A Community Underfoot: Density and Diversity of Invertebrates in Soil or Ground Cover

SYNOPSIS FOR CORE EXPERIMENT

Students will construct Berlese-Tullgren funnels from plastic bottles to extract the invertebrate fauna from soil or ground cover samples. They will determine the effects of specific environmental variables on the diversity of fauna taken at regular intervals away from a major roadway.

APPROPRIATE BIOLOGY LEVEL

Introductory or advanced

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Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 131. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

At the end of this lab, students will be able to:

- Construct a Berlese-Tullgren funnel to sample invertebrate fauna in soil or ground cover samples.
- Estimate the population density and diversity for different environmental conditions.
- Describe the effects of specific environmental variables on a target population or community structure.

MATERIALS NEEDED

For the teacher-led demonstration, you will need the following for a class of 24:

- 1 2-L plastic soda bottle
- 1 pair scissors
- 1 10-cm^2 square of 1/4 or 1/8-inch mesh hardware cloth or plastic needlepoint backing
- 1 pair tin snips
- 1 pair pliers
- 1 10 x 10 x 5-cm soil-surface sample
- 1 trowel or pancake turner
- 1 pair of gloves
- 1 3.8-L (1-gallon) sealable, plastic bag
- 50.0 mL ethanol
- 1 500-mL glass jar with 7.5-cm diameter opening and tight lid
- 1 piece of newspaper
- 1 9-watt colorless light bulb and a socket in a string of holiday lights or a 25-watt shielded light
- 1 10-cm^2 square of aluminum foil or dark paper

You will need the following for each group of two students in a class of 24:

- 3 2-L plastic soda bottles
- 1 pair scissors
- 3 10 x 10 x 5-cm soil-surface samples

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Introduce the Berlese-Tullgren funnel and key. Practice using the dichotomous key to identify the invertebrate organisms from the demonstration extraction.

Day 2 (45 minutes)

- Analyze data from demonstration sample. Use class totals to estimate population density and diversity. Discuss environmental variables that might influence the density and diversity of these populations.

Day 3 (30 minutes)

- Develop the question, hypothesis, and rationale. Assemble materials for the funnels.

Day 4 (30 minutes)

- Collect sample and place in funnel.

Days 5 to 7

- Allow samples to dry.

Days 8 & 9 (45 minutes)

- Collect and analyze data from the students' extractions.

PREPARATION TIME REQUIRED

30 minutes

- Assemble the demonstration Berlese-Tullgren funnel.

60 minutes

- Collect soil or ground cover sample.

60 minutes

- Cut the screen squares with tin snips and remove the bottoms from bottles.

TEACHER'S NOTES

(continued from p. 117)

- 1 pair of gloves
- 3 1-L (1-quart) sealable, plastic bags
- 3 10-cm² square of 1/4 or 1/8-inch mesh hardware cloth or plastic needlepoint backing
- 3 500-mL glass jars with 7.5-cm diameter openings with tight lids
- 3 9-watt colorless light bulbs and 3 1-socket sections of a string of holiday lights or 3 25-watt shielded lights
- 1 stereoscope
- 1 eyedropper
- 1 forceps
- 1 9 x 14-cm (8.5 x 11-inch) sheet each of black and white construction paper
- 3 10-cm² squares of aluminum foil or dark paper
- 5 sheets of newspaper
- 150.0 mL 70% isopropyl alcohol
- 1 garden trowel

SAFETY PROCEDURES

Students should wear gloves whenever handling soil samples and wash their hands at the beginning and end of the lab.

Alcohol is flammable and should not be used near an open flame.

Students should not drink the alcohol or inhale its fumes.

The classroom should have adequate ventilation.

Vapor levels can be reduced by using a collecting jar that fits snugly with the funnel and by keeping collecting jars and petri dishes covered.

If you use a 25-watt light bulb as a heat source over the Berlese-Tullgren funnel, keep the bulb at least 15 cm away from the sample to prevent the ignition of any dry leaves in the sample.

When students collect their soil-surface samples, remind them to disturb as little of the area as possible.

DIRECTIONS FOR SETTING UP THE LAB

Berlese-Tullgren Funnel Assembly

- Cut the bottom from the plastic bottle at the edge of the colored bottom or at the ridge where the sides of the bottle straighten. When the bottle is inverted with the mouth facing downward, it becomes a funnel. See Figure 1. Cut 1-cm slits on opposite sides of the bottle's cut as shown in Figure 1.

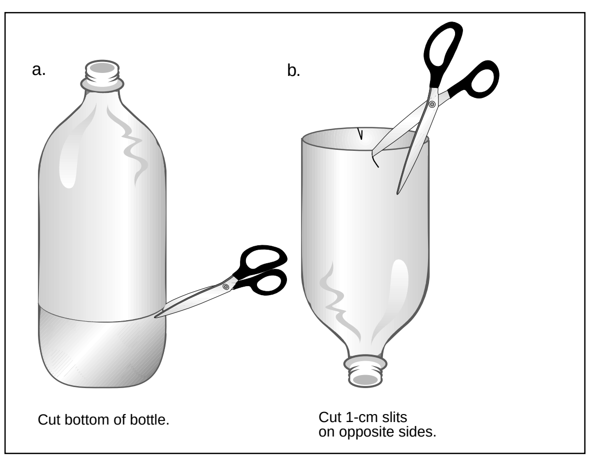


Figure 1. Extraction funnel assembly.

