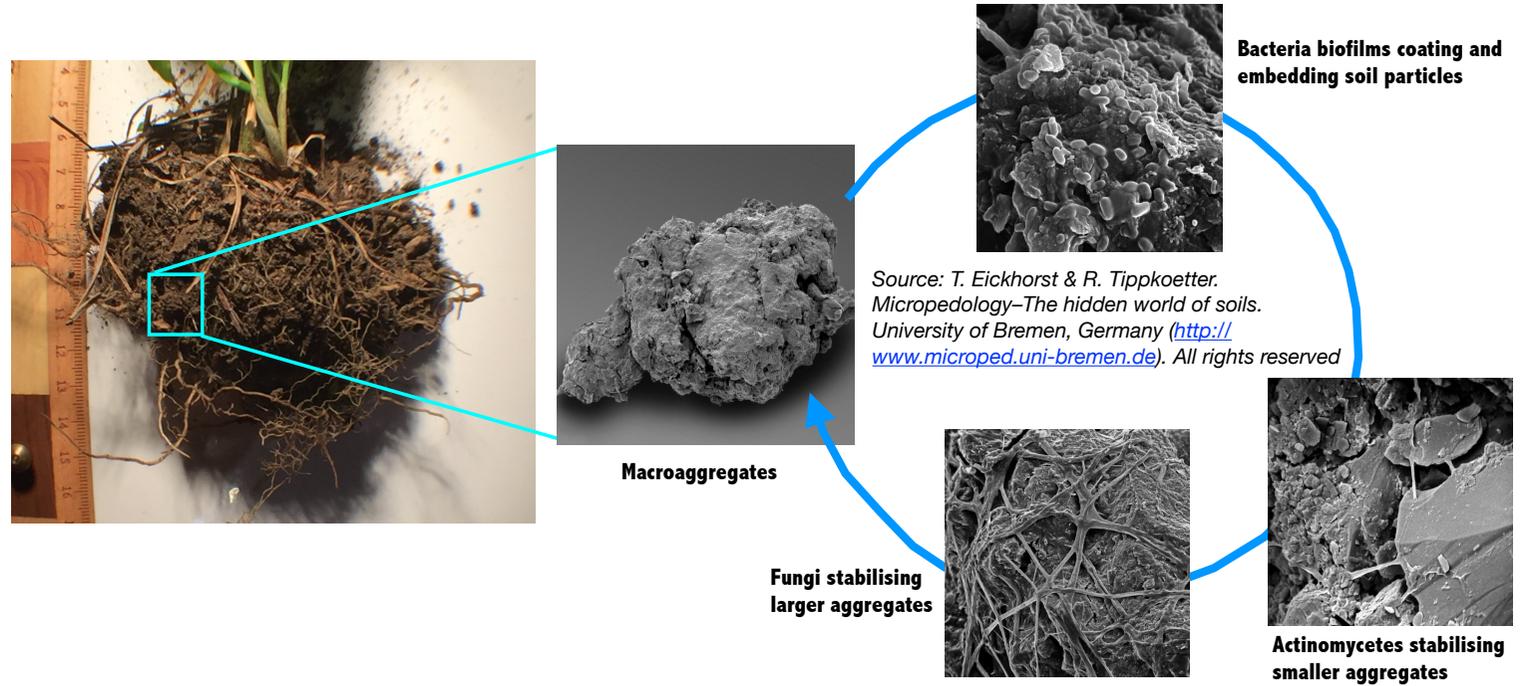


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# 2. THE CONTEXT: Impact of soil biota on soil structure and microhabitat

## MINERAL AND BIOLOGICAL COMPONENTS



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.



