

Quick, Easy Method to Show Living Soil Organisms to High School or Beginning-Level College Students

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ABSTRACT

The living component of soil is difficult for students to learn about and understand because students have difficulty relating to things they cannot see (beyond sight, beyond mind). Line drawings from textbooks help explain conceptual relationships but do little to stimulate an active interest in the living component of soil. Alternatively, movies, videotapes, or more recently web-based videos show motion of the organisms but the environment is artificial compared with direct observations on a soil the student has collected. Inexpensive, easy-to-prepare media dishes can be used for growing organisms from soil brought into the classroom by the student. The techniques described in this article are relatively simple and require little expertise to grow the organisms. An inexpensive microscope (ideally up to 500× magnification but acceptable to 100× magnification) and light source are needed but an autoclave and aseptic conditions are not required. The organisms are grown in mixed culture in petri dishes on low-energy medium. Although bacteria grow prolifically on the dishes, their small size makes individual organisms hard to see at 500× but they serve as a major food source for larger organisms that can be observed. Different organisms will develop based on dish moisture. Wetter conditions encourage protozoa and rotifer growth, whereas drier soil conditions favor fungi, nematodes, and springtails. With several dishes at different moistures, fungi, protozoa, mites, springtails, nematodes, rotifers, potworms, and perhaps a nematode-trapping fungus or tardigrada can be commonly observed. Web resources should be used to provide beginning students with high quality images and video to confirm observations from the culture dishes.

Because many organisms are invisible to the unaided eye, small life forms are difficult for students to comprehend. Limited time, expertise, and equipment normally prevent use of living organisms for student observation. Thus, students often learn about soil organisms by using still images or line drawings from textbooks. These images and line drawings do little to stimulate the student's excitement and interests, especially for students who

routinely view TV, play animated games, and are engrossed with interactive video.

Organisms less than 100 μm , commonly called *microorganisms*, are too small to be seen by the unaided eye. These include viruses, bacteria, actinomycetes, fungi, and the microfauna protozoa. Most people's unaided eyesight can see an image at 100 μm with good contrast and lighting, but detail is lacking. Mesofauna, represented by nematodes, acarina, collembola, and mites, are between 100 and 2000 μm (Sylvia et al., 2005). Many in this group are important in the food web of soil. Although less numerous than microorganisms, members of this group are large enough with magnification to see considerable detail. Of this group, the nematodes are expected to be most numerous.

Why Interest in Soil Organisms?

The organisms present in soil are ubiquitous in most environments. Thus, the morphology and physiology of soil organisms are similar to those in water, on food, in the air, or even in the mouth. Unlike animal or plant pathogens, many soil organisms are beneficial organisms upon which human life depends. Some of these organisms (earthworms, beetles, millipedes, and slugs) are large and readily seen with the unaided eye. But most can only be viewed in any detail with the aid of a microscope. Undoubtedly, the best way to view organisms is by using live specimens, but because of expertise, media requirements, and preparation time, this often is impractical (Heckman and Strick, 1996). Other options include use of line drawings, still images, movies, videotapes, CD-ROM, or video from the internet. There is a rapidly growing interest among biologists, conservationists, environmentalists, and sustainable agriculturalists in the fates of the diverse array of "good" organisms in soil because:

1. Soil life controls nutrient cycling in C, N, S, and other cycles. Without this cycling of nutrients, plants could no longer grow and animals that consume plants would cease to exist.
2. Soil life involves a food web where smaller organisms are food sources for larger organisms. Microbes are the starting point of most food chains with organisms such as protozoa, nematodes, and rotifers feeding on microbes, and then larger organisms feeding on these smaller organisms. The process continues until the top of the food chain is reached.
3. Soil life controls nutrient flow to plants through

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J. Nat. Resour. Life Sci. Educ. 35:202-208 (2006).
Article
<http://www.JNRLSE.org>
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symbiotic relationships, such as nitrogen fixation (bacterial–plant symbiosis), mycorrhizae (fungal–plant symbiosis), and lichens (fungal–algal symbiosis).

4. Soil life is centrally involved in degrading and reducing organic pollutants from the environment, such as pesticides, human organic wastes, petroleum spills, and many others.