- Use tin snips to cut a 10-cm^2 square from 1/4 or 1/8-inch hardware cloth. See Figure 2a. (Plastic needlepoint backing may be substituted for the hardware cloth. Cut with scissors.)
- Use pliers to bend the edges of the wire into a circle just slightly larger than the funnel's inside diameter. See Figure 2b.

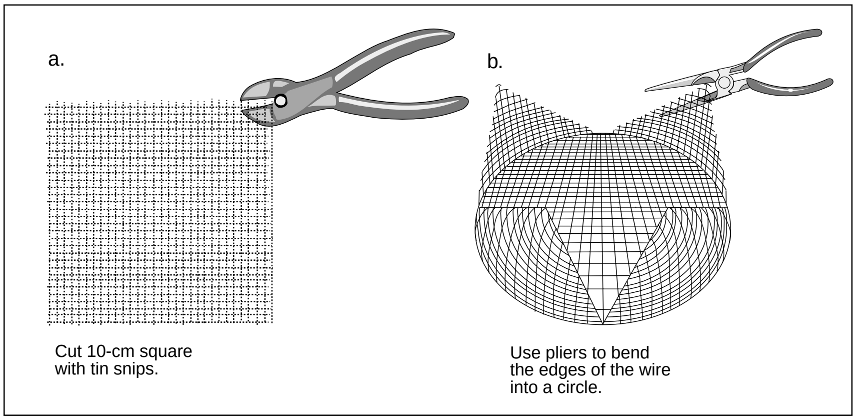


Figure 2. Cutting the hardware cloth.

- Make a shelf inside the inverted bottle by pushing the hardware cloth into the funnel until it rests on the sides of the bottle where the bottle begins to narrow as shown in Figure 3.

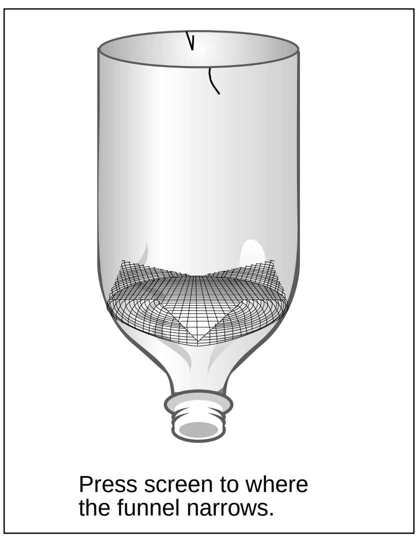


Figure 3. Placement of the hardware cloth.

Sample Collection

Collect a soil-surface sample of moist leaf mold, compost, or rotting wood. Choose a site that is 5 to 6 cm thick. Use a trowel or pancake turner to scrape everything in a 0.5 meter square area above the soil surface into a plastic bag.

Sample Extraction

- Pour about 50.0 mL of alcohol into a 500-mL jar. The alcohol should be about 1.5 cm deep.
- Working over a piece of newspaper, place the soil-surface sample into the funnel. As the fine sample particles fall through the screen onto the newspaper, return

TEACHER'S NOTES

them to the funnel. After all the loose material has stopped falling, set the funnel on top of the alcohol jar.

- Center the light over the funnel and anchor the cord in the slits that were cut. Set the bottom of the funnel over the light. This will provide a shield to keep insects attracted by the light from contaminating your sample. See Figure 4.

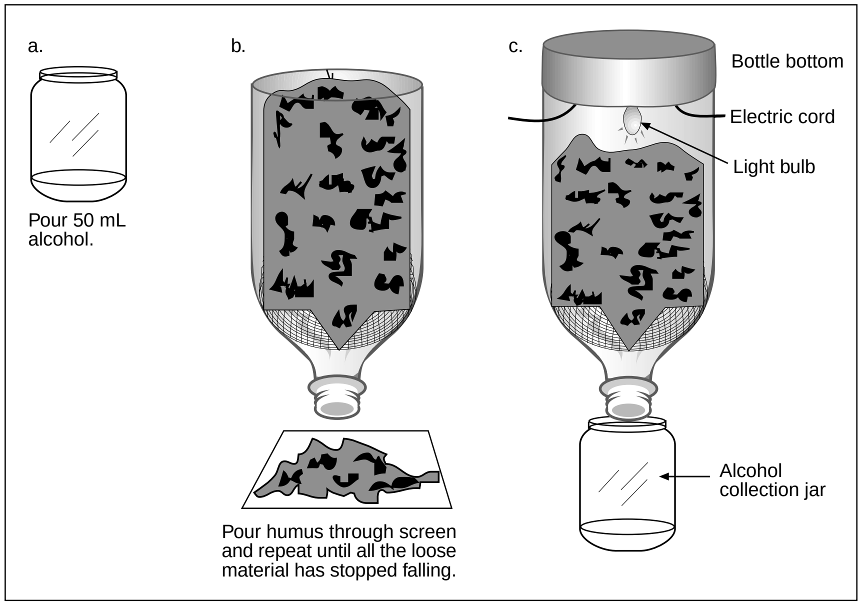


Figure 4. Extraction funnel setup.