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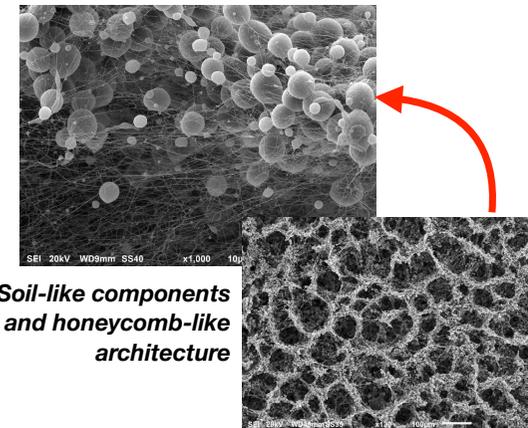
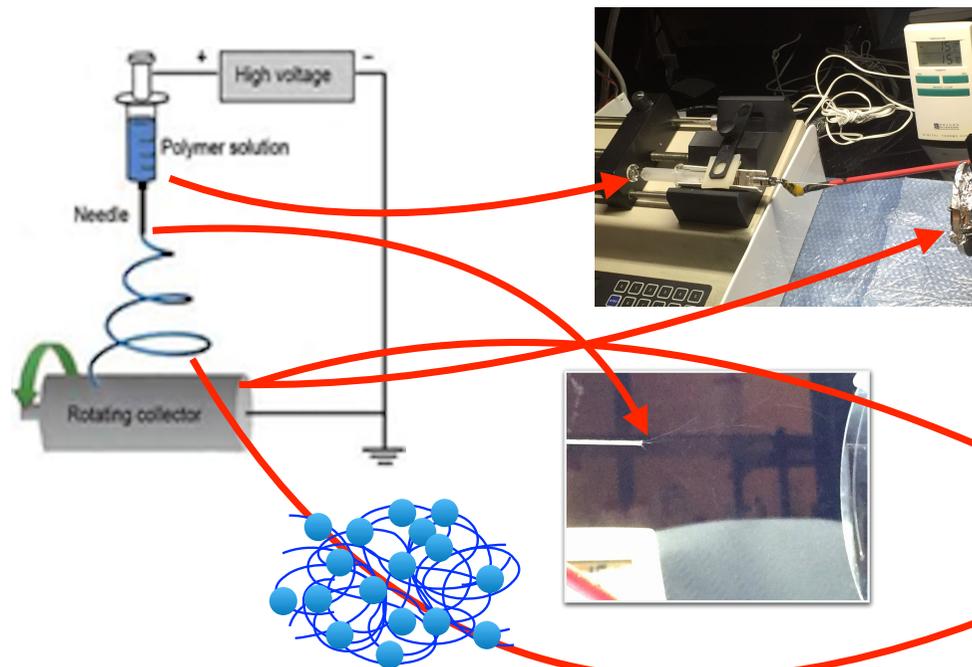


### 3. AIM: Development of a 3D polymer nanostructured fabric mimicking the soil architecture as a model substrate for studying the soil-microbe microhabitat at micro- and nanoscale

As a model system, a self-standing 3D biodegradable polymer 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 (i.e. organic matter and mineral particles) and the relative spatial architecture and porosity at the micro- and nanoscale were created by electrospinning.

**Electrospinning** is a nanotechnology producing 2D and 3D nano- and microfibrils scaffolds from polymer solutions under an electric field.

Here, the resulting NS were characterised by considerable porosity and extensive surface area.



