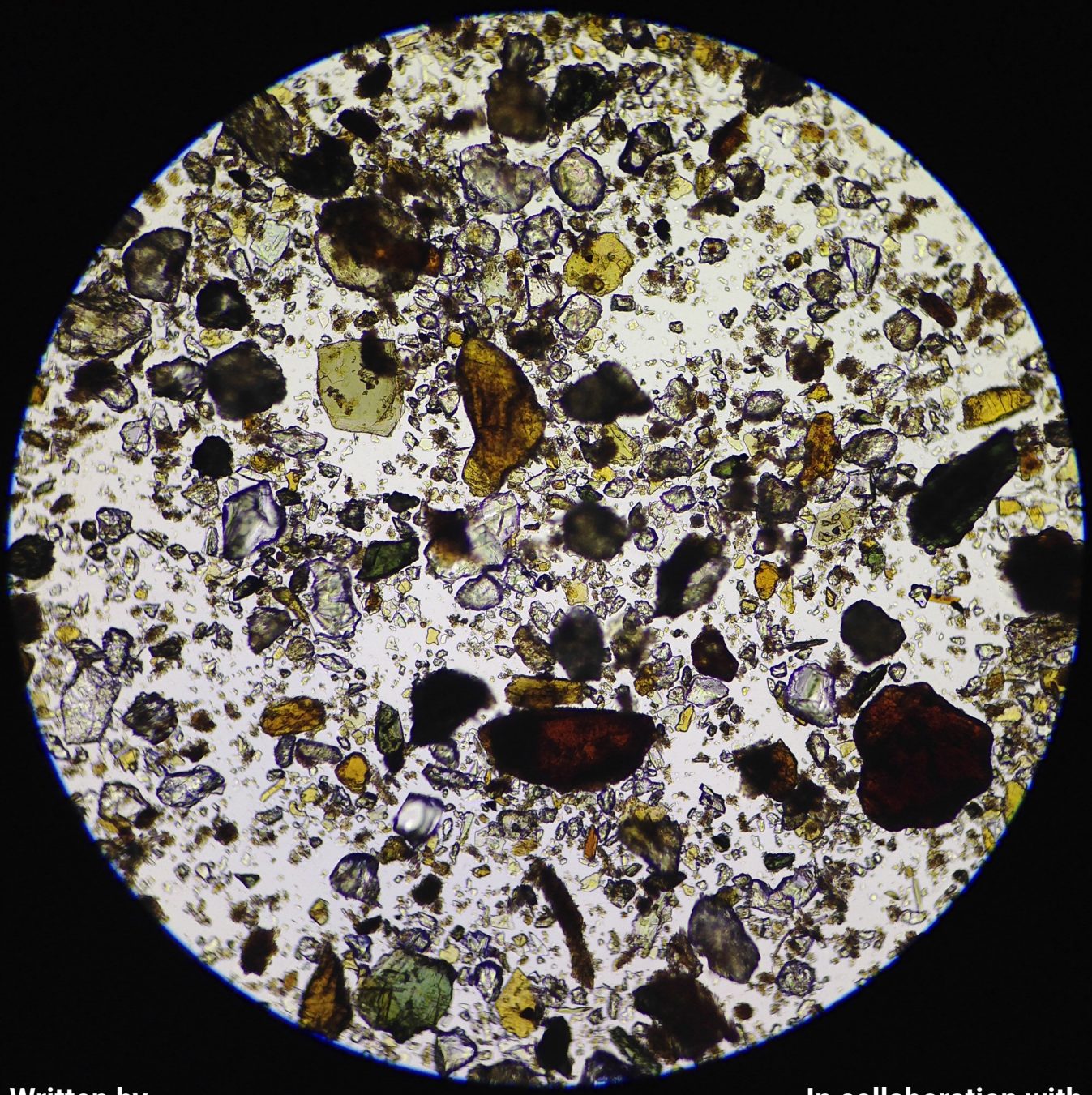
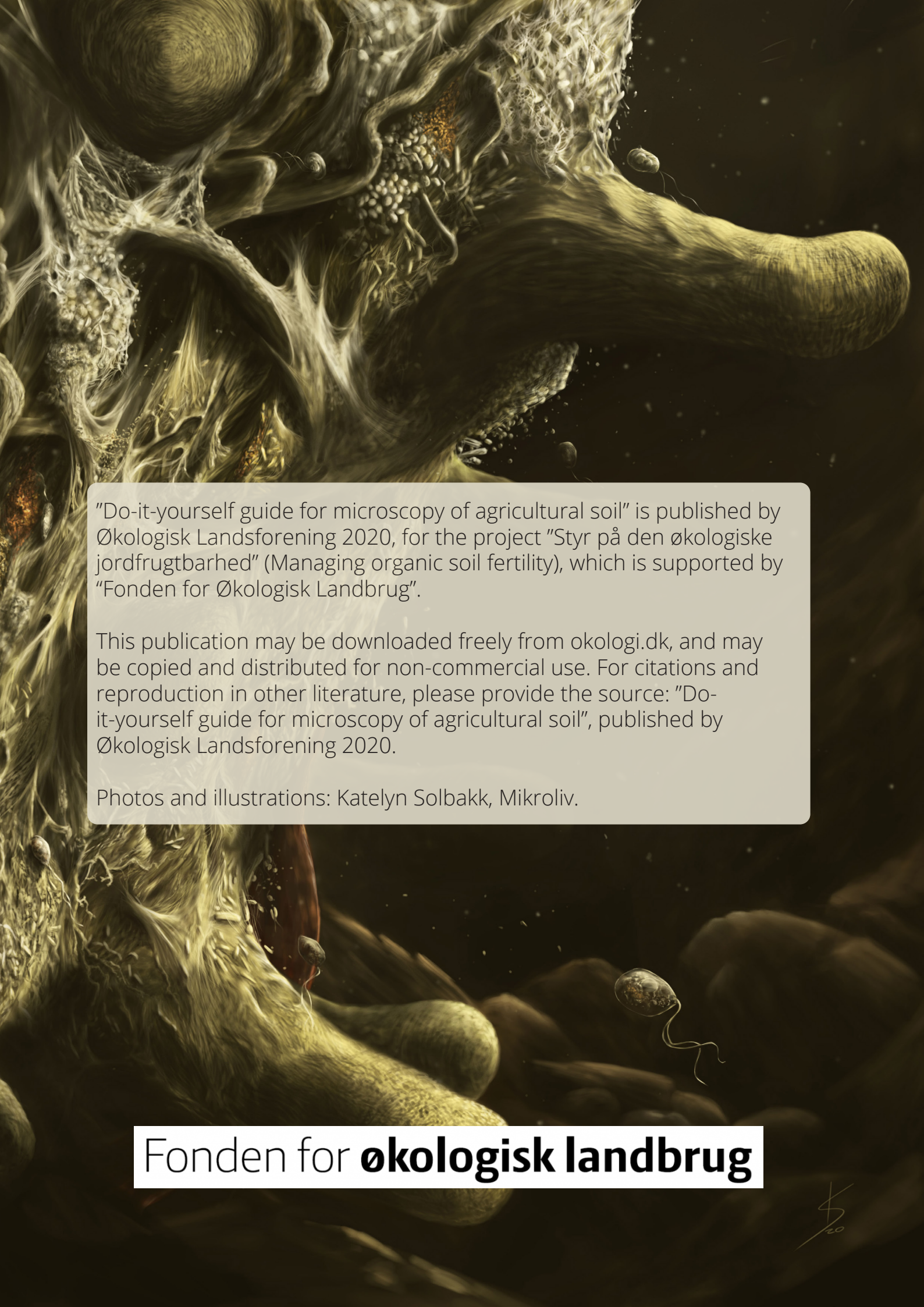
DO-IT-YOURSELF GUIDE FOR MICROSCOPY OF AGRICULTURAL SOIL



Written by
Katelyn Solbakk,
Mikroliv

In collaboration with
Janne Aalborg Nielsen,
Økologisk Landsforening

ØKOLOGISK LANDSFORENING



”Do-it-yourself guide for microscopy of agricultural soil” is published by Økologisk Landsforening 2020, for the project ”Styr på den økologiske jordfrugtbarhed” (Managing organic soil fertility), which is supported by “Fonden for Økologisk Landbrug”.

Photos and illustrations: Katelyn Solbakk, Mikroliv.