- Wrap foil or dark paper around the jar to make a dark refuge for the invertebrates that are negatively phototropic. See Figure 5a.
- As the sample dries on the top, the animals will move downward through the screen into the alcohol. Check the alcohol level daily and add more if it evaporates. After 4 or 5 days, carefully remove the alcohol collection jar and screw on its lid. See Figure 5.

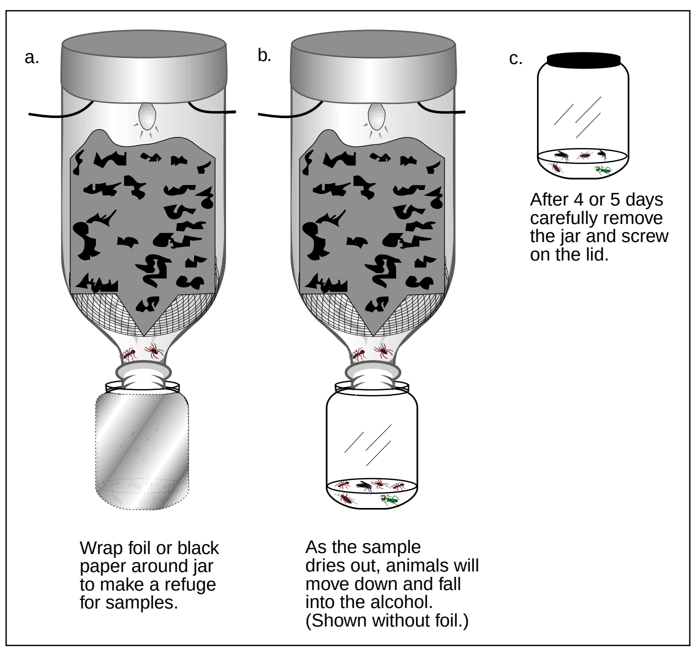


Figure 5. An extraction funnel with a sample.

TEACHER'S NOTES

TEACHER'S NOTES

TEACHER BACKGROUND

Content Information

Beneath our feet lives a world of organisms most people have never seen. Every major animal phylum, except the Cnidaria and Echinodermata, has members in the below-ground ecosystem. Numerous Protista and Monera are also abundant there. From a single 30-cm^2 sample of the 2.5 to 5.0 cm of humus that covers a typical temperate deciduous forest or grassland, representatives of 15 to 20 major taxonomic groups may be collected. The population density of some species may exceed one million individuals per acre.

Among these soil organisms are representatives of every trophic level including producers, primary and secondary consumers, and decomposers. Population growth is limited by environmental conditions and the amount of resources available. Invertebrate animals may be herbivores, predators, parasites, or saprophytes. They may consume roots, seeds, fungi, dead wood, decaying leaves, or each other. The soil community is a microcosm of the complex ecological interactions among living organisms. The density of the population is not fixed, but varies with climate, season, and environmental factors, such as light, water, space, and nutrients.

Even though the soil surface is densely populated, many of its inhabitants can be found only by extraction and observed only under magnification. At the end of the 19th century, the Italian entomologist, Antonio Berlese, developed the first funnel-type extraction system for separating small arthropods from the soil. Hot water circulated between double brass walls of his funnel. A sample of soil or leaf litter was placed in the cavity of the funnel that was then suspended over a jar of alcohol. Heat, conducted from the water through the metal walls of the funnel, caused the moisture in the sample to evaporate. Arthropods and other small invertebrates moved downward through the funnel to escape desiccation and eventually fell into the alcohol where they were killed quickly and preserved.

Fillippo Silvestri, a student of Antonio Berlese, used this extraction system in 1907 while he was studying the soil fauna of the woodlands near Syracuse, New York. Some of his samples contained an undescribed arthropod species that proved to be the first identified representative of an entirely new taxonomic order, the Protura. These tiny insects are found only in moist leaf litter and are considered to be the most primitive group of Hexapods. So far, 20 species have been discovered on the North American continent.

In 1918, Tullgren modified Berlese's original extraction method by replacing the water jacket with an electric light bulb. All commonly used extraction funnels depend on the differential light and moisture created by placing a water-based collection vat beneath a funnel and an electric light and heat source over the funnel. You will find literature references to both Berlese funnels and Tullgren funnels. We honor both innovators by calling the extraction apparatus a Berlese-Tullgren funnel.

Collembola (springtails) are probably one of the most abundant animals collected in Berlese samples. Approximately 1,500 species of the order Collembola have been identified (Lovett, 1993) and their population density often exceeds 1 million per acre. In some grasslands, they have been found in densities as high as 300 million per acre (Wallwork, 1970). This primitive, wingless insect has a unique "springtail" near the end of its abdomen that it can snap against the substrate, propelling itself through the air. Some Collembola can jump up to 20 centimeters, a distance 50 to 100 times their own body length. Unlike most other arthropods, springtails appear to have evolved in cool climates and their relative abundance in the soil tends to increase as the mean annual temperature decreases. Today, these animals are even found in the Arctic and Antarctic (Lovett, 1993).

Many of the animals are so tiny that you easily could overlook them even if they were separated from their surrounding litter or soil. Ninety-five percent of them are less than 5 millimeters long and nearly 37% of them are even smaller than 1 millimeter (Birch & Clark, 1953).