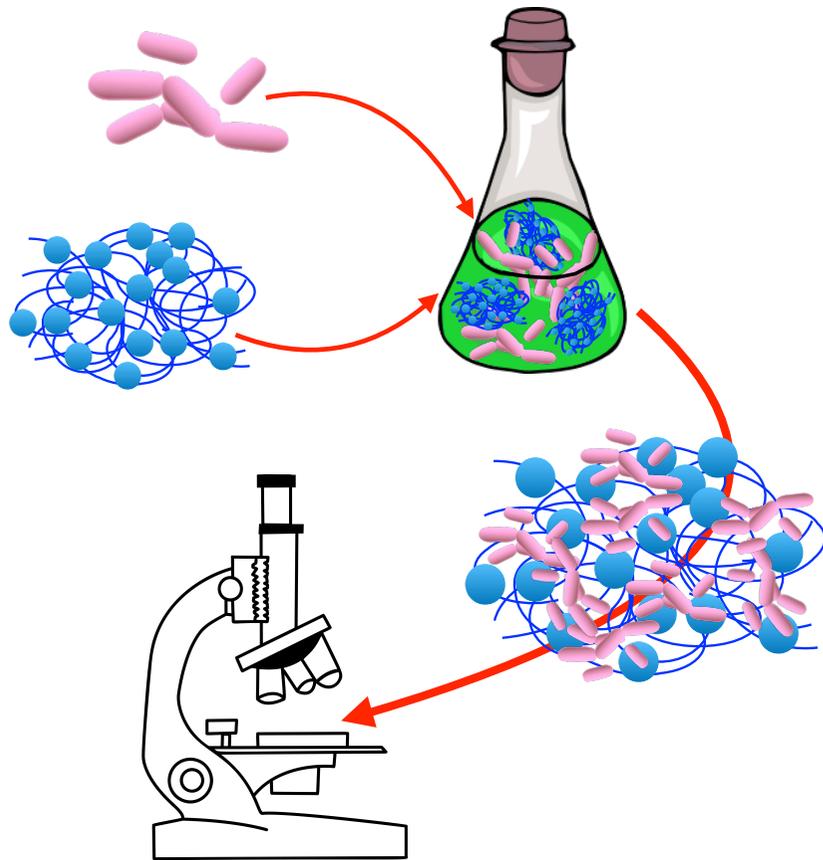
Soil-like components and honeycomb-like architecture

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## 4. MATERIALS and METHODS to study the interactions between microbes and soil components in a simulated soil-like architecture



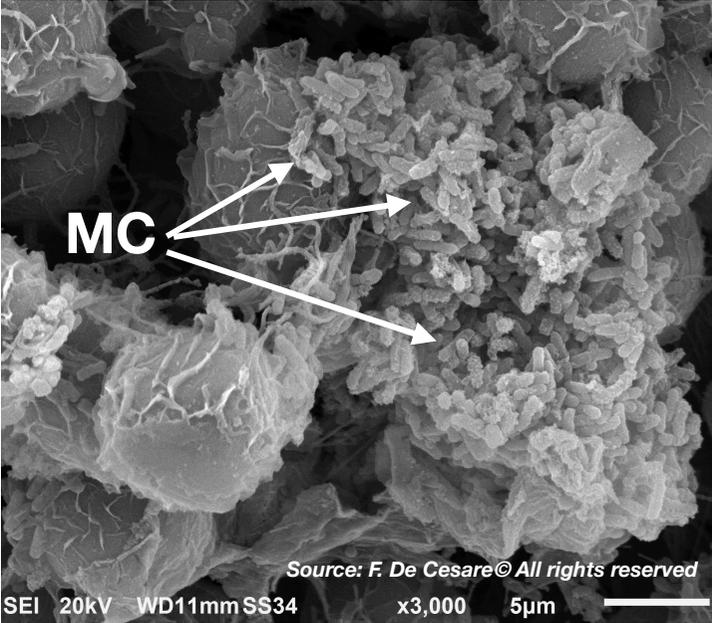
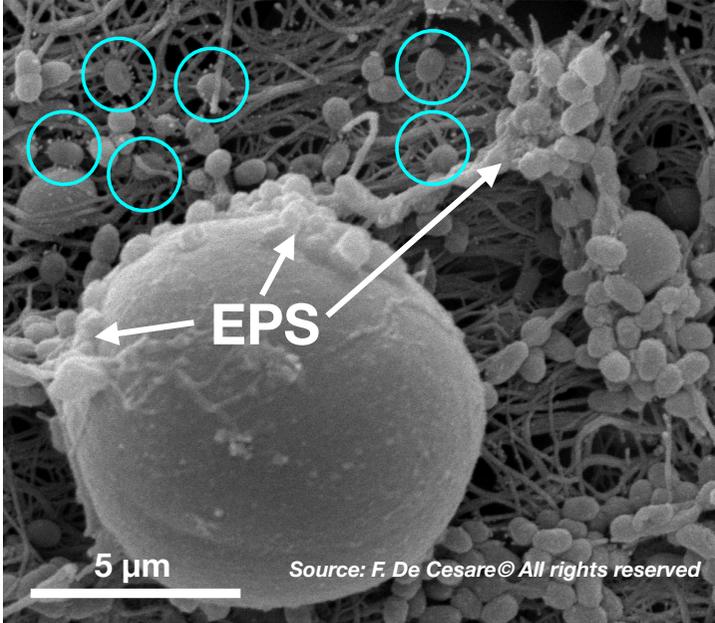
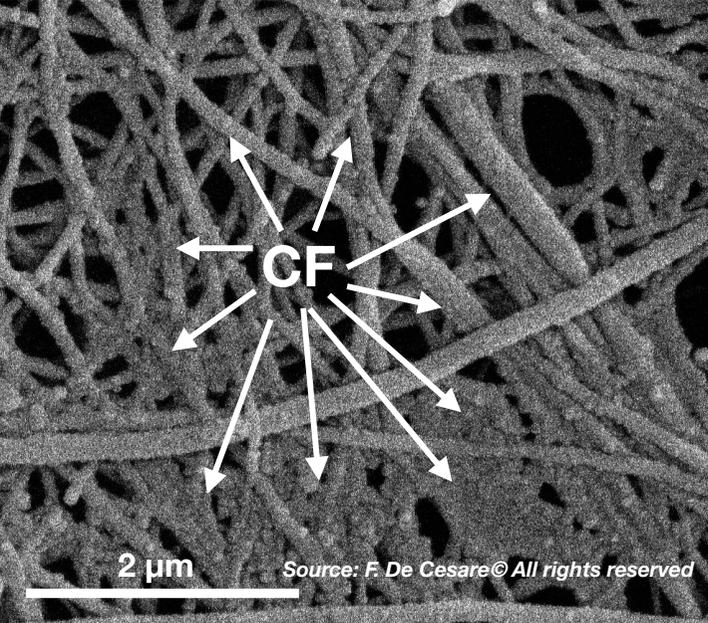
A PGPR species was employed as a model microbial type to test the capacity of similar NS of supporting microbial growth until biofilm development.

