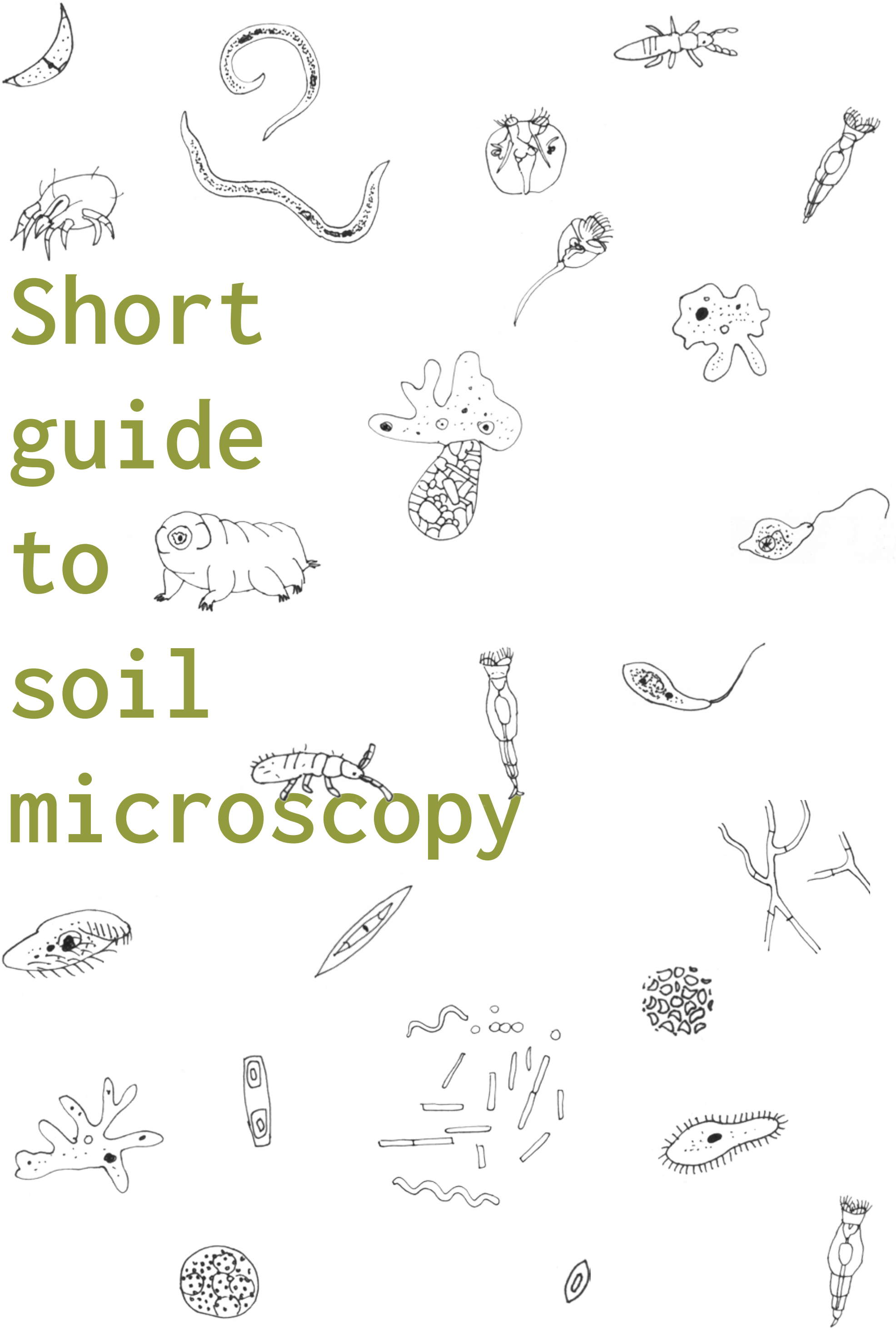
Short guide to soil microscopy



Introduction

Microbial ecosystems create the conditions for our survival on planet earth: they establish and modulate all our habitats, our climate as well as our health and mood. Cooperation with microbes is the only reasonable strategy for all larger form of life – including humans.

Fertile soil is the basis of civilization. Empires rise and fall with the cultivation and degradation of land. Industrialized agriculture leads to a loss of soil structure, nutrient degradation and contamination – ultimately breaking down ecosystems and eroding the soil to infertile desert.

Let's see who actually lives down there. Maybe they are still willing to collaborate.