Fonden for **økologisk landbrug**

CONTENTS

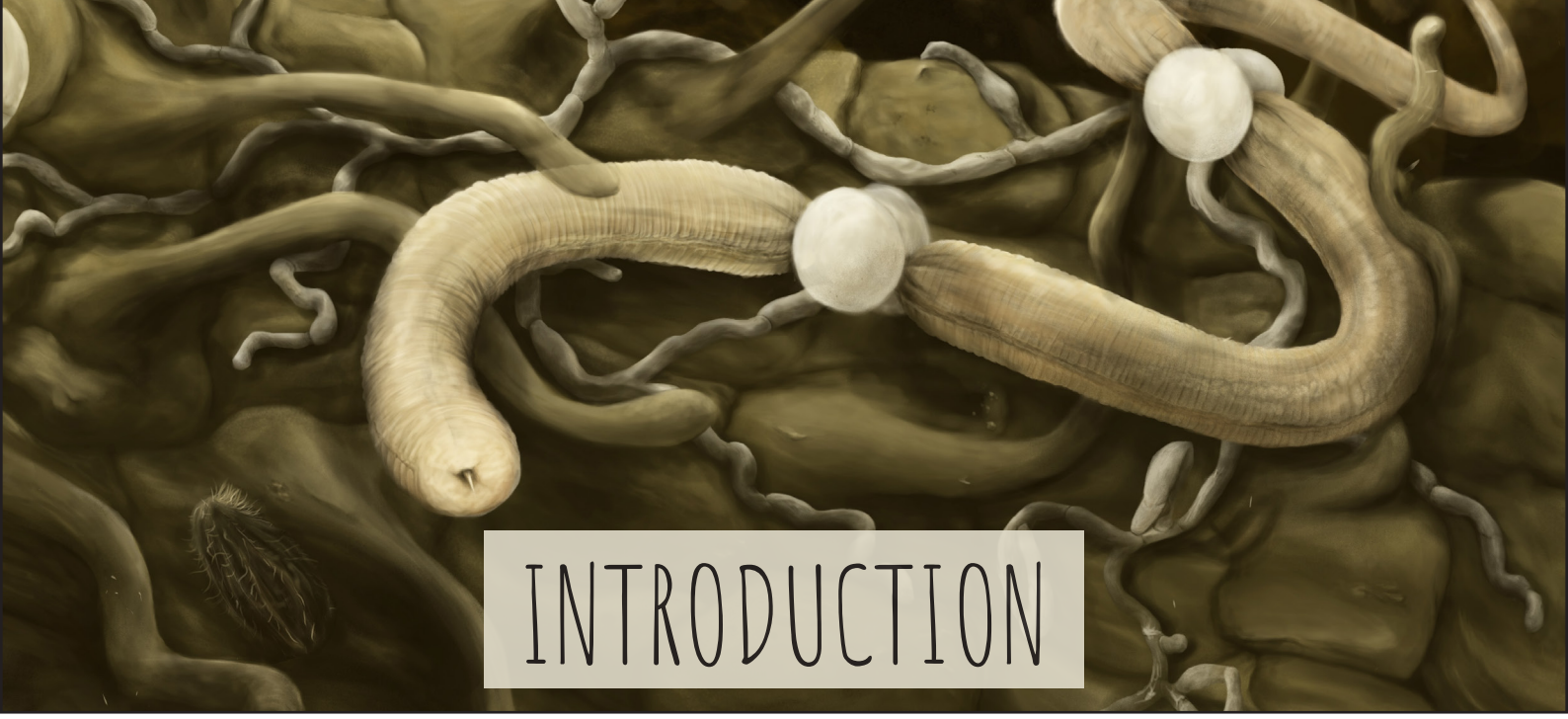
Introduction 4

Part 1
Collecting and preparing samples 5

Part 2
What can you see?
Bacteria 11
Fungi 12
Protozoa 13
Microscopic animals 14
19

Part 3
Evaluation and next steps 20

Quick reference table 24



INTRODUCTION

The soil ecosystem is a highly complex web, and each organism has a unique role to play. Science has barely begun to untangle the connections and functions of the extremely diverse array of life in soil, which is why it is so important to protect biodiversity in the ecosystem as a whole, and ensure that as many different organisms as possible are able to thrive and ultimately support the growth of healthy plants.

Some key functions of a healthy soil ecosystem include:

- Good water retention and drainage
- Healthy structure and resistance to erosion
- Nutrient cycling
- Improved plant health
- Carbon storage
- Resilience against pest and disease outbreaks

Many of these functions are the direct result of microbial activity in soil. Microscope analysis offers you a unique window into the life of your soil, giving you the chance to directly check in on your own soil ecosystem using simple equipment. There are even attachments that enable you to use your smartphone as a microscope!

It does take some practice to learn how to identify organisms, but this guide will help you get started and perform a basic evaluation of your own soil, and introduce you to some of the organisms that may be living there.

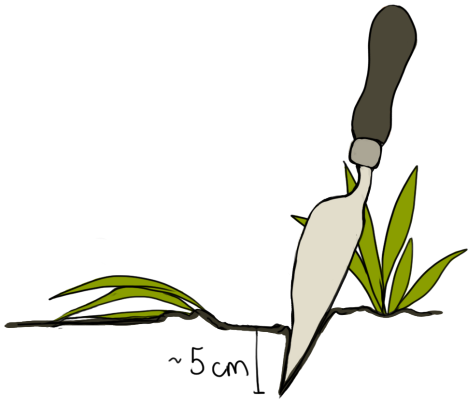
Part one

Collecting and preparing samples

Step one Collect samples

1

Clear any vegetation from the sample area.

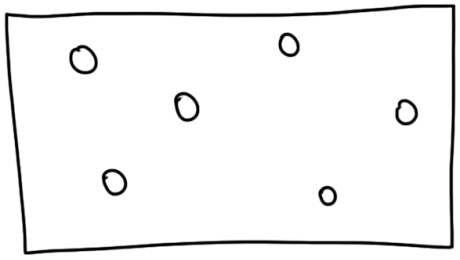


2

Collect soil 0-5 cm deep.

3

Repeat several times around the test area and mix the soil to create one sample that represents the whole plot.



Step two Prepare samples

Materials required:

- Kitchen scale
- Glass jar(s) with lid
- Spoon
- Tape
- Marker
- Water (145 ml per sample)

1. Use tape and a marker to label jars with the sample location/ID and collection date.

2. Mix 5 g of soil with 145 g of water in each jar.

3. Close the jars tightly, and shake gently for five minutes.

4. Loosen the lid(s) and let the samples rest undisturbed for two days.

Note: You can look at the sample immediately, but you will likely find more organisms after the waiting period. This is because many protozoa will rest in a dormant state called a “cyst” when conditions are dry. Allowing the sample to rehydrate gives them a chance to “wake up”.

Tip: If you have more than one sample, remember to label jars before filling them, and clean the spoon between samples.

Step three Meet your microscope

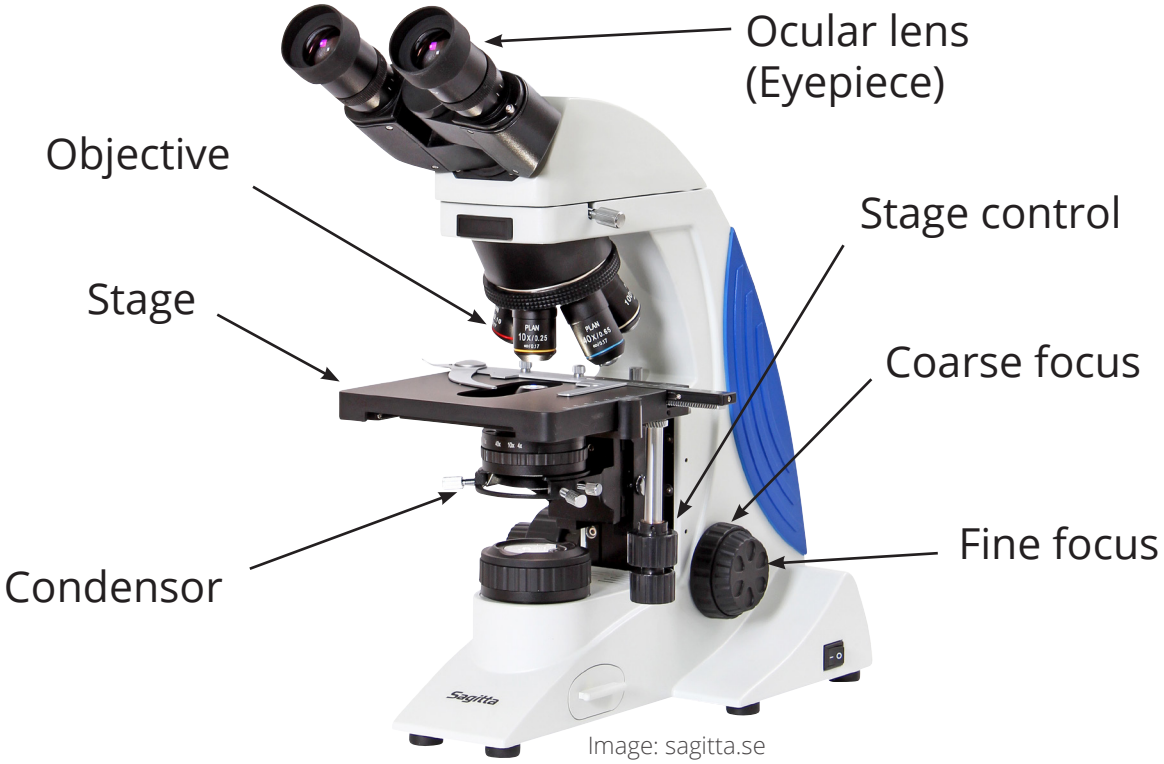


Image: sagitta.se

This is an example of a typical compound light microscope. There will be some variation between different brands and types of microscopes, and you can even use completely different types of microscopes, such as phone attachments. Consult your user manual to learn about your specific microscope if it does not match the image above.