5. Soil life can produce pollutants, both inorganic and organic. Inorganic products such as nitrate (nitrification) can enter groundwater and nitrous oxides (N_2O) (nitrification or denitrification) can diffuse into the stratosphere where they react with ozone (O_3), leading to enhanced UV exposure to humans and increased skin cancer potential. Organic products such as geosmin—produced by actinomycetes and cyanobacteria—can alter the taste of drinking water.

6. Soil life may be altered by genetically engineered organisms that potentially enter the soils and waters of the world. What are their long-term impacts to the environment?

How Students Visualize Soil Organisms

Line Drawings and Still Images

Idealistic line drawings, as seen in Fig. 1, can be used to observe detailed features of an organism. These are extremely valuable to a first-time viewer to give a sense of what is supposed to be observed.

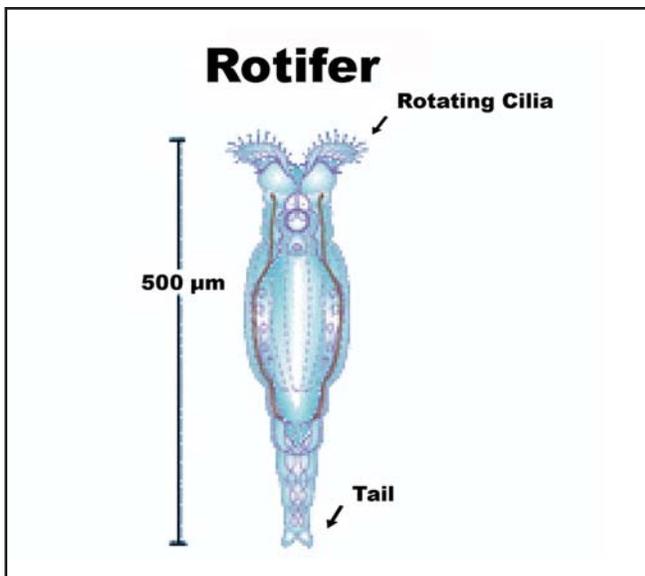


Fig. 1. Line drawing of a rotifer.

It sometimes takes imagination, however, to see the features in a real organism. A still image is better for the student to see actual features. Both the line drawings and still images, although useful in identification, do little to excite the interests and enthu-

siasm of the viewer. Motion is more apt to grab the student's attention.

Viewing Moving Organisms by Visual Media

Students may view moving organisms whose action has been captured photographically or electronically. Two common options exist. One is to use commercial videotapes or movies. The major limitation for this option is the cost of the media and a severe lack of quality materials available. Few teachers use this option. Another option, becoming more widely available, is video or video clips on the internet. Streaming technology allows video images to be collected and distributed electronically worldwide. Video files are usually several megabytes in size (even with compression) and consequently take a long time to download. Narrow bandwidth limited early length and size of the videos (Loynachan, 1998a), but this limitation is rapidly changing. The author has recently updated a website (www.agron.iastate.edu/~loynachan/mov) that uses macromedia flash player and allows video streaming for viewers who have wide bandwidth connections (current streaming technologies allow the video to begin playing while the remaining file is being downloaded, which allows the entire file to be played without apparent interruption). To accommodate a range in bandwidth, the site offers viewers three options. For those with slower connections, short clips can be viewed (approximately 500 Kb, 256 by 192 pixel format, without audio). For those wishing to view in a streaming mode, the clips are about 2 to 3 minutes long, 1 to 2 Mb, 360 by 240 format, and with audio. A third option allows the viewer to download larger versions of approximately 10 Mb, 720 by 480 format, and with audio. The latter size and quality are adequate for classroom projection. Many classrooms now have computers and projection equipment for viewing digital media.

Computer-based technologies have several advantages. Someone else (presumably an expert) prepares the medium, cultures and classifies the organisms, captures the digital images, explains what is being observed, and makes the material available in a readily useable format on the internet. Minimal expertise of the teacher and minimal laboratory equipment are required. If teachers wish to download materials from the internet and incorporate all or part into their own creations, they should be familiar with Copyright Law and the Fair Use Doctrine. Teachers are given wide latitude in face-to-face teaching activities in the classroom but less latitude where materials are copied and distributed. Stanford University (2005) maintains a website entitled Copyright and Fair Use (<http://fairuse.stanford.edu>) that provides guidelines on use of copyrighted materials including multimedia in teaching.