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 for future applications.

Combination of imaging techniques such as optical, SEM and TEM microscopy were used to shed some light into the nexus between microorganisms and soil structure and the reciprocal influence and in particular 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.



# 5. RESULTS: Insights on the impact of soil microhabitat on soil biota and vice-versa



The typical phases of conditioning film (*CF*) release, initial and stable adhesion mediated by appendages (*rings*) and *EPS* release, and micro- and macrocolony (*MC*) 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 bacterial growth rate, the overall respiratory activity and its spatial distribution throughout the NS were recorded.



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## **6. CONCLUSIONS & OUTLOOKS**

### **STRATEGY**

Creation of a 3D NS framework mimicking the soil structure architecture based on soil component morphology to provide a more “natural” environment for microorganisms (e.g. bacteria).

### **OUTCOMES**

Studying of microbial community organisations where microbes can develop more “natural” attitudes and physiological traits, hence reliably reproducing the spatial and temporal dynamics of microbial populations in specific soil contexts like hotspots typical of the rhizosphere, which is of central importance for the entire soil ecosystem functioning, or specific treatments.

### **BENEFITS**

These 3D NS can also provide the opportunity of zooming in microbial lifestyle, from the dynamics of interactions with organic matter and particle surfaces to their spatial distribution and colony formation, and link biological processes in particular conditions to specific physical and chemical features of soil and vice-versa by observing microbes at work at different scales (from mm down to nm).

### **POTENTIALS**

Such 3D NS could be used to develop functional products for several applications, from agriculture to environment, industry until medicine, where certain microbial species playing distinct functions can be hired and hosted.