Part	Function
Objective	Adjusts magnification
Coarse focus	Moves the stage a large amount to roughly adjust focus
Fine focus	Moves the stage a small amount to finely adjust focus
Ocular lens (Eyepiece)	This is where you look
Stage	Where you put the sample. Use the metal clip to hold it in place
Condensor	Adjusts contrast
Stage control	Use these controls to move the sample when viewing

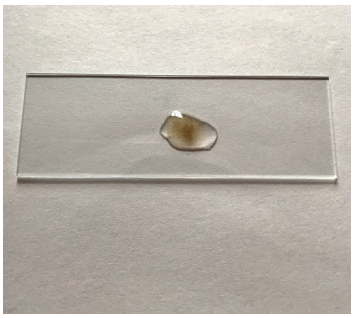
Step four Observe

Materials required:

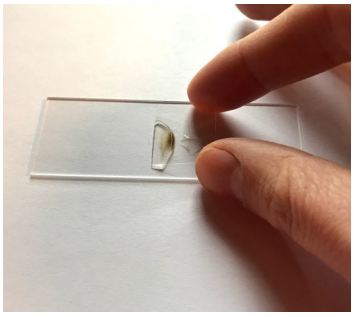
- Microscope
- Glass slides
- Glass covers
- Pipette
- Rinse water
- Empty jar for waste water
- Prepared samples

1. Close the lid tightly and gently shake the sample. Use the pipette to draw up some of the solution. Allow the heaviest particles to settle and drip out from the pipette (large debris will not fit properly under the cover slip).

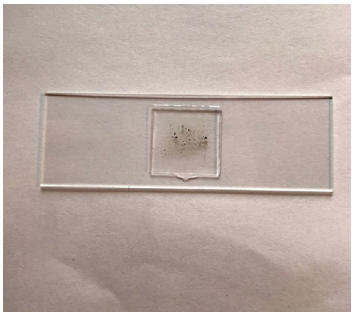
2. Place one drop of the sample on the glass.



3. Hold the cover glass gently by the edges (beware: cover glass is very thin, sharp, and fragile!). Place one edge on the glass next to the drop and slide it over so the solution spreads along the glass edge (see photo).



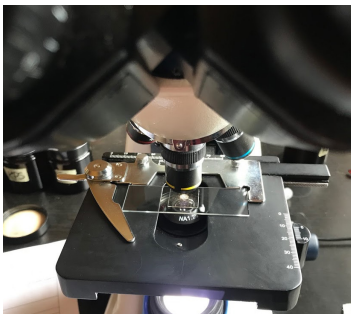
4. Carefully set the cover glass down so the sample is spread evenly.



Step four Observe

5 Place the sample on the microscope stage, using the metal clip to hold the slide in place.

6 Begin with low magnification and use the focusing knobs to make the image sharp, then switch to a higher power. Use the stage controls to explore the slide, and see what you can find!



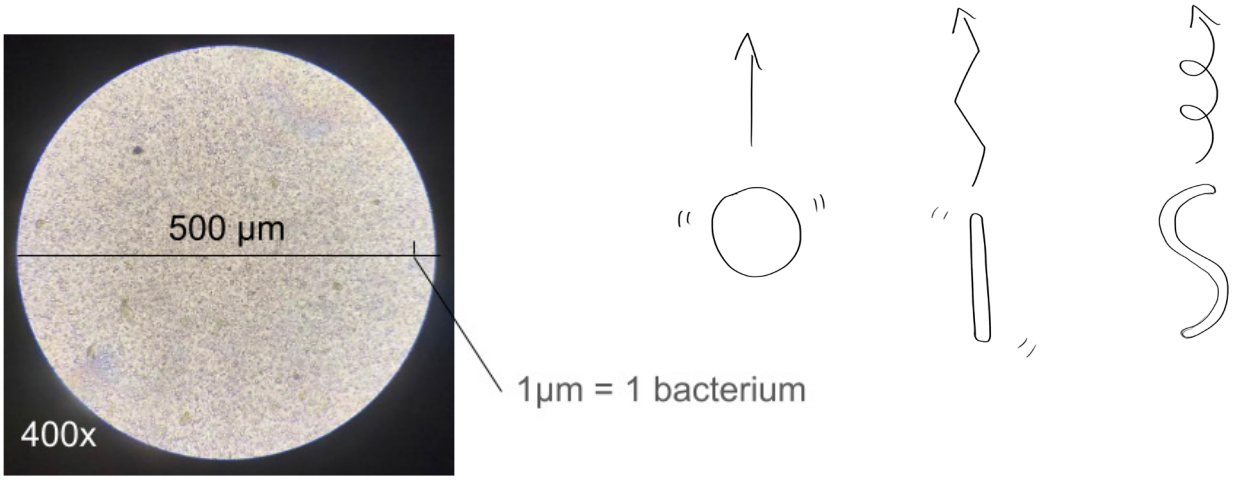
Tips:

- **Do not use the coarse focusing knob on high magnification**! It moves the stage too much and can crush the slide against the objective.
- When taking a drop with the pipette, avoid taking material from the very bottom of the jar as this will be too dense to look at.
- The microscope has a very narrow depth of field. Keep one hand on the fine focusing knob and continuously adjust the focus “up and down” while viewing samples.
- Analysis is typically performed at 400x, but 100x lets you see see larger areas at once and get a better view of larger, fast moving organisms.
- If you get “lost” and can’t seem to bring the sample into focus, switch back to the lowest magnification and use the coarse focusing knob until you can see the sample again. Then switch back to higher power and try again.
- If you have difficulty finding something to focus with (ie. it’s just white), try moving to the edge of the glass first and make that line sharp, then try exploring the sample again.

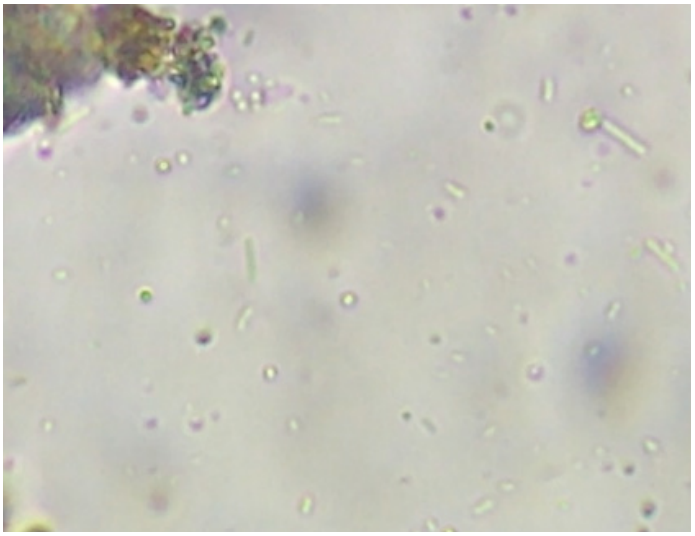

Part two
What can you see?
2016

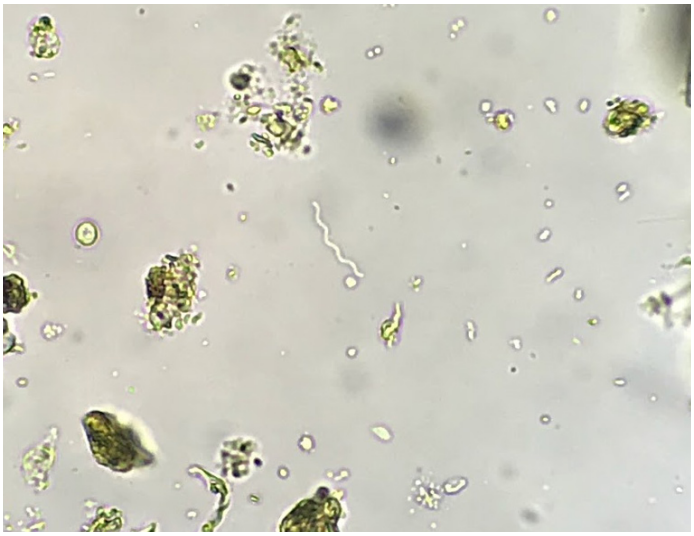
Group one Bacteria

Bacteria are very small; often just 1 µm in diameter (0.001 mm). The image below demonstrates how tiny they are at 400x magnification in a microscope with a field of view 500 µm (0.5 mm) across.