Viewing Live Organisms

So, if good resources are available on the internet, why would teachers want to provide live specimens for viewing to their students? Viewing live specimens is a hands-on activity involving participatory learning. The National Science Education Standards (Seymour and Hewitt, 1997) called for inquiry-based learning in the teaching of sciences. All too often, science education in high school biology classes and at the college undergraduate level is passive. Ledbetter (1993) found that inquiry-based classes helped students learn and retain information and had a positive effect on students' attitudes toward doing science. Hurst (2001) pointed out that many educators believe there is an increasing need to provide education to students on their own terms, coining the phrase "student-controlled learning." Learning occurs through a process in which the student plays an active role in constructing the set of conceptual structures that constitute his or her own knowledge base. So, the challenge becomes of how best to involve students in active inquiry-based or student-controlled learning where the students play a part in the process. Calling up and reviewing websites, even videos, involves the student more than the resources being provided by the teacher, but still is artificial to the student. Taraban et al. (2004) recently pointed out that live specimens are more effective than web sources for learning plant materials. The same argument presumably can be extended to the viewing of soil organisms. The procedures that follow describe an inexpensive, relatively simple technique that requires little expertise to grow the organisms and involves the students in collecting the soil, selecting conditions for growth, and identifying the organisms cultured.

Procedures

The teacher should challenge the student to collect an interesting soil sample from the field, note the location and past management of the soil, evaluate the morphological features of the sample, and then follow that sample in the laboratory to determine the living component.

To view the living component, most organisms must be separated from the soil matrix, and opportunities must be provided for the organisms to grow. The procedures described in this article do not require sophisticated equipment or knowledge and are a modification of procedures first reported by Pramer and Schmidt (1964). A petri dish becomes a microcosm, which provides conditions for a broad group of organisms to grow and develop. The initial organisms then serve as a food source for secondary and tertiary organisms that develop later. Depending on when the dishes are viewed, different groups of organisms are active. The excitement for students is that they collected the samples and set the conditions for growth. It is live before their eyes and they

are in control of the growing conditions that determine which group of organisms develops.

Oxygen Levels

The oxygen status of the dishes is critical for development of different groups of organisms. Two main features control the oxygen level. If the medium used in the dishes is too readily available (high energy), the rapid growth of organisms uses the available oxygen in the water films. Thus, a low-energy medium (such as cornmeal extract medium) is needed. A richer medium (such as nutrient agar often used in microbiology laboratories) is not satisfactory because anaerobic conditions develop in the water films. The second factor that controls oxygen status is thickness of water film on the surface of the medium. A thicker water film limits oxygen diffusion, whereas a thinner film allows rapid oxygen movement to the medium surface (the oxygen diffusion constant through air in $\text{cm}^2 \text{sec}^{-1}$ is 0.189 and through water is 0.0000256) (Papendick and Campbell, 1981). Fortunately, the water film thickness is a variable easily controlled by the student. As dish moisture varies, different organisms proliferate. Wetter dishes allow for protozoa and rotifers to development. Drier dishes allow for fungi, nematodes, mites, springtails, and potworms to develop.

Step-by-Step Details

1. Obtain a soil sample.
 - a. A soil in good condition for plant growth will provide a myriad of organisms. Soils that are aerobic with good organic matter content, or to which organics have been recently added, will yield high populations of diverse organisms. Compacted soils or soils devoid of plants will have fewer living organisms. Avoid soils that have been waterlogged for an extended time because major groups of organisms will be absent. The sample may come from a lawn, garden, pasture, forest, stream bank, or cultivated field. Encourage different students in class to sample diverse areas.
 - b. During sampling, surface soils are usually better for organismal numbers and diversity than subsoils. Collections may be made in small, nonsterile, plastic bags because there is little concern about aseptic conditions (only looking for a source of organisms). A quantity of soil equaling about 100 grams should be adequate for an individual sample. A small trowel or spade can be used to cut a vertical slice into the soil, the bag is positioned in the void next to the soil face, and soil is scraped into the bag. Be sure students carefully note the location of the sample site (the teacher can develop a questionnaire for students to describe the site in the field if desired).
 - c. A fresh soil sample is best. If the sample must be stored, minimize storage time. The best way to store the sample is to maintain ambient soil temperature and moisture. For short-term storage (less than a week), store in the plastic bag in a cool, dark

location such as a basement. Some groups of organisms may be lost with refrigeration.