Extractions of humus collected from a temperate deciduous forest are likely to contain individuals from some or all of the following taxonomic groups:

- Nematoda (roundworms)
- Annelida (segmented worms)
- Mollusca (snails)
- Arthropoda (spiders, ticks, mites, daddy longlegs, pill bugs, sow bugs, woodlice, centipedes, silverfish, fleas)
- See the *Kwik-Key to Soil-Dwelling Invertebrates* (Figure 7a & b) for detailed information.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts:

Correct Concept	Misconception
• The soil surface is densely populated with animals. Soil animals are hard to see.	• The soil surface contains only nonliving material. • Very few animals live on the soil surface.
• Many different types of animals live on the soil surface.	• All soil-surface animals are the same. Only worms live in the soil.
• The organisms living in soil form a microcosm of the larger ecosystem and are also part of that ecosystem.	• The soil-surface environment is not an ecosystem or part of the larger ecosystem. • All soil-surface organisms fill the same trophic level and make no contribution to the larger ecosystem.

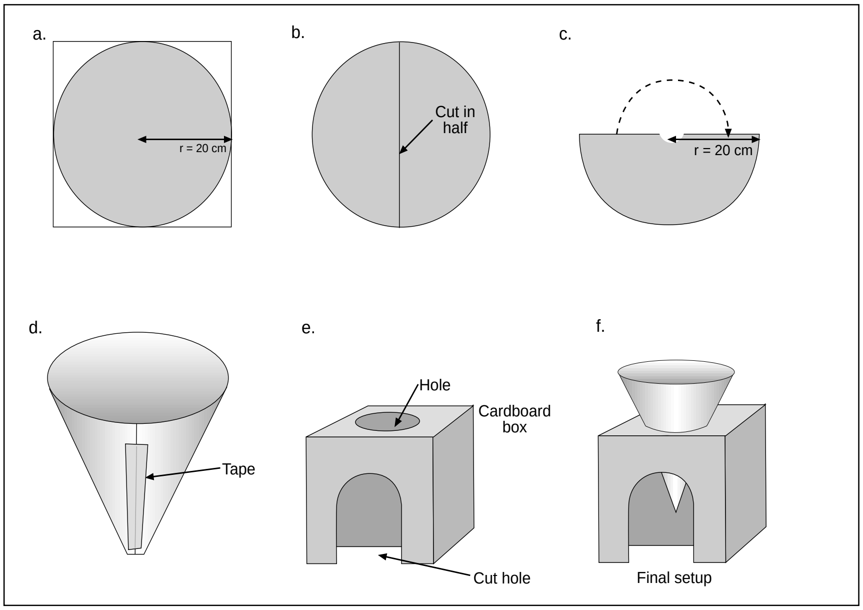


Figure 6. Extraction funnel and stand made from acetate or sheet vinyl and a cardboard box.

TEACHING TIPS

- Selection of the sample sites is critical to the success of this laboratory activity. More organisms will be found in samples from sites that are somewhat sheltered and not subject to periodic flooding or desiccation. High population density and high species diversity usually can be found in the following materials:
  - leaf mold associated with neutral soils in deciduous forests
  - leguminous ground covers such as clover, vetch, alfalfa
  - compost piles with a mixture of vegetable matter
  - mulch from vegetable gardens or organic farms.
- Prior experience with dichotomous keys and/or cladistics is helpful.
- Samples that are too damp or dense do not work well. In some cases, a larger sample (25 cm^2) may be needed to see a good cross-section of animals.
- All Berlese-Tullgren funnels should be set up in a location where they will not be disturbed. Excessive agitation will cause the dirt to loosen and fall into the alcohol. This will cloud the final sample and make identification of the organisms more difficult.
- May is the time suggested by field testers for this lab. The soil is warmer and invertebrates are more active.
- Berlese-Tullgren funnels that can accommodate larger soil-surface samples can be constructed from inexpensive materials such as sheets of clear acetate or scraps of vinyl flooring. Cut the material into a half-circle, as illustrated in Figure 6. Then, join the radii and use cellophane shipping tape or vinyl duct tape along the seam to form a funnel. Make the radius of a half-circle equal to the desired diameter of the finished funnel. A 40-cm diameter funnel will be large enough to hold a 30 x 30-cm sample. Make a stand for the funnel by cutting a circle in the top of a cardboard box.
- A Berlese-Tullgren funnel for a small humus sample can be made using the above procedure with 15-cm diameter filter paper. Punch a small hole at the point of the funnel. A cone-shaped coffee filter may be substituted for the filter paper.

(continued on p. 125)

Figure 7a. *Kwik-Key to Soil-Dwelling Invertebrates* (Steps 1 through 4).

Figure 7b. *Kwik-Key to Soil-Dwelling Invertebrates* (Steps 5 through 8).

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Have the Berlese-Tullgren funnel set up in front of the class when students arrive. Ask them to describe the apparatus and explain how you set it up. Challenge students to determine the function of the apparatus.

Discuss the soil-surface sample and describe its collection site. Show them a map of the site if one is available. Allow students to inspect the sample briefly, then ask the following questions:

- What organisms do you think live in the environment of the soil-surface sample?
- What would affect the number or kinds of organisms in a sample?
- What are the organisms eating?
- What evidence do you find that supports your answers?