Bacteria are grouped into three broad categories based on the shape of the cells. These are round (cocci), rod-shaped (bacilli) or spiral shaped (spirilla). The arrows in the diagram above demonstrate the type of movement that is often seen with these different types of cells. Cocci often just vibrate, or move with a roughly straight motion. Bacilli sometimes move in a straight line as well but often have a rocking or zig zag motion. They can sometimes be seen linked together in chains. Spirilla have a characteristic corkscrew motion that is easy to spot.

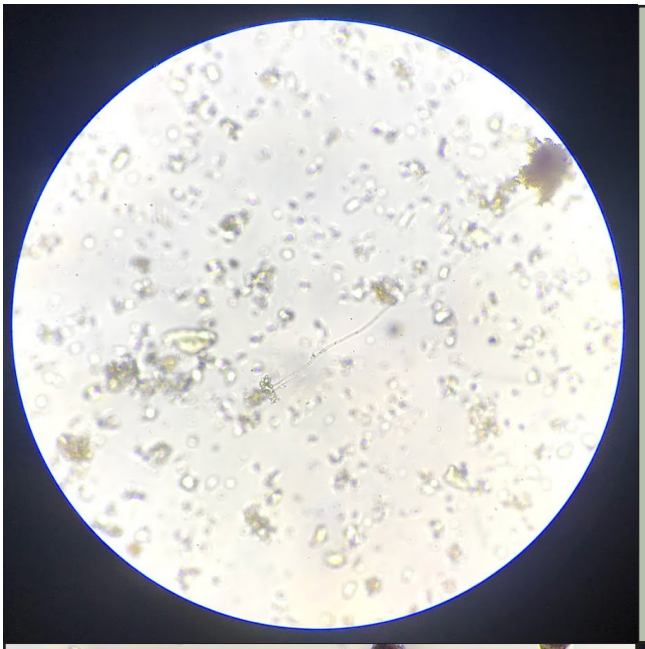




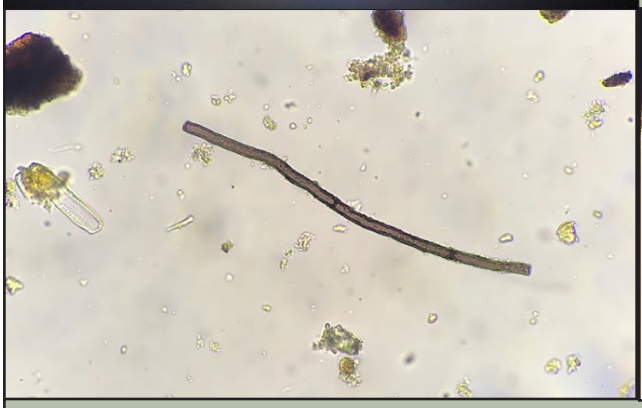
Group two Fungi

Healthy soil typically has robust networks of diverse fungal threads called “hyphae”. In the microscope, these look like clear or brown strands, typically between 2-6 µm in diameter. They can be small fragments or long strands that cover large areas of the microscope slide. Soil with many robust fungal hyphae typically has stronger structure and better aggregation. When disturbance is minimal, fungal networks weave through soil, binding particles together into strong aggregates, and they provide significant benefits to plants. Because fungi grow slowly and are easily disturbed, they can be good indicators of ecological succession. Agricultural soil often has low fungal populations and forest soil tends to be very rich in fungal networks.

Below are some examples of fungal hyphae that you can compare with your soil sample.



The fine horizontal line in the center of this picture (left) is a very narrow, thin fungal hypha. If you are seeing mostly or only strands like this, it could be a sign that conditions in the soil are not optimal.



The brown threads in the images above are examples of robust fungal hyphae fragments. Note the distinct smooth edges and clear segments. Hyphae like this are considered a positive indicator of soil health.

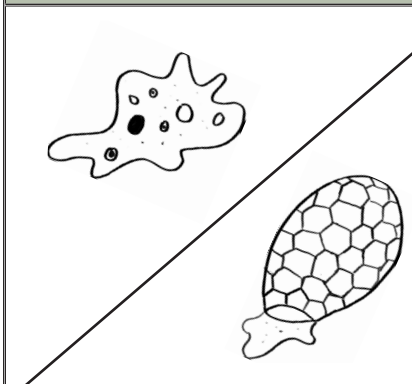
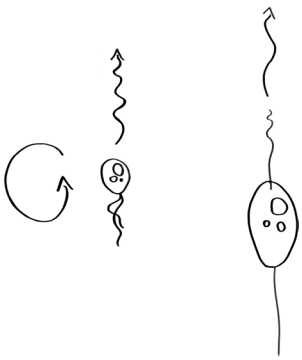
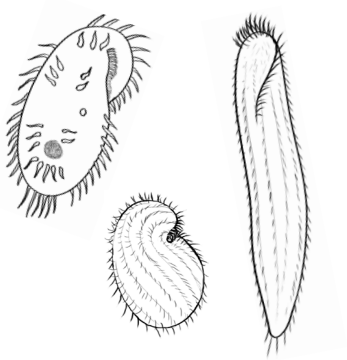
Group three Protozoa

Protozoa are incredibly diverse single-celled organisms. They are usually larger than bacteria and easier to see in the microscope. They tend to be very active, and sometimes will notice the movement of soil particles before you see the organism itself.

When protozoa graze on bacteria, they free up nutrients that have been immobilized in bacterial cells, and make them available to plants again. This makes them incredibly important members of the soil ecosystem. They have also been found to promote plant health and improve growth independently of nutrients.

Protozoa are known to be picky eaters. Each species hunts for specific types of bacteria. That means diversity in protozoa may be useful as an indicator of bacterial diversity in the soil. The more unique types of protozoa you can find in your soil, the better!

There are three main categories of protozoa, based on their physical characteristics and movement:

Amøber 10-50 µm	Flagellater 3-10 µm	Ciliater 10-80 µm
		
Amoebas are protozoa without a fixed shape. They move with a slow oozing motion, extending temporary “false feet” or pseudopods to slide across surfaces or engulf their food.	Flagellates have one or two long, whip-like appendages that they use to move. They often have a twitching, spinning, or rocking motion.	Ciliates come in many diverse shapes and sizes. They have many smaller hairs (called cilia), which allow them to move much faster and often more smoothly than flagellates.