2. Obtain plastic petri dishes containing cornmeal extract agar.

a. A minimum of two dishes per student will allow students to maintain different moisture levels. (For larger classes, the teacher may wish to divide the students into groups who work together on a common soil.)

b. The cornmeal extract agar can be purchased commercially from BBL DIFCO (www.vgdllc.com/BBL_dehydrated_media.htm) (Becton, Dickinson and Co., Franklin Lakes, NJ). It comes in 500-gram containers and costs about \$135US. Seventeen grams are added to 1 liter of tap water and brought to a boil (to dissolve agar). The hot medium is then dispensed 15 milliliters into 15-centimeter petri dishes (sterile conditions are not needed other than to control gross contamination because soil will be inoculated onto the surface of the medium).

c. Alternatively if on a tight budget, add 50 grams of ground yellow corn (or corn meal from the grocery store) to 1 liter of tap water and heat at 80°C for 30 minutes (Pramer and Schmidt, 1964). Filter through paper or cheesecloth and reconstitute with tap water to 1 liter volume. Solidify with 15 grams agar. The agar can be purchased from several microbial vendors (or ask your microbiology colleagues for a small quantity).

3. To each dish, sprinkle a small quantity of soil to one side and add pieces of decomposed wood, dried manure, bits of moss or surface duff, or partially decomposed grass or leaves. Sprinkle soil and bits of the debris so that no more than one-sixth of the dish is covered. A small piece of wood, 1 to 2 cm², that is moist and was in contact with the soil is good. (It is through the clear area that you will observe organisms and the soil will disperse as you wet it, so minimize the area covered with soil and debris.)

4. Add tap water to each dish. In one dish, add about 1 milliliter, and to the second dish, add about 3 milliliters. In the first dish, the surface should be moist but have *no* free water when tilted. In the second dish, water should run when the dish is tilted. More or less water may be needed, based on drying conditions of the medium after the dishes were poured. Place the dishes up-right in a plastic bag and close with a rubber band.

5. *Check dishes weekly* and add tap water as needed to maintain starting moisture conditions.

6. Observe dishes weekly by placing the petri dish on the microscope stage and using the low-power objective (usually 4 or 10×). (This objective strength times the ocular strength, usually 10×, gives total magnification.) You should see a host of organisms such as arthropods, fungi, and protozoa that develop and then die back. If the light source is coming from the base of the microscope, light will not pass

through larger organisms (such as mites, springtails, or tardigrada) and they will appear opaque. To see surface detail, an additional light source is needed to shine from the top onto the specimens. Smaller organisms such as rotifers, protozoa, and nematodes are sufficiently transparent that bottom lighting is adequate.

Note: It is critically important not to ram the objective into the medium containing soil particles during observations (quartz particles in the soil have similar hardness as does the glass objective and damage can occur if the objective comes into contact with the soil). To avoid damage, always be increasing the distance between the objective and agar surface when viewing through the microscope. After placing the dish on the stage of the microscope and while looking from the side, reduce the distance between the objective and agar surface to about 5 millimeters. Then look through the objective while *increasing* the distance between objective and agar surface until the surface is in focus. Look for movement when viewing through the microscope. If you go through the focal plane, repeat the process. If an oil objective is available (ca. 100×), one can place a cover slip on the agar surface, add a drop of immersion oil, and carefully focus the objective. Because the light is coming from the bottom and passing through the agar, the iris diaphragm must be opened wide. The small organisms darting around are bacteria. (Because it takes expertise to focus the oil objective and sloppy technique will ram the objective into the agar, use of the 100× objective should be limited to advanced students or the teacher).