Identification & evaluation

Getting to know the creatures that live in your soil does not necessarily mean to know all their names and count their number per gram. If you are just starting to do microscopy, it is of course much more interesting to watch how they move around and do their thing. Take your time to observe. Make friends.

Whenever you are ready for a more systematic approach, begin by formulating a precise research question: e.g. is there a difference between the part of the garden that I covered with mulch and the part that I left exposed?

Recommendations:

- make a time-plan – when to take samples & when to do microscopy
- take at least 3 samples of each plot that you want to compare
- be very precise in the preparation – treat all samples exactly the same
- make sure that the 'waiting-time' before microscopy is always similar
- when you prepare the slides, try to pipette the same amount and density
- scan the slide systematically and note each organism (sometimes you have to estimate)
- if the 3 samples of the same plot are not similar at all, do more rounds of counting

How can I improve my soil?

There is no universal recipe for soil regeneration, since every garden or farm has it's unique conditions and problems. Making earth a better place to live requires observation, lot's of time and mindful interaction. Diversity is often the key to health: when you transform a monocultures into an ecosystems, your soil life will thrive and create feedback loops that accelerate humus building. Often it is enough to use regenerative techniques like reduced tilling, cover cropping, mulching and especially the integration of trees and bushes on your farmland. Sometimes the soil food web is severely damaged and you have to (re)introduce specific groups of organisms (e.g. via compost) to accelerate the desired transformation.

This guide aims to show you some basic techniques of soil microscopy, how to take samples and how to identify the organisms you find. Use it as a starting point for your own explorations, compare your samples from plot to plot or year to year, draw your conclusions and implement the techniques that work on the piece of land you are taking care of.

Here are some examples of our observations:

Generally low diversity (often lot's of bacteria)
Typical for industrial agriculture. As above so below.
Thorough regeneration needed.

Lot's of ciliates
Indicator of low oxygen conditions. Often found in compacted soil and anaerobic compost.

Lack of fungal mycelium
Indicates a lack of organic matter and/or frequent disturbances (e.g. plowing or chemicals).

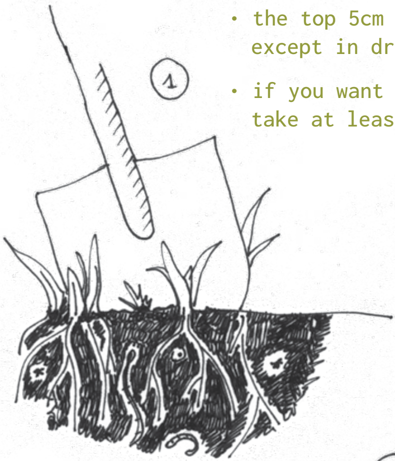
Almost all groups of organisms are present
Congratulation. You are doing a great job!!

If you want to dive deeper into the microbial world, there is a great variety of videos, books and workshops available online. You can also check out our website, where we are starting to collect content on microbes, soil biology and regenerative agriculture: *mikrobiomik.com/humussapiens*

PART 1

Preparing a sample

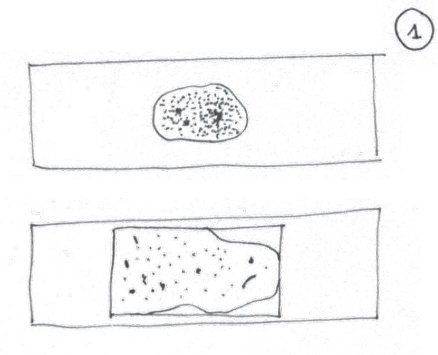
- collect a handful of soil (without big stones or roots)
- the top 5cm are often very alive, except in drought conditions
- if you want to compare locations, take at least 3 samples from each place



- fill you sample-container (e.g. glass jar) without compressing the soil
- add water and gently mix the sample until the soil is saturated but not ‘swimming’ *(when you tilt the container, a small drop of water should form on the side)*
- if you tap water contains chlorine, use non-carbonated bottled water instead

- let the soil soak for at least 1 hour (better 1-2 days) *(the dryer your samples were during collection, the longer you have to wait for the microbes to ‘wake up’)*
- the samples should stay at a warm (not hot) place with their lids slightly open
- sometimes you have to add a little bit of water after the waiting period
- use a pipette and gently suck in the drop of water that forms when you tilt your container