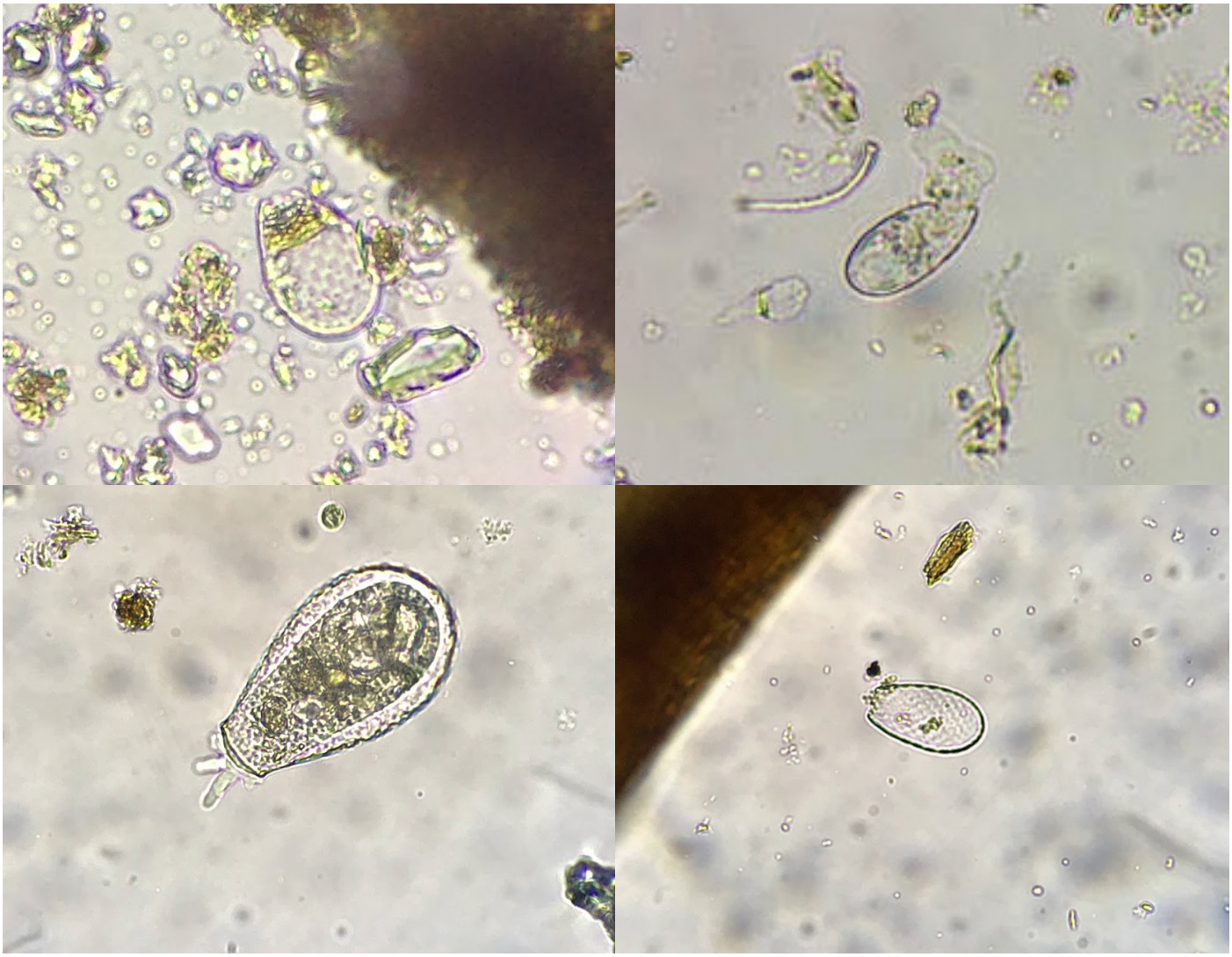
Group three Protozoa

Amoebas are a type of protozoa without a fixed shape. They move with a slow oozing motion, extending temporary "false feet" or pseudopods to slide across surfaces or engulf their food. Amoebas are particularly voracious predators of bacteria. Their flexibility makes it possible for them to reach bacteria in the tiniest soil pores that other predators can't access.

There are two main types of amoebas: **testate** (with a shell) or **naked** (without a shell).

Testate amoebas are common and easy to identify in soil. The shell has a distinct almond or balloon-like shape with an opening on one end. You can sometimes see a subtle pattern of tiny scales on the shell.

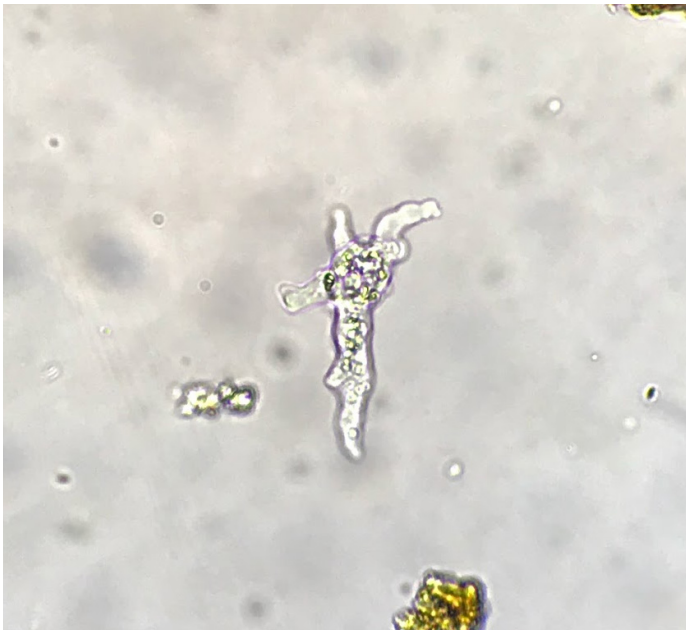
Here are some examples of testate amoebas in the microscope:

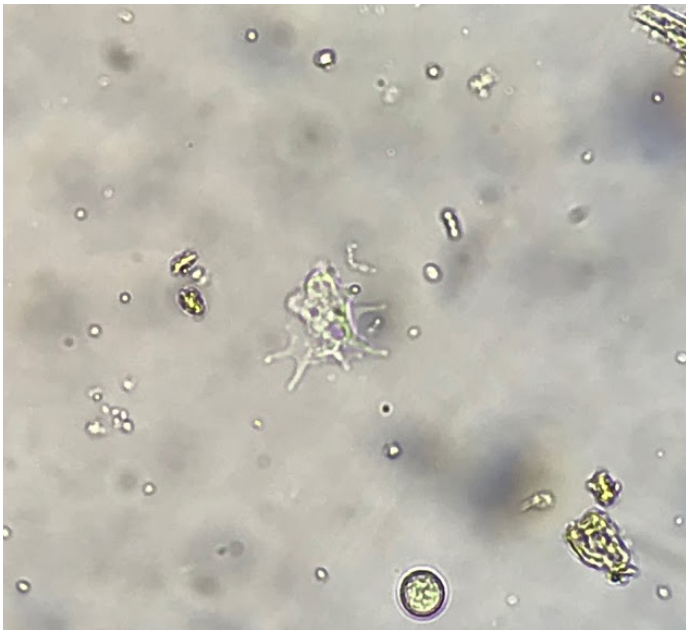


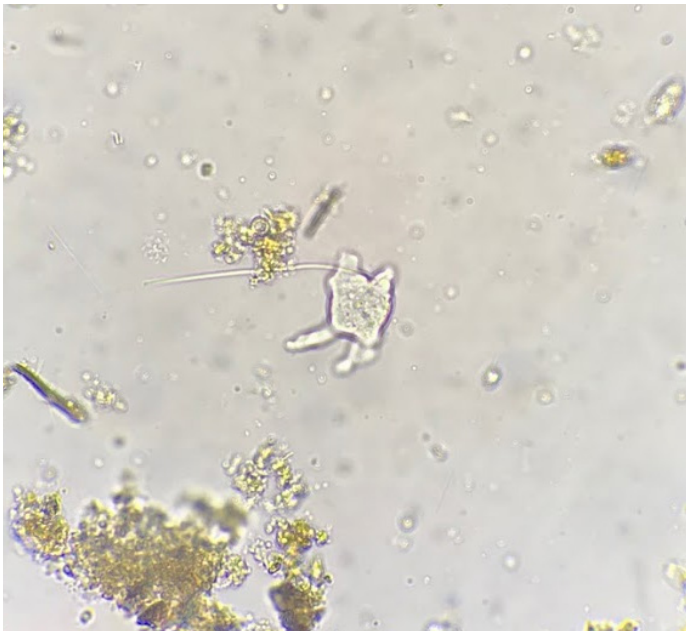
Group three Protozoa

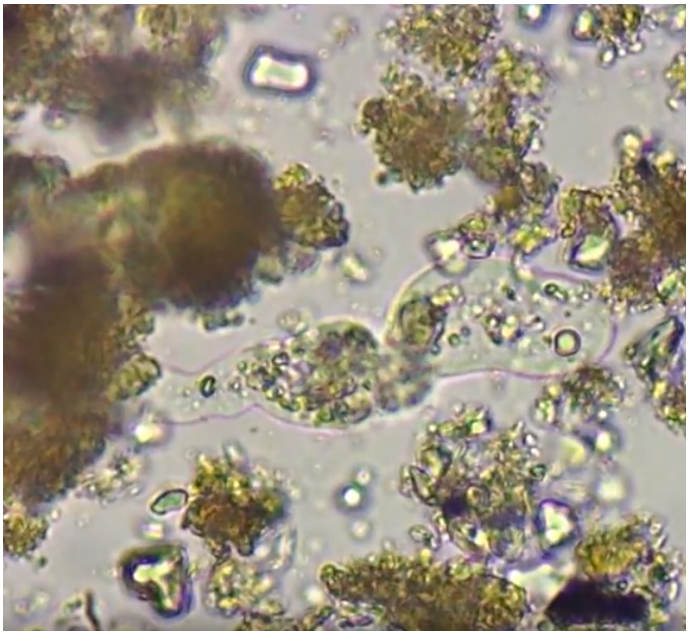
Naked amoebas have no shell. They can be more difficult to spot than other protozoa because their movement is often slow. They can be very tiny or quite large, very active or barely moving at all.