7. Sketch the more interesting organisms observed. What are their notable features? What is their color? What is their relative size (be sure to indicate magnification at which observed)? What do the organisms appear to be living on? How do they move? How does the diversity of organisms change with time? How does the moisture level affect groups of organisms observed?

8. An interesting alternative to sketching organisms, which may seem boring to some students, is to have capabilities to capture images by a digital camera.

Observations

It's Alive and It's Moving...What Is It?

Movement is one of the first things to catch the student's eye. The organism may be walking, swimming, crawling, or slowly changing shapes. If walking, the organism is probably meso- or macrofauna (considered by most to be >200 μm). If swimming, it may be meso- or microfauna. Nematodes often swim through water film and rotifers move about on water films. At 100× total magnification, both are easy to see. If swimming and at the approximate resolution of the eye at 100× (i.e., looks like a small

Sample Study Questions

1. What types of living organisms do you expect to find in soil? How do they differ in size and appearance? What activities and transformations do they accomplish?
2. What is a food web in soil? What organisms are at the bottom of the food web? What organisms are near the top of the food web?
3. What organisms in soil are expected to be most numerous? What organisms are expected to be least numerous? Why do the most numerous organisms tend to be small and the less numerous organisms tend to be large?
4. What organisms in soil are expected to have the most biomass (living tissue)? What organisms are expected to have the least biomass?
5. How would you expect differences in water and oxygen contents, organic matter, and pH to affect the organisms present in soil?
6. What is an ecosystem? Explain how the petri dish used in this procedure represents an ecosystem? Are conditions in the petri dish characteristic of conditions in the field? Explain.
7. Do organisms in the dishes change ecosystem conditions as they grow? Explain.
8. How would growth differ if you incubated the dishes in a refrigerator rather than at room temperature?
9. Would you expect soils obtained from different regions of the world to have similar or different organisms present? Explain.

moving speck when looking through the microscope), the organisms are likely protozoa. If crawling, the organisms may be tardigrada (water bears), which are relatively uncommon in soil. Few individuals have seen these organisms but their pseudo feet are fascinating to watch under a microscope.

So, how does the first-time viewer determine what is being viewed? The internet provides a good format for investigative learning where the student enters a tentative name in a search engine and discovers more about that organism. Even if a wrong name is entered, the student learns characteristics of the organism while eliminating it as the unknown. Some sites have still images, line drawings, and video clips to help make visual comparisons.

Internet Resources

Most students use and enjoy the internet. Materials can be combined to form new resources or examined from multiple perspectives as learners think critically and evaluate information. Thompson et al. (2003) stated that the real promise of technology in education lies in its potential to facilitate fundamental, qualitative changes in the nature of teaching and learning. Internet resources are rapidly becoming a first choice as students seek supplemental information. Although this listing is not intended to be complete, it indicates the types of sites available that have useful information about soil organisms, both still and video images. These include:

- The MicrobeLibrary (American Society for Microbiology, 2004, www.microbelibrary.org) visual collection contains stills and some video (especially of bacteria, short, small format, mainly between 500 Kb

and 4 Mb); also contains some extremely nice still images and video of nematophagous fungi.

- A good site is the Microbiology Video Library (University of Leicester, 1999, www-micro.msb.le.ac.uk/video/video.html). The site has smaller formatted video on the web at 240 by 180 pixels and larger CD versions 320 by 240 on CD, which sells for \$79US.

- Tree of Life web project (University of Arizona, 1997, <http://tolweb.org/tree>) contains good still images and organizational relationships of organisms, including soil organisms.

- Soil Health is located at the University of Western Australia (2005, www.soilhealth.segs.uwa.edu.au) and has still images.

- Soil Biology Information Resources (Natural Resources Conservation Service, 2003, http://soils.usda.gov/sqi/concepts/soil_biology/sbinfo.html) is a very nice portal of informational resources, includes links to biological images.

- Microbe Zoo (University of Otago, 1999, <http://microbes.otago.ac.nz/scifest/micrzo/home.html>) is well done with incorporation of text and animation, with some good still images. It contains limited video. (The site has a very nice animation on the opening page showing size relationships between macro- and microorganisms.)