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

Students should identify a site to collect a sample of soil surface or leaf litter and list possible factors that may affect the sample. Then, they should form a hypothesis about the effects of a variable that was present at that site. If necessary, you may suggest examples from the following lists.

Study sites might include:	Variables might include:	
	Natural	Artificial
• Agricultural fields • Organic farms • Compost piles • Grasslands or pastures • Orchard ground cover • Hardwood forest leaf litter • Coniferous forest pine straw • Wetlands (bogs or swamps) • Lawns or golf courses	• Time of year • Grazing • Shading • Elevation • Slope face (north vs south) • Degree of slope (flat to steep) • Location on a slope (top vs bottom)	• Physical disturbance • Addition of chemicals • Mulching • Mowing • Nearby disturbance • Introduction of exclusion of organisms

As students develop a hypothesis, they should decide what they will measure to test it. Possible measurable elements include:

- population age structure determined by:
  - measuring the head
  - counting the body segments
- species diversity calculated by using diversity indexes
- population density of a selected species determined by:
  - extrapolating from sampling counts
  - using statistical analysis
- rate of development or change determined by observing population dynamics
- trophic structure determined by:
  - developing a pyramid of numbers or biomass
  - counting the relative numbers of predators and preys

TEACHING TIPS

(continued from p. 122)

- Provide black and white construction paper for students to place under their petri dishes while examining the extracted sample. Light-colored organisms should be more visible with a dark background; darker organisms should be more visible with a white background.
- Scratch a grid on the bottom of the petri dish to help students keep track of their search progress. A dissecting needle or straight pin are good tools for this. A piece of graph paper attached to the inside of the petri dish will provide a guide for your grid pattern. Five squares to 2.5 cm (1 inch) is fine enough for most searches.
- Gently swirl the petri dish of alcohol containing the sample on the microscope stage. Most of the animals will concentrate near the center of the dish.
- Most animals will sink and can be seen easily on the bottom of the petri dish, but Collembola float and can be found on the surface. Wash down the sides of the collection vial to dislodge any organisms adhering to the sides of the dish.
- Students can estimate population density for each taxon using the factor-label method. For example, 36 mites collected in a sample measuring 15 x 15 cm (225 cm^2) would use the following conversion:

  $$\frac{36\ \text{mites}}{225\ cm^2} \times \frac{10{,}000\ cm^2}{1\ m^2}$$

  $= 1{,}600\ \text{mites}/m^2$
- Pool class data and average all values from similar habitats to give a more accurate estimate of population density. Extrapolate this estimate to number/acre or number/hectare by using the appropriate conversion factors:

  1 hectare = 2.471 acres
  = 10,000 square meters
  1 acre = 4047 square meters
  = 0.4047 hectare
- An illustrated key for identifying soil-dwelling invertebrates to class or order is used in this lab. The *Kwik-Key to Soil-Dwelling Invertebrates* illustrated in Figures 7a & b includes a synopsis of the life history and ecology for each taxon. This key and 2 x 2 color transparencies of various soil-dwelling organisms may be purchased from Vision Press, PO Box 5554, Raleigh, NC 27650.

TEACHER'S NOTES

Sample Hypotheses

- Leaf litter or a soil-surface sample collected from a suburban area treated with pesticides will have fewer living organisms than a sample from an untreated suburban area.
- Leaf litter or a soil-surface sample collected from the schoolyard will have less diversity of organisms than a sample collected from an isolated wooded area.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. *Additional test questions are found on page 129.*

Question

Does a roadway have an effect on the soil communities living nearby?

Hypothesis

Species diversity and density increase with increasing distance from a highway.

Rationale

Motor vehicles will produce a variety of pollutants that will be introduced to adjacent communities. The farther a community is from the road, the less likely it will be exposed to these pollutants.

Procedure

1. Construct 3 Berlese-Tullgren funnels following the demonstration instructions.
2. Collect leaf litter or soil-surface samples at regular intervals from the roadway.
3. Place the sample in the funnels.
4. Allow the samples to dry out for 5 days. Make observations each day of the number of organisms that have fallen into each collection jar.
5. After 5 days, remove the funnels. Count and identify all the organisms that have fallen into each collection jar.
6. Analyze data to measure density and diversity and compare various samples.
7. (*Optional*) Analyze diversity using Simpson's Diversity Index.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 1. The diversity of organisms at three distances from a highway.

Taxonomic group	Number of species present at a distance of:		
	1 Meter	2 Meters	3 Meters
Subphylum Chilicerata	7	6	5
Subphylum Crustaceae	0	0	1
Subphylum Uniramia: Classes Chilopoda and Diplopoda	3	6	6
Subphylum Uniramia: Class Insecta	5	14	24

To analyze these data calculate the total number of species at each distance from the highway.