Here are some examples of naked amoebas in the microscope:





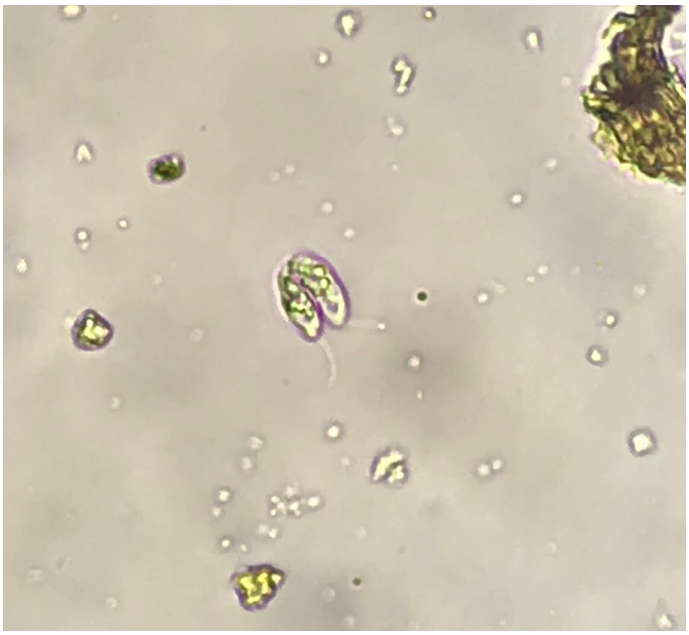


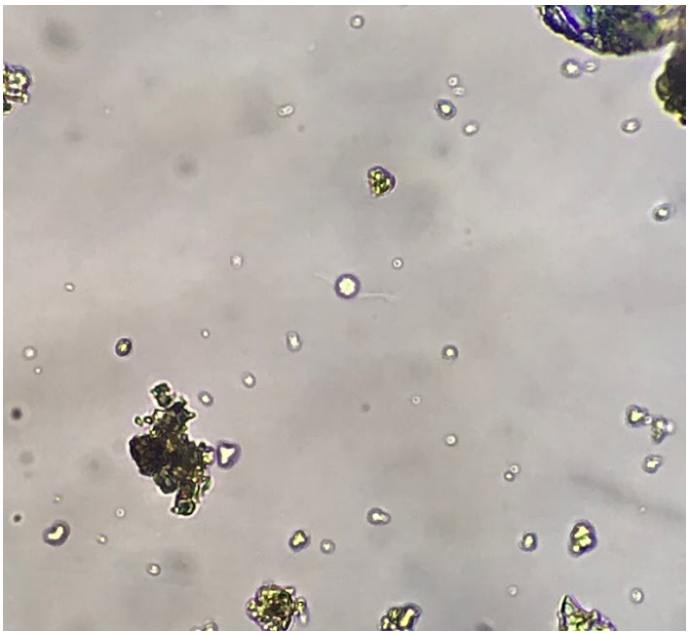


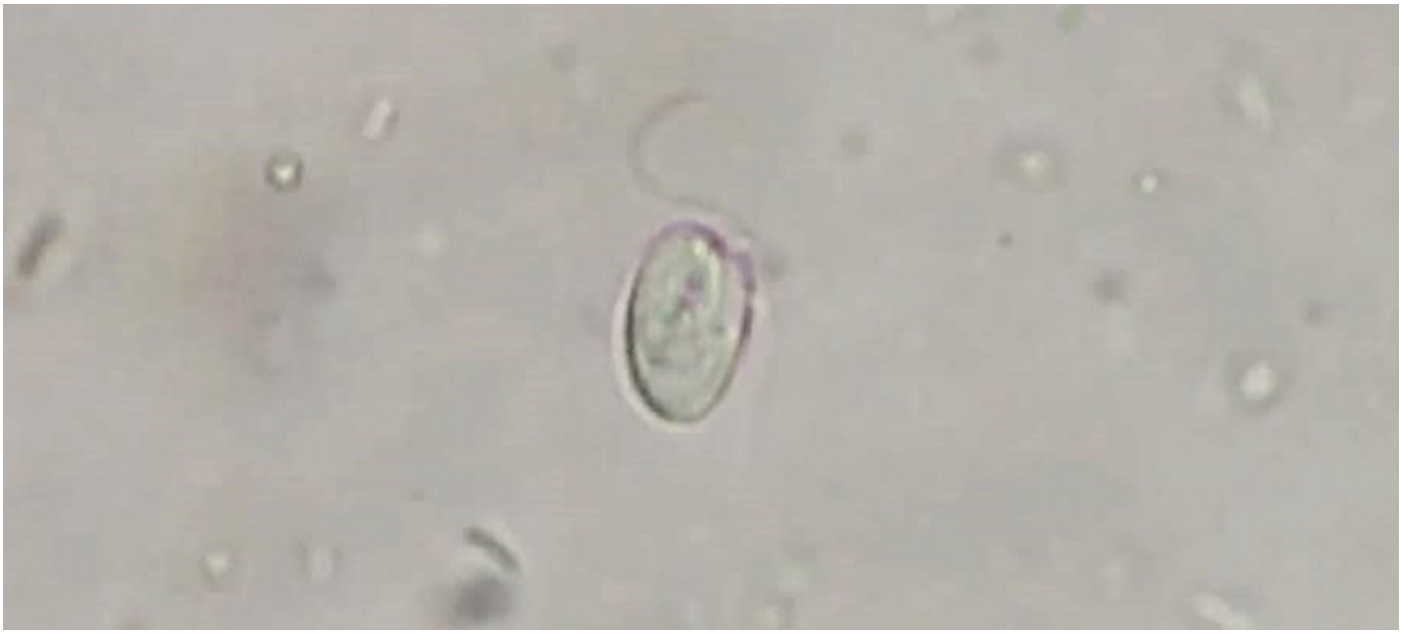
Group three Protozoa

Flagellates are the most abundant protozoa you will see in soil samples. They are hardier and more pioneering than larger organisms, and are better able to withstand tough conditions. They are usually smaller than ciliates. The main difference between flagellates and ciliates is that flagellates have one or two long, whip-like appendages that they use to move. They sometimes have a smooth movement, but they most often have a twitching, spinning, or rocking motion and they tend to be slower than ciliates It could be compared to paddling a boat with one or two oars, versus many people paddling together in perfect unison.

Here are some examples of flagellates in the microscope:



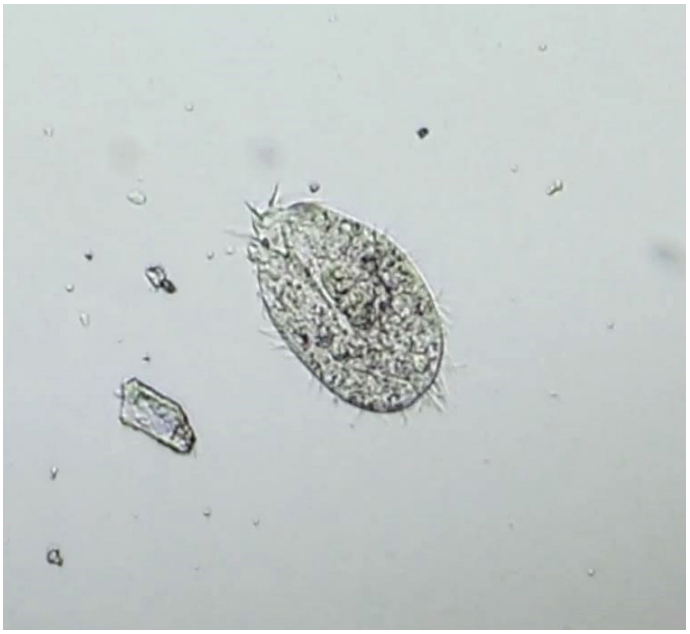


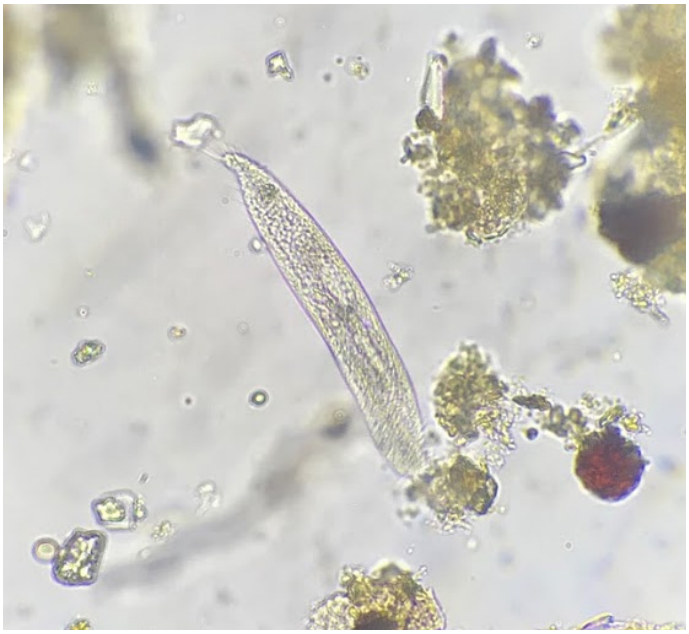


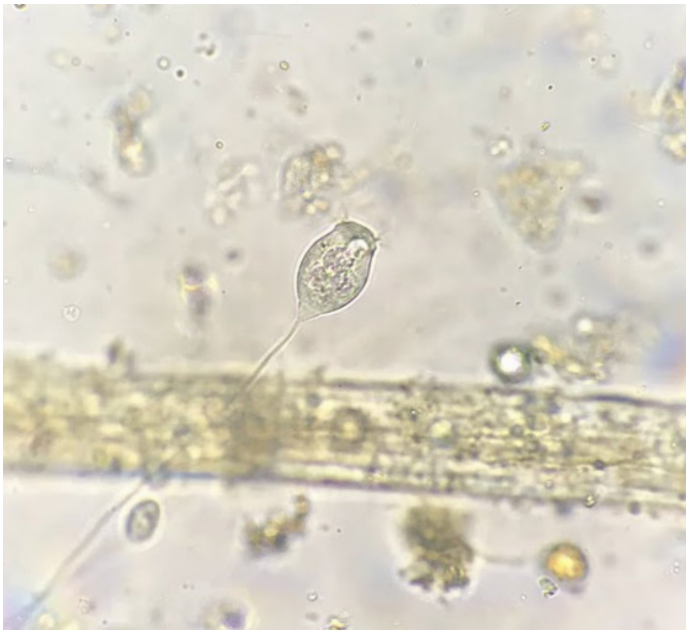
Group three Protozoa

Ciliates are less common in agricultural soil samples, but can be seen occasionally. They are also the most fun to watch, because they are usually very active and come in a huge variety of shapes and sizes. Remember that the main difference between flagellates and ciliates is the number of “hairs” they have. Ciliates have many smaller hairs (called cilia), but flagellates just have one or two long ones (called flagella).

Here are some examples of different ciliates that you might find in soil samples:



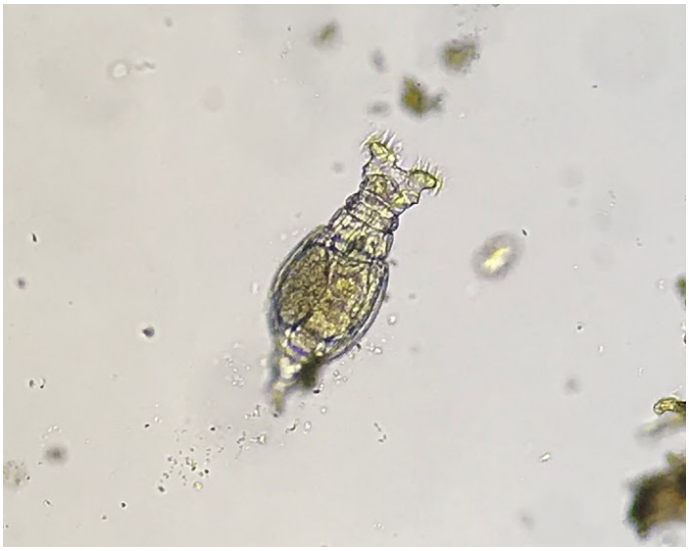


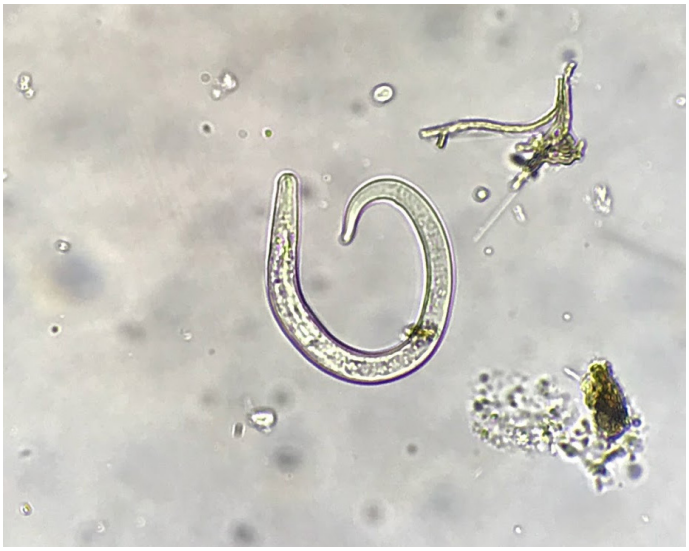


Group four Microscopic animals

Microscopic animals are highly diverse multicellular organisms. They have organs including a complete digestive tract, but they are so small that a microscope is needed to see them. In soil and compost samples, you may see rotifers (left photo) and nematodes (right photo). Nematodes are well known as plant parasites, but in fact they are extremely diverse and most soil nematodes are actually beneficial. Both nematodes and rotifers, like protozoa, consume food (mainly bacteria and fungi), and release nutrients that plants can use.

In forest samples, especially mosses, you may even find tardigrades, also known as water bears or moss piglets, like the one illustrated below.





Part Three

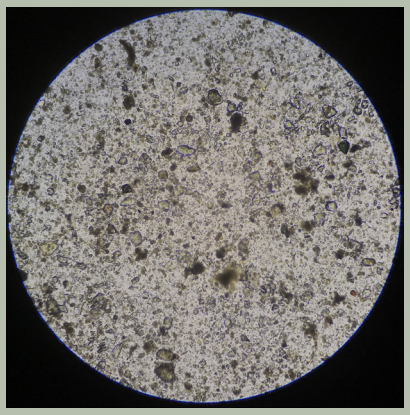
Evaluation and Next Steps

Evaluation Ecological succession

Soil progresses through stages of ecological succession just like other ecosystems. In early stages the habitat tends to be relatively "simple" and dominated by resilient pioneering organisms that reproduce and spread rapidly. Soils in this stage are mainly populated by bacteria, and perhaps some small flagellates and thin fungal hyphae. Early stage soils are often dominated by plants we refer to as "weeds". As succession progresses, the habitat becomes more complex, allowing more diverse organisms with more nuanced functions to thrive.

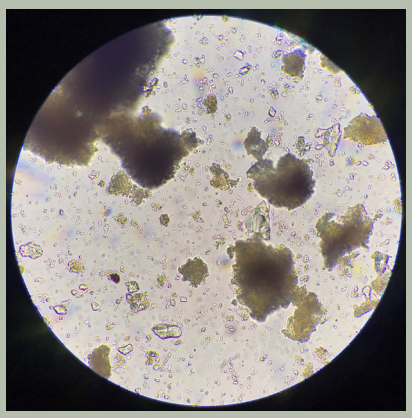
Agricultural activities have a direct influence on the ecological status of the soil. When soil is disturbed (eg. by mechanical or chemical interference), it is pushed back to an earlier stage of succession. If the soil is frequently reset to an early ecological stage and dominated by pioneering organisms, it becomes more vulnerable to erosion and there is a greater risk of pest, weed, and disease outbreaks for crops. It is very common for agricultural soil to be in the pioneer category, and this is something that should be addressed in order to achieve better soil health and sustainability in agriculture.