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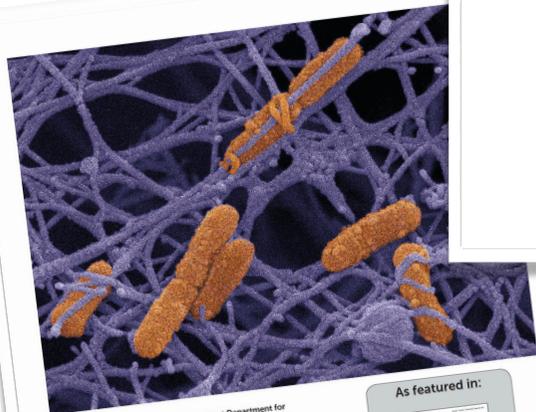
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6, 778

## A study on the dependence of bacteria adhesion on the polymer nanofibre diameter

Fabrizio De Cesare, <sup>1</sup>ab Elena Di Mattia, <sup>1</sup>c  
Eyal Zussman <sup>1</sup>d and Antonella Macagnano <sup>1</sup>ab

CONTACT: [decesare@unitus.it](mailto:decesare@unitus.it)



Featuring work from Dr. Fabrizio De Cesare at Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Viterbo, Italy, and Antonella Macagnano at Institute of Atmospheric Pollution Research (IIRA), National Research Council (CNR), Monterotondo, Italy.

A study on the dependence of bacteria adhesion on the polymer nanofibre diameter

Adhesion of bacteria to fibres is size-dependent with a preferential attachment to 100 nm wide nanofibres. The interaction mechanisms are investigated here. The presence of a conditioning film coating the nanofibres is found to be crucial for adhesion.

As featured in:



See Fabrizio De Cesare et al., *Environ. Sci.: Nano*, 2019, 6, 778.

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### Fishing bacteria with a nanonet Development of a bacteria biofilm on an electrospun scaffold of beads and nanofibres

F. De Cesare,<sup>1,2,3,\*</sup> E. Di Mattia<sup>3</sup> and A. Macagnano<sup>1</sup>

<sup>1</sup>Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Viterbo, Italy  
<sup>2</sup>Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, Viterbo, Italy  
<sup>3</sup>Institute of Atmospheric Pollution Research (IIRA), National Research Council (CNR), Monterotondo (RM), Italy



\*This image is a runner up in the Materials Today cover competition. There has been an increasing interest in recent years on improving crop productivity to fulfil the food needs of an ever-increasing

## Uncovered

world population. The damage and risk to the environment (crops, forests and water sources) and human health through impacts on agricultural practices (illage, excessive fertilisation and pesticide treatments, and deforestation) to satisfy these needs, however, have enhanced the belief that such practices should be urgently replaced by more environmentally friendly processes and compounds [1].

In recent decades, conservation agriculture practices like the Integrated Crop and Soil Management (ICM & IFM) and organic farming have been aimed at preserving natural resources (soil, water, fish) and limiting the use of agrochemicals [2]. However, the short-term reduction of crop yields, the low effectiveness and high cost of commercially available low-impact fertilisers (slow-release fertilisers - SRF) and the time-consuming research necessary to develop effective biopesticides (pesticides employing organisms and their products) have so far limited the application of these practices by farmers.

New strategies and tools are thus necessary to fill the gap between intensive and conservation agriculture.

Nanomaterials have recently been proposed in a multitude of contexts as groundbreaking materials to deal with a broad range of problems and surpass the limits posed by traditional approaches [3]. In agriculture, novel and green groundmaking tools have been developed employing nanomaterials to deliver agrochemicals to plants (nanopesticides), but reducing the impact of these compounds on the environment and human health by reducing the global amount provided and improving the efficiency of their actions [4].

Similar results can be obtained, however, following a more 'green' and sustainable approach based on microorganisms. Microbes preferentially live in complex structures, adhering to surfaces that are termed biofilms [5]. Such a particular lifestyle is extremely advantageous for these organisms to resist to harsh environmental conditions, toxic substances (pollutants), biocidal agents (antibiotics, immune systems) and predators [6, 7]. Such presence of harmful and detrimental microorganisms can cause damage (distortion and disease, corrosion and fading of metal substrates) [8]. Conversely, the same properties can be extremely

\*Corresponding author: Dr. Cesare, F. (decesare@unitus.it)