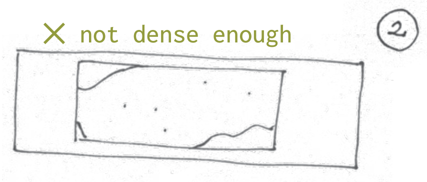
Preparing a slide



- drop the liquid on your glass slide until a little puddle forms (around 3-5 drops)
- carefully lay the thin ‘coverslip’ on the drop without any pressure

  (the space between the glass should ideally be completely filled with your soil extract)

✕ not dense enough



✕ too muddy

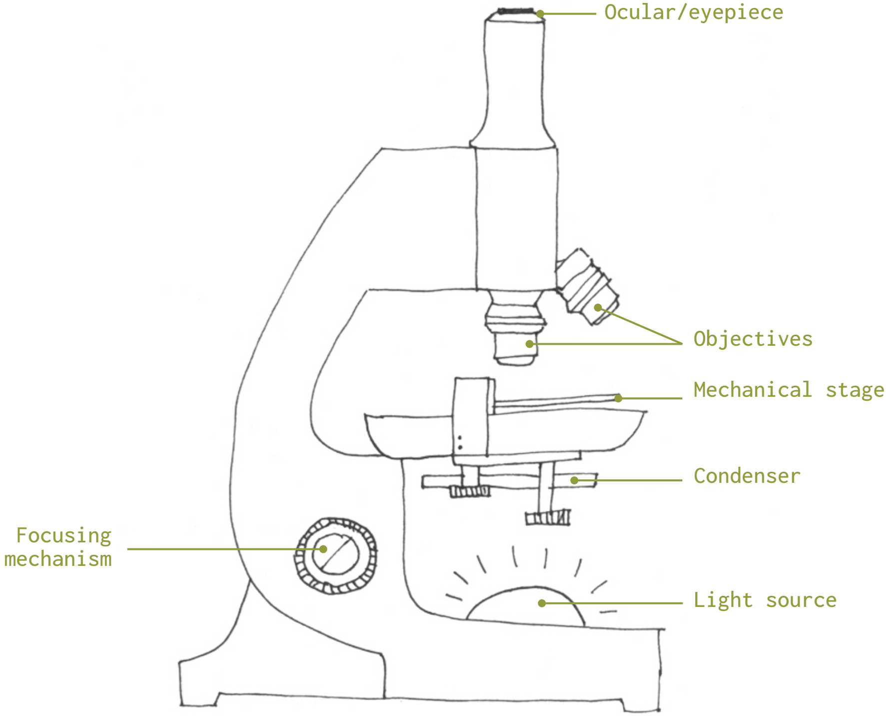
- with a bit of practice, you will find the right amount and the right ‘density’ of the extract - each soil sample is different and the sample preparation is often intuitive
- generally, the slide should not be completely transparent and also not too muddy

  (it is ok if some larger crumbs are present, but the cover should be laying flat)

The typical design of a „classic“ microscope allows direct observation of specimens by eye. In addition, a camera can be attached to take photographs and video recordings. In self-built microscopes (see next page), the specimen can usually be viewed exclusively via a screen.

Using the microscope

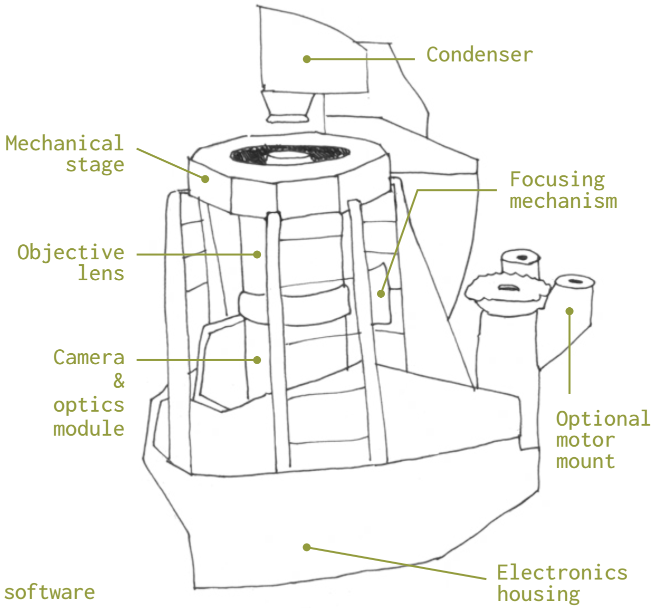
“Classic” microscope



Features:

- mechanical sample-stage (you can move the slide around with 2 knobs)
- precise focusing and several objectives for different magnifications
- condenser lens and other options to change the lighting (filters, etc.)