Ecological succession in the microscope



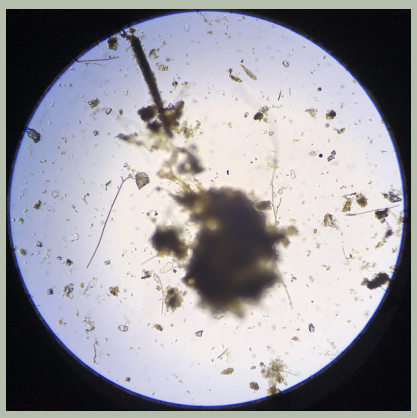
Pioneer

- Bacteria dominant
- Small flagellates
- Highly mineral
- Little organic matter
- Few or no fungi
- Low biodiversity
- Poor aggregation



→

- More diverse bacteria
- Bacteria and fungi in balance
- Greater biodiversity
- Microorganisms are more noticeable
- Better aggregation



Climax

- Fewer visible bacteria
- Fungi dominant
- Very high biodiversity
- More complex organisms
- Strong aggregation; the sample looks "clean"

Evaluation Ecological succession

You can use the following characteristics as a guide to evaluate the ecological status of your soil samples.

Pioneer	Middle succession	Climax
There may be dense bacteria, but few signs of diverse cell types or active movement.	Bacteria and fungi are in balance. It will likely take some experience before you can recognize this.	Bacteria are less dense, but with more types of cells and they may be more active.
Very few fungi. Mostly thin, pale or clear fragments.	Fungi are more noticeable. Fungal hyphae are larger, more robust, more numerous, and often darker in colour.	Fungi are clearly dominant compared to bacteria. It is easy to find large, robust networks of fungal hyphae of various colours and sizes.
Large amount of loose material. Very cluttered and messy, with few or no large aggregates. Much of the non-living material is pale or clear.	The appearance is cleaner and more “organized” looking, with stronger, darker aggregates and some open space between aggregates.	Physical structure is dominated by large, dark aggregates with plenty of clean, open space.
Few protozoa. Mostly small flagellates with low diversity (eg. 1-4 types).	Protozoa are easier to find. They may be larger and more active, and there should be better diversity (eg. 5-10 types).	Protozoa should be easy to find, active, and highly diverse.
Low overall biodiversity. Need to search for signs of life.	Overall biodiversity is moderate. It is easy to see signs of life.	Overall biodiversity is high and there are many obvious signs of life.

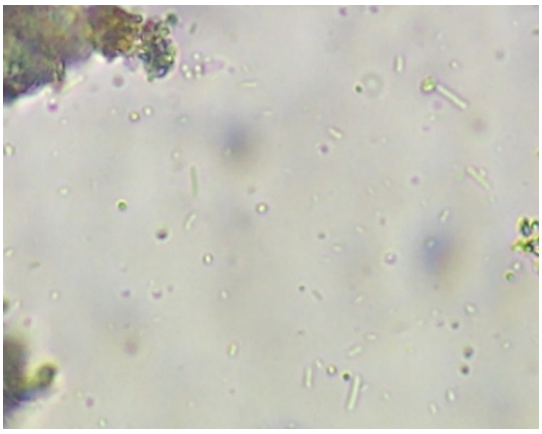
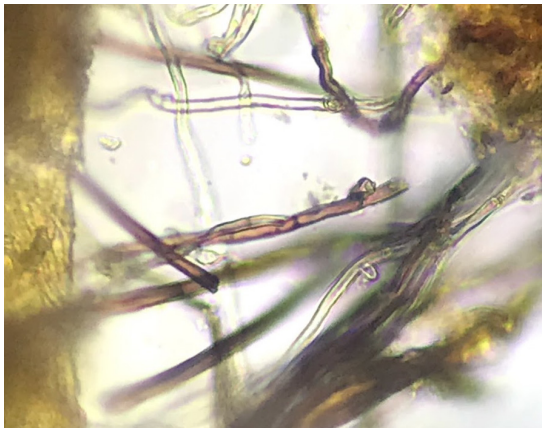
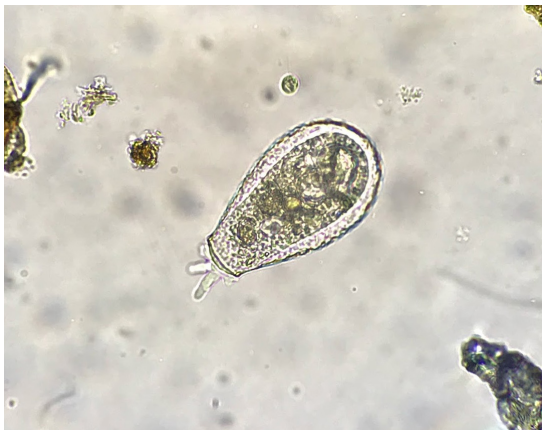
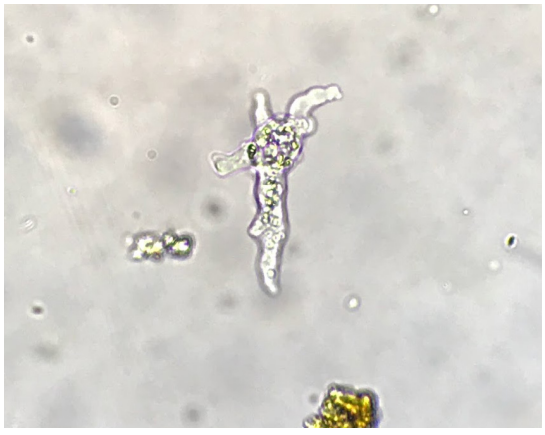
How can we support soil life?

Here is a summary of the types of conditions that favour microbial activity in soil, and some basic principles for how you can provide them.

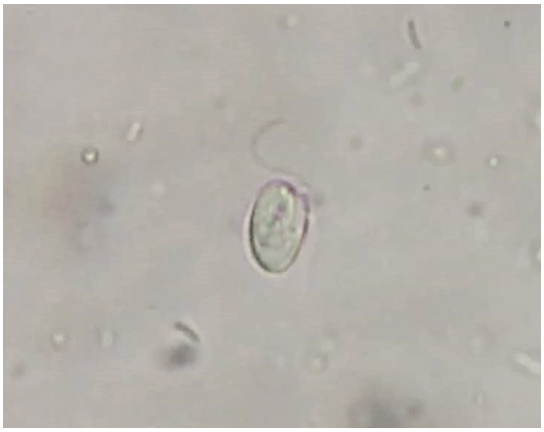
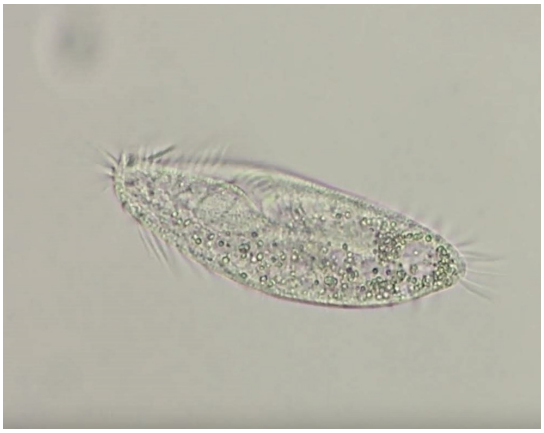
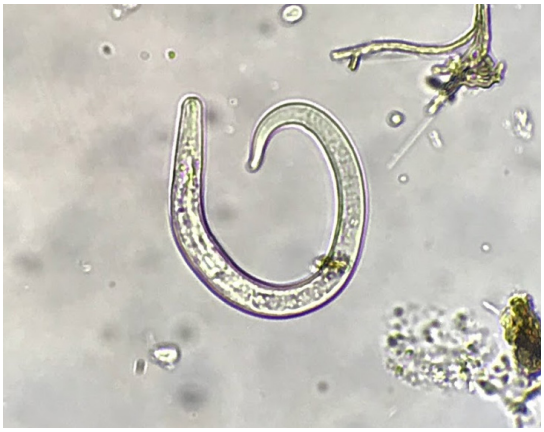
Microbes need:	You can provide it by:
Moisture	Keeping the soil covered as much as possible.
Oxygen	Allowing natural structure to develop and avoiding compaction.
Energy and nutrients	Maintain cover with living plants and mulch, with as much diversity as possible.
Shelter	Keeping the soil covered as much as possible.
Reduced disturbance	Minimizing tillage, driving, and chemical interference.
Earthworm activity*	All of the above.

*Earthworms are known as “ecosystem engineers”. Their activity improves soil quality and creates conditions that support beneficial microorganisms.

Evaluation Quick reference table

Organism	Example
Bacteria	
Fungi	
Testate amoeba	
Naked amoeba	

Evaluation Quick reference table

Organism	Example
Flagellate	
Ciliate	
Nematode	
Rotifer