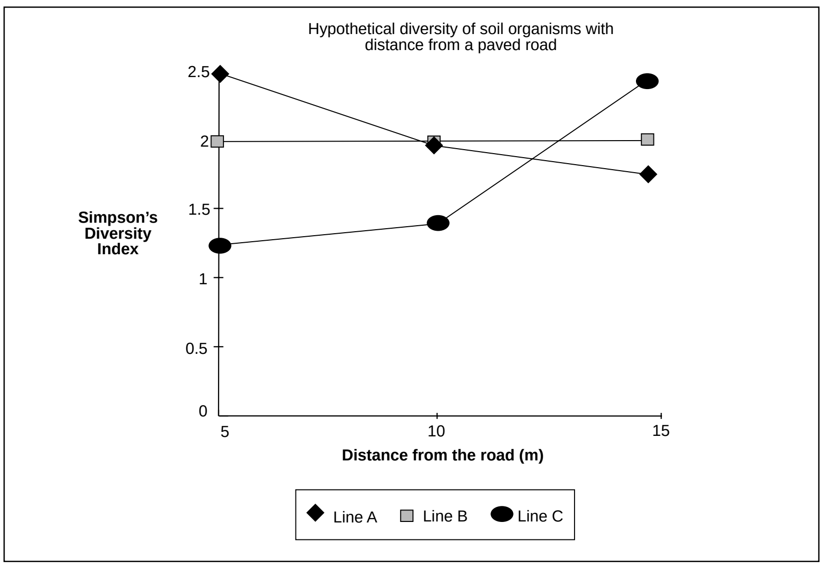
Advanced students can compute an Index of Diversity for comparing the samples. One index they are likely to have appropriate data for is Simpson's Diversity Index. The formula for calculating this index is:

$$N_2 = \frac{N(N-1)}{\sum n\,(n-1)}$$

where: N_2 = *Simpson's Diversity Index*
N = Total number of individuals of all species
n = Number of individuals of a species

TEST QUESTION

Which of the following lines represents an increasing species diversity with increasing distance from the highway?



Graph A. Hypothetical diversity of soil organisms with distance from a paved road.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think about questions that occurred to them as they conducted their first experiment. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- What did you find out about the density of soil communities near a roadway?
- What did you find out about the diversity of soil communities near a roadway?
- What do you think you would find if you selected a different roadway?
- What are some of the factors that might have affected the density and diversity in soil communities?
- What other factors might affect the density and diversity in soil communities, e.g. soil moisture, pH, mowing, light, seasons, animal grazing, disturbed vs. undisturbed, developed vs. undeveloped?

Interpretation

The hypothesis should be accepted if the number of species collected decreases in samples closer to the highway. The hypothesis should be rejected if the number of species is similar in all the samples or if the number increases closer to the highway.

Answer to Test Question

Line A.

TEACHER'S NOTES

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

- Excuse blind or severely visually impaired students from this experiment due to the complicated nature of identifying microscopic organisms. Organisms are too small to be identified by touch. Their sounds are not audible enough for identification. There is also the chance that the student may be bitten by one of the insects. An alternate experiment would be to do a behavioral study by changing the size and species of animals.

Deaf or Hard-of-Hearing

- Provide for easy communication within the lab team, especially when participants are in the field.

Gifted

- Challenge students to explore more complex habitats and to design multivariate experiments.
- Distinguish between measures of frequency, abundance, constancy, and fidelity.
- Discuss the difference between abundance or population per unit area and relative abundance or percent of the total sample.
- Use binomial confidence intervals to illustrate how reliability of a population estimate increases as the number of samples increases.
- Look for a relationship between frequency and abundance by plotting these statistics on opposing axes. Use classroom data for several taxa from a series of similar Berlese-Tullgren samples.
- Compare several diversity indices and determine which ones are most sensitive to changes in sample size.
- Identify species associations by using correlation statistics on Berlese-Tullgren data collected from different habitats.

Mobility Impaired

- Equip the wheelchair with a sturdy tray to allow the mobility impaired student to work on soil samples supplied by lab team members. "Dirt tires" make many areas accessible for the wheelchair-bound student.
- Encourage mobility impaired students to assist with the construction and use of the Berlese-Tullgren funnel.
- Encourage lab team members when asked to assist physically and mobility impaired students with observations of the invertebrates that are separated from the soil samples and with locating them under the microscope.
- Provide physically impaired students with the *Kwik-Key to Soil-Dwelling Invertebrates* to assist in identifying the small organisms.
- Place organisms under a magnifying glass mounted on a stand.
- Have other students photograph the site for later reference. The photo should include students collecting samples.
- Select a site that will facilitate mobility for students in wheelchairs.
- Use a video microscope camera to display organisms on a large screen.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. Describe how a Berlese-Tullgren funnel works and its use. Provide an illustration in your answer.
2. Write a simple key to separate and identify the following four types of organisms.

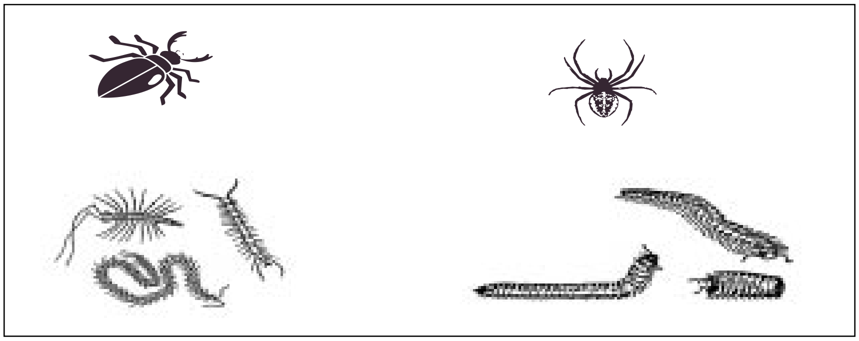


Figure 8. Four common types of arthropods.