“Openflexure” microscope

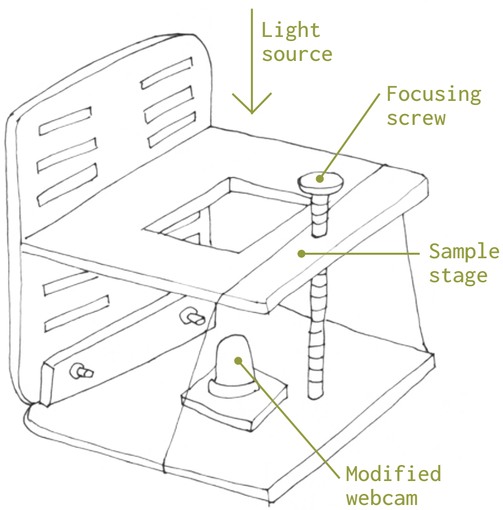


Features:

- precise focusing & mechanical sample-stage
- open-source 3D-printed design with inverted geometry (the objective is below the sample)
- uses Raspberry Pi with open software
- can be upgraded with professional optics

More information: *openflexure.org/projects/microscope/*

“Do-it-yourself” microscope



Features:

- extremely cheap and can be built from almost any available material
- uses a modified webcam, with inverted geometry, can be equipped with a light source
- detailed instructions on different design-options are available online

More information: *hackteria.org/wiki/DIY_microscopy*

PART 2

Soil organisms are often grouped by size and traditionally by their appearance as more animal-like ("Fauna") or plant-like ("Flora"), although these classifications often don't make any sense in the microbial world. Unfortunately, also many other commonly used terms like "protozoa", "amoeba" or "algae" are a result of historic developments and are often based on morphological appearance instead of biological relationship. We have to call those creatures by some name and it makes a lot of sense to sort them into groups, but be aware of the fact that "taxonomy" - the science of naming & classifying organisms - is a complete mess when it comes to microbes. If you are not a fan of fancy Latin, just use the general terms and don't bother with the names of individual species.

This is not a complete list of soil organisms and for sure not a taxonomic guidebook. The creatures described here, are the ones that you will likely encounter in your soil samples and the images are meant to help you with their identification. We assume that you can identify larger creatures like earthworms, insects, snails and moles if you find them in your sample.

We chose to list 11 common soil-dwellers and sort them roughly by size in a descending order:

1. Collembola
2. Acari
3. Tardigrades
4. Nematodes
5. Rotifers
6. Amoeba
7. Ciliates
8. Flagellates
9. Microalgae
10. Fungi
11. Bacteria & Archaea

For the images, we used a "classic" microscope (older model from Zeiss) and a cheap ocular camera (from Bresser). For most images the magnification is around 50x. For the ones of ciliates, flagellates and baceria it's 100x. The quality of the micro-photographs is intentionally on amateur-level, to make them comparable to images that you can obtain during a beginners workshop or with home experimentation. The images are modified (e.g. higher contrast) for printing purposes.

Collembola (springtails)

- their body size is usually 1-3 mm and they have 6 legs (but are not considered insects)
- found in humus layers of not too dry soils and on decaying plant material
- omnivorous and important for the control of soil microbial communities
- about 9 000 known species

Acari (mites)

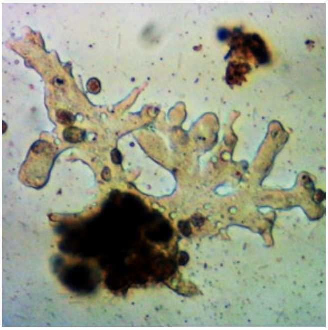
- soil dwelling mites are usually smaller than 1 mm and have 8 legs (like spiders)
- very widespread and sometimes predatory on other mites, springtails or nematodes
- play an important role in decomposition and help spreading beneficial bacteria and spores
- about 50 000 known species

Nematodes

- species found in the soil are usually between 0.3 and 1 mm long
- most abundant in grasslands and forests; have been found in a depth of up to 3.6 km
- usually specialized on eating either bacteria, fungi, other nematodes or plant-roots
- about 20 000 known species (1 million estimated)