- The Microbe Zoo (Michigan State University, 1999, <http://commtechlab.msu.edu/sites/dlc-me/zoo>) is an education resource about ecology and microbiology. Use of video is limited but the site has nice still images and good habitat descriptions, including DirtLand.

- Underground Adventure was produced by The Field Museum (2003, www.fnmh.org), Chicago, IL,

and is an especially interactive site dealing mostly with larger fauna. This would be a fun site for the junior high-age student.

• The author's video homepage is Soil Biology Movies (Loynachan, 1998b, www.agron.iastate.edu/~loynachan/mov), which contains good video of a number of organismal groups. It is available at narrow bandwidth (for those with phone modems) and wide bandwidths with streaming. Additionally, larger (and higher quality) downloadable files are now available, which are best for the teacher who wishes to project video in the classroom.

Specific Fungal–Nematode Relationships

The student may see a fungus and see a nematode but not understand their relationship. As originally described by Pramer and Schmidt (1964), the basic procedures described in this paper were used to observe nematode-trapping fungi. In the author's experience, approximately one-third of the dishes from diverse soil samples will develop these fascinating relationships. In positive dishes, students can observe nematode-trapping fungi as early as 4 weeks (6–8 weeks more common). The fungi have adapted creative structures to capture nematodes and use the nematodes as a food (and probably N) source. The capture structures are hyphal "traps" produced in the presence of their prey (Fig. 2), and are rather widespread in nature. The four basic trapping mechanisms are: (1) sticky branches and networks, (2) sticky knobs and clubs, (3) nonconstricting rings, and (4) constricting rings.

1. Once nematodes appear, look carefully for the trapping fungi. Look for dead nematode carcasses on the agar surface and carefully examine for evidence of fungal hyphae and the organelles of capture produced by the nematode-trapping fungi. Verify by observing multiple-capture structures along the fungal hypha—a single, nonrepeating structure may be an artifact.

2. A trapped nematode may struggle for 24 to 48 hours. After this time, the fungal hyphae will penetrate the nematode and the nematode will soon die. If in doubt whether a nematode is trapped, circle the location of the nematode on the bottom of the dish with a Sharpie or grease pencil and come back later to view again.

Conclusions

Requirement for sophisticated equipment and specialized expertise likely limit use of living soil specimens in high school biology and college-level soil science courses. Use of cornmeal extract dishes is an inexpensive means of providing living materials obtained from a soil provided by the student. Culture conditions will alter groups of dominant organisms present. Ecology and food chains can be easily demonstrated as groups of organisms change with time. These dishes allow excellent opportunities for experi-

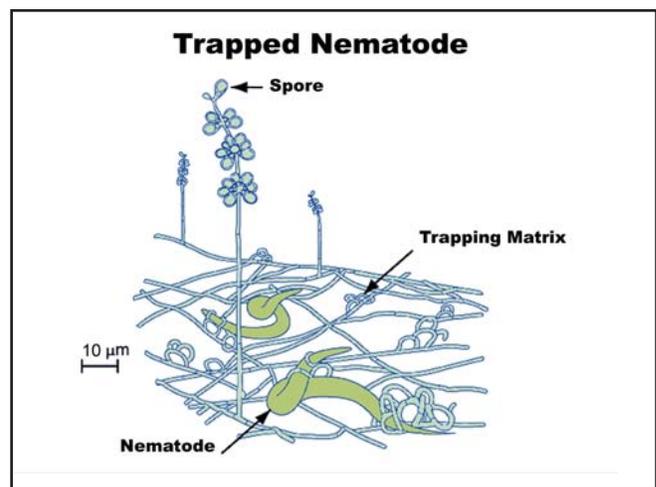


Fig. 2. Line drawing of the matrix of a nematode-trapping fungus.

mental learning and provide students with a better understanding of the fascinating life of the soil.

Acknowledgments

I would like to thank the many students who have taken Agronomy 485, Soil Microbial Ecology, at Iowa State University during the last 28 years and have shared ideas on improving this exercise, which has always been one of the students' favorites. Much of the video in Soil Biology Movies (www.agron.iastate.edu/~loynachan/mov) was collected by using these dishes with materials being discovered by students. The enthusiasm of these students has been contagious and has helped to maintain the enthusiasm of the instructor.

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