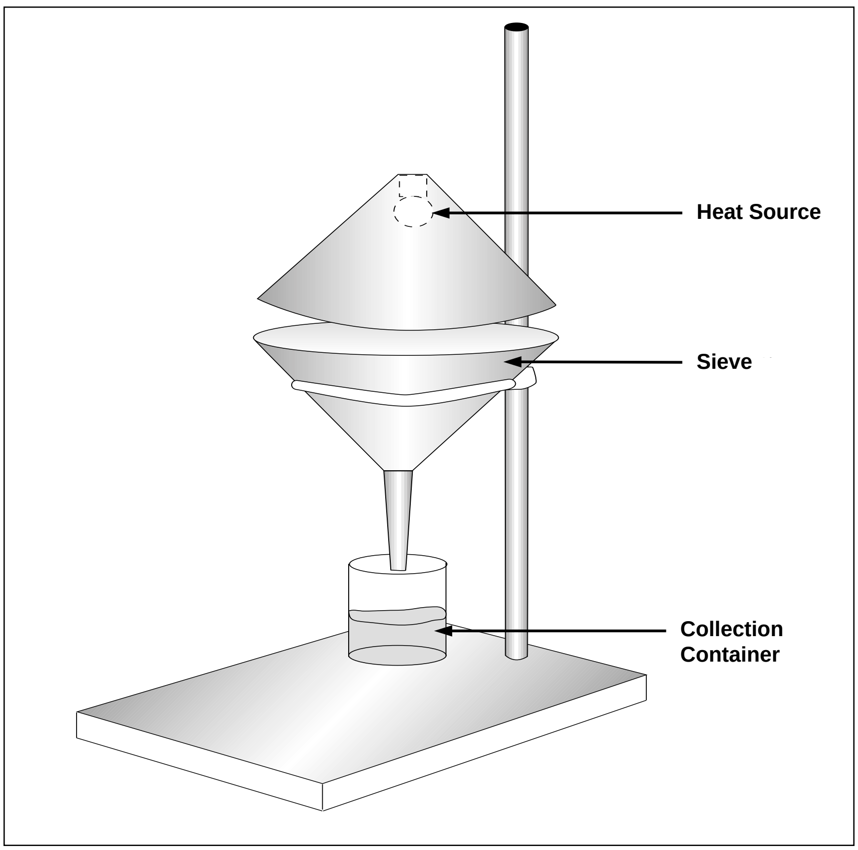


Figure 9. A Berlese-Tullgren funnel has a heat source, a sieve to support the samples, and a container of alcohol to collect specimens.

Answers to Additional Test Questions

1a. A Berlese-Tullgren funnel is used to extract invertebrate animals from a leaf litter or soil-surface sample. It works in the following way:

- Place a sample of soil in the funnel and suspend it over a jar of alcohol.
- Place a light over the sample so that the sample will dry out slowly from the top down.
- The invertebrate animals in the sample will move downward to escape the dryness and fall into the alcohol where they will be preserved.

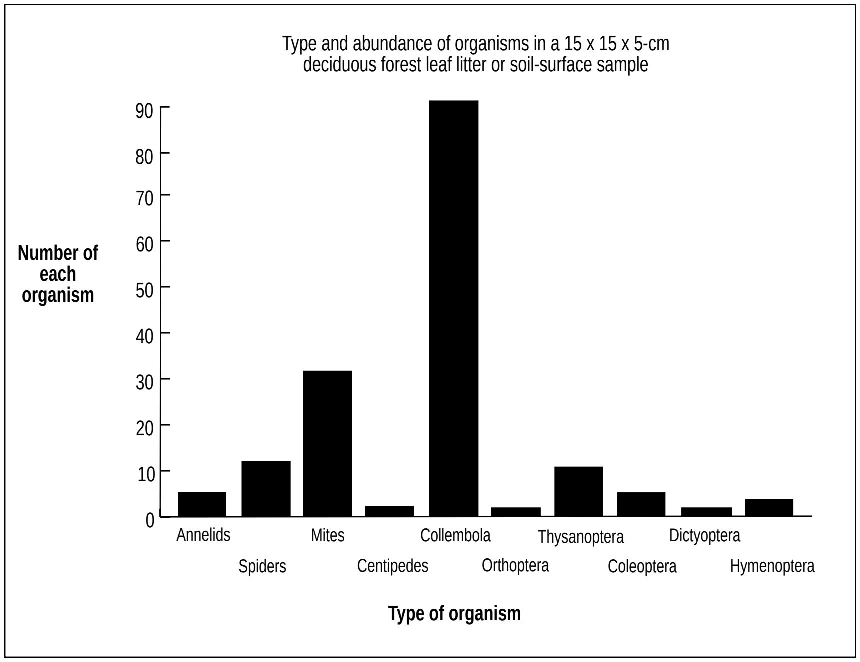
1b. See Figure 9 for a sample setup.

