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Investigating the invisible: The unseen life below our feet

Der Boden - Lebensraum für unzählige Mikroorganismen, Bild: Pixabay, CC0

It's alive! Numerous microorganisms are responsible for the structure and fertility of soil, which is so much more than a field of crops or dirt underneath one's fingernails: It is home to a microscopic world teeming with bacteria, fungi, protists and viruses.

Soil microorganisms: Important for healthy environment

With an estimated one trillion species, there are more microbes on Earth than stars in the galaxy. The thin layer of material covering the earth's surface, commonly known as soil, is especially rich in microscopic organisms: Every teaspoon of soil is assumed to be home to 200 meters of fungal hyphae and one billion bacterial cells [1]. Many of them actively break down and recycle nutrients and ensure that our ecosystems are resilient to environmental changes.

As with other ecosystems on Earth, it is important to understand the many types of organisms and their functions, conserve them and thus contribute to the protection of our environment. For this reason, the multidisciplinary field of soil ecology developed. Soil ecologists are interested in the interaction of soil organisms with their physical and chemical environments [2]. They investigate the diversity of the seemingly invisible organisms that live in soil and try to find out more about their important roles in keeping our environment healthy.

What is soil?

Soil – also referred to as earth or dirt – consists of plant residues, microbial and animal remains and minerals. These components are stuck together by sugars, which are secreted by microorganisms themselves or by plant roots, and form aggregates. The spaces between these aggregates can become filled with water or air.

In this complex maze, insects and earthworms burrow, further changing the structure of the soil and leaving their waste behind as food for microorganisms. Long fungal hyphae stretch through the soil and provide highways for bacteria to move from one place to another to access food. The structure of the soil environment also provides protection from predators.

As conditions in soil can change quickly with weather patterns, seasons or even fluctuations throughout the day, soil is also a very challenging environment for microorganisms to survive.

The importance of microscopic life in soil

Because soil is a difficult habitat to live, microbes have developed many different strategies for obtaining the energy they need to survive. Microorganisms decompose organic matter by producing enzymes that break down dead plant, animal, and microbial remains into their simplest components. This way they recycle nutrients and make them easily usable for other living microorganisms and plants. In addition to this, microorganisms in the soil take up greenhouse gases such as carbon dioxide (CO_2), dinitrogen monoxide (N_2O) or methane (CH_4) or release them into the atmosphere. This makes them also important players in global warming.

In addition to the role of bacteria and fungi in carbon cycling, there is growing evidence that viruses are a vital, yet under-studied part of it. Some viruses live inside bacterial or protist cells and control the way carbon is recycled in soil environments. By lysing their host cells and causing their death or by using their own genes related to carbon cycling, tiny viruses can thus play important roles in the global carbon cycle [3]. Discovering the diversity of bacteria, fungi, protists and viruses that live in soil will help us to better understand and protect the health of our ecosystems.

Common techniques for studying soil microorganisms

Soil is not only a highly dynamic and challenging habitat for microbes to live, but also a very complex environment for scientists to study. Given the porous structure of soil, its microbial composition can vary from centimeter to centimeter. The types of microorganisms found in a location at a particular time can also change rapidly, as microbes grow, reproduce, die and migrate from one place to another. On top of that, the enormous number of different microorganisms also requires many different methods to study them.

Soil ecology has a long history. Major advances have already been made towards our understanding of the organisms living there and the important roles they play. Much of what we know about microorganisms in soil has been discovered using the following experimental techniques:

- **Microscopy**: Microscopes were used for the first time by Antonie van Leeuwenhoek in 1675 for the observation of bacteria. At that time, microscopes only had a single lens, but they have come a long way since their humble beginnings. Today, small sections of soil samples can be analyzed by adding a dye, which sticks to the DNA of the organisms. When stimulated with lasers built into the microscopes, the dye becomes fluorescent and allows to view the organisms directly in the soil. Microscopy has thus made it possible to count individual cells, investigate their different shapes and structures and detect patterns of cell growth in their natural environment.