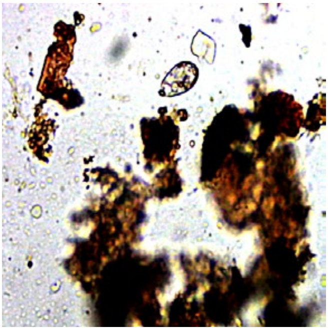
Tardigrades (water bears / moss piglets)

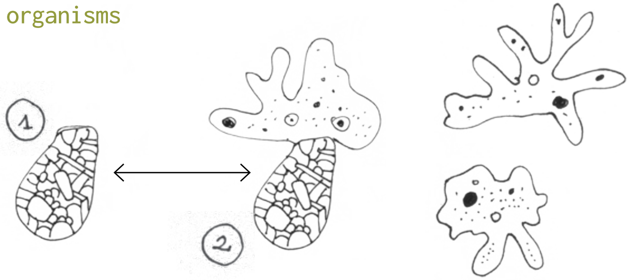
- adults are around 0.5 mm and can often be found in wet moss or leave litter
- able to survive extreme conditions (even exposure to outer space)
- feed mainly on plants and bacteria, but predatory species are also known
- about 1 300 known species

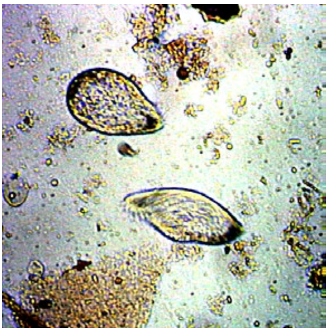


Amoeba

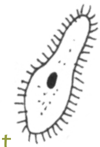
- multiform group of single-celled organisms that do not have a fixed body shape
- usually between 0.1 and 1 mm in size; sometimes with shells (like snails)
- capture their prey by flowing around them and thereby enclosing them with their bodies
- the term "amoeba" describes a lifestyle, therefore it is a very diverse group of organisms







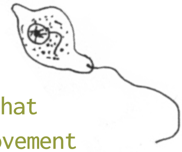
Ciliates



- single-celled organisms (0.01 - 1 mm) that use cilia (little hairs) for movement
- common in nutrient rich soil or compost samples (especially if not well ventilated)
- often very fast moving and feeding mainly on bacteria and algae
- about 4 500 known species (40 000 estimated)

Rotifers (wheel animalcules)

- multicellular organisms with body-sizes between 0.1 and 0.5 mm
- they have feeding organs with cilia that give the impression of a spinning wheel
- usually eats bacteria, single-celled algae and dead organic material
- about 2 000 known species



Flagellates

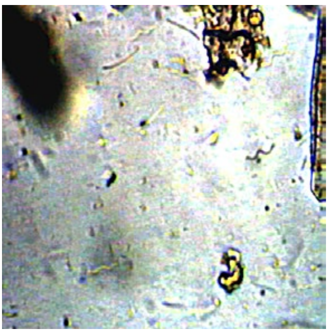
- group of single-celled organisms that use flagella (long ‘whips’) for movement
- usually between 0.003 and 0.01 mm in size (3-10 µm) and found in almost all soils
- often show twitching or twisting movements and typically slower than ciliates
- the term describes a form of movement, therefore it is a very diverse group of organisms

Microalgae

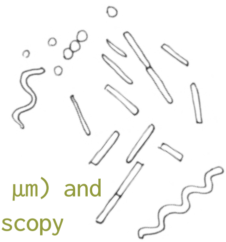
- general term for microscopic organisms that use sunlight to generate energy (photosynthesis)
- large size-range of 0.1 to 1 mm and very diverse shapes & colors (often green/brown)
- single celled but often form groups and chains or slowly swim around
- can usually only be found in the surface-layer during moist conditions

Fungi

- form their own kingdom of life including yeasts, molds and mushrooms
- single-celled species (e.g. yeast) and mushroom mycelium can be found in soil
- the mycelium often looks like thin ‘roots’, characterized by frequent branching
- very important for nutrient distribution and decomposition of organic matter



Bacteria & Archaea



- round, rod-shaped or spiral-shaped single-cells without nucleus
- very small 0.001 to 0.003 mm (1-3 µm) and difficult to see with light microscopy
- often move chaotically or in zig-zag motion, spiral-shaped cells usually like a corkscrew
- essential for mobilizing and transforming minerals as well as nitrogen fixation

Acknowledgements

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Links

(1) hackteria.org/wiki/UROS
(2) mikrobiomik.org/humussapiens
(3) openhardware.science
(4) instagram.com/ayllucoope
(5) gt22.si
(6) kons-platforma.org

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