2. Possible student key:

1a. fewer than 10 legs....................2
1b. more than 10 legs....................3
2a. 6 pairs of legs....................insect
2b. 8 pairs of legs...................spider
3a. 1 pair of legs per segment................................centipede
3b. 2 pairs of legs per segment.................................millipede

Answers to Questions and Analysis on Student Page

1. The answer will be a bar graph based on the students' data. The graph will vary depending on these data. The graph title and axes labels should be similar to those of Graph B.
2. Yes.
3. Answers will vary.
4. Number of organisms in the sample the teacher set up will vary because each group analyzes only a part of the sample. In samples collected by students, answers will vary also due to natural variation within a habitat and variation across habitats. For example, within a habitat, dry and wet areas in the same location will provide different results.
5. The more samples you have, the more your data will represent the habitat accurately.
6. No.
7. Location, season, weather conditions, and time of day are some examples of differences.
8. Diverse organisms exist in the soil-surface sample. Sampling is very important in a scientific experiment for accurate results. Lots of different variables will affect the results of these data. Answers will vary based on class results.
9. Answers will vary. One possible answer is the following: Many invertebrates inhabit the soil surface. Some examples are springtails, earthworms, and pill bugs. Some invertebrates are carnivores, some are herbivores.



Graph B. The abundance of diverse soil organisms in a spring sample collected by high school students.

REFERENCES AND SUGGESTED READINGS

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VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

VARIATION 1
The Effect of Cattle Grazing on the Population Density of Oribatid Mites

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from a pasture where cattle are grazing and from adjacent land where cattle are excluded. They will count the number of oribatid mites in each sample.

HYPOTHESIS GENERATION

Question

How will cattle grazing affect density of mites in a pasture?

Sample Hypothesis

Grazing cattle reduce the density of oribatid mites in a pasture.

Rationale

Students should develop their own rationale for the hypothesis.

Sample Experimental Procedure

Collect soil-surface samples from cattle-grazed and nearby ungrazed pasture. Follow the sample procedure from the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 2. The density of oribatid mites in adjacent grazed and ungrazed pastures.

Replicate	Treatment (grazed)	Control (ungrazed)
1	25	19
2	17	14
3	34	32
4	21	28
5	13	6
Mean	22	19.8

Analyze these data using the mean and the variance for the treatment and the control. Use average values to estimate the population density of the mites.

TEACHER'S NOTES

Interpretation

Accept the hypothesis if the mean number of mites in the control is higher than in the treatment. This suggests that grazing influences the population size of oribatid mites. Reject the hypothesis if the mean number of mites in the control is equal to or lower than in the treatment.

Answer to Test Question

The hypothesis is supported. There are fewer Collembola after clear cutting than before clear cutting.

VARIATION 2

TEST QUESTION

A student hypothesizes that Collembola density will decline after clear cutting a forest. Do the following data support this hypothesis?

Table 3. Density of Collembola before and after clear cutting in a hypothetical location.

	Number of Collembola	
Sample number	Before clear cutting	After clear cutting
1	25	19
2	34	30
3	17	14

VARIATION 2
The Density of Millipedes on North- and South-Facing Slopes

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from north- and south-facing slopes near the peak of a dome-shaped hill or mountain. They will identify and count all Diplopoda.

HYPOTHESIS GENERATION

Question

Is millipede density affected by exposure to north- or south-facing slopes?

Sample Hypothesis

Millipedes are less dense on north-facing slopes than on south-facing slopes.

Rationale

Students should derive their own rationale for the hypothesis.

Sample Experimental Procedure

Collect soil-surface samples from the north- and south-faces of adjacent hillsides. Follow the sample procedure from the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 4. The hypothetical density of millipedes on north- and south-facing slopes.

Millipede	North	South
Brown species		
Replicate 1	3	7
Replicate 2	5	8
Replicate 3	2	10
Ivory species		
Replicate 1	5	5
Replicate 2	3	7
Replicate 3	6	5
Banded species		
Replicate 1	8	2
Replicate 2	10	3
Replicate 3	9	0

Analyze these data by calculating the mean and the range for the numbers of individuals belonging to each species in each location.

TEST QUESTION

A student hypothesizes that fewer spiders will be found on a north-facing slope than on a south-facing slope. How could the student design an experiment to test this hypothesis?

VARIATION 3
The Seasonal Changes in the Age Structure of a Centipede Population

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will collect and process Berlese-Tullgren soil-surface samples from the same site at monthly or seasonal intervals. They will count the number of body segments and/or measure head capsule width for all members of one centipede species.

HYPOTHESIS GENERATION

Question

Is the age of a generation of centipedes similar?

Sample Hypothesis

If there is only one generation of centipedes per year and centipedes live less than one year, all members of the population should be similar in age.

Interpretation

The hypothesis should be accepted if a higher millipede density is found in samples collected on south-facing slopes. This may suggest that the warmer micro-climate favors millipede growth and development. Reject the hypothesis if similar populations were found in both areas or if higher densities were found in north-facing samples. This may suggest that millipedes are not sensitive to the temperature of the micro-climate or that they prefer conditions that are cooler and more humid.

Answer to Test Question

Construct an experimental setup with samples taken from north-facing and south-facing slopes. Count the number of spiders in each sample and compare these data.

TEACHING TIPS

- Do not suggest a Chi-Square Goodness of Fit analysis unless there are at least 5 individuals in each count.
- External calipers will be useful for measuring the width of the head capsule.

Rationale

Students should derive their own rationale for the hypothesis.

Sample Experimental Procedure