- **Cultivation of microorganisms:** By culturing bacteria and fungi in the laboratory, scientists can investigate their morphology and growth. Liquid medium in tubes or solid medium in petri dishes provides all the nutrients they need. However, many soil microorganisms do not survive outside of their natural environment, as they need to exchange nutrients with other soil microbes to thrive. This explains why up to now, only about one percent of the different types of microorganisms can be cultured in the lab [4]. Growing microorganisms in the lab thus remains a big challenge.

- **DNA Sequencing:** DNA sequencing represents a good way to study microorganisms from soil without growing them in the lab. It allows scientists to match the genetic code of soil samples with the code of known soil microbes and classify them.

Samples for DNA sequencing can be collected from soils from different ecosystems, ranging from rainforests to deserts and from farms to alpine mountain peaks. In a first step, the DNA of the samples – which usually contain millions of bacterial and fungal cells as well as viral particles – is extracted and prepared in the lab. The DNA sequence, i.e. the genetic code of the soil microbes contained therein is then analyzed by sequencing machines. The use of DNA sequencing has helped to discover a large number of new organisms and to decipher their important functions in environmental processes.

New ways of investigating soil microbes

Studying the diversity of microorganisms is still a difficult and exciting venture for many scientists. Today, highly sophisticated microscopes and microscopy techniques are used to look at the growth and spatial patterns of microorganisms.

- **Fluorescence in situ hybridization, also known as FISH,** is one of them. It allows researchers to mark specific gene sequences in microbial cells with fluorescent tags. Under the microscope, microorganisms can be differentiated this way. FISH is used for example to analyze the growth of soil microbes and their interaction with one another.
- **The 'isolation chip' or 'iChip'** represents a creative solution to provide suitable growth conditions for microbes [5]. Instead of trying to grow single strains on liquid or solid medium, soil is diluted so that only a few cells remain. These cells are suspended on a plate in agarose, which is then buried under the soil to create more natural conditions for the organisms to

grow. After a few weeks, bacteria and fungi start to grow that could otherwise be overlooked using more traditional culturing methods.

- **Sequencing of organisms from soil i**s a rapidly advancing area of research. To generate improved DNA sequences from soil, individual or small numbers of cells can be separated from the community of soil organisms. This allows analyzing bigger parts of their genetic code, and the community of microbes can be pieced together like a puzzle according to its sequence. This way of sequencing also helps to discover new organisms and to decipher how they contribute to the larger ecosystem.

The use of these novel techniques has contributed to an increased knowledge about specific types of bacteria, fungi and viruses that live in soils. It has helped to get insight into important processes controlled by soil microorganisms and revealed microbial functions affected by environmental change. Understanding microorganisms in soil will be essential for protecting farmland to grow the food we need, and for predicting how ecosystems around the world will withstand climate change. Scientists have already made enormous progress into this direction, but there are still gigantic numbers of microbes waiting to be discovered and analyzed.

The soil below our feet may only seem like a bunch of dirt, but it is a dynamic microbial world with large impacts on the world above-ground.

This text is a guest contribution from Dr. Lauren Alteio from the Department of Microbiology and Ecosystem Science, University of Vienna

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List of references

[1] FAO, ITPS, GSBI, CBD and EC. 2020. State of knowledge of soil biodiversity - Status, challenges and potentialities, Report 2020. Rome, FAO.

[2] Zuckerman MAP: Soil Ecology (2008). Encyclopedia of Ecology.

[3] Trubl G., Jang HB, Roux S. et al.: Soil Viruses Are Underexplored Players in Ecosystem Carbon Processing (2018). mSystems Oct 2018, 3 (5) e00076-18

[4] Martiny AC: High proportions of bacteria are culturable across major biomes (2019). ISME J 13, 2125–2128 (2019).

[5] Nichols D., Cahoon N., Trakhtenberg EM et al.: Use of Ichip for High-Throughput In Situ Cultivation of "Uncultivable" Microbial Species (2010). Applied and Environmental Microbiology Apr 2010, 76 (8) 2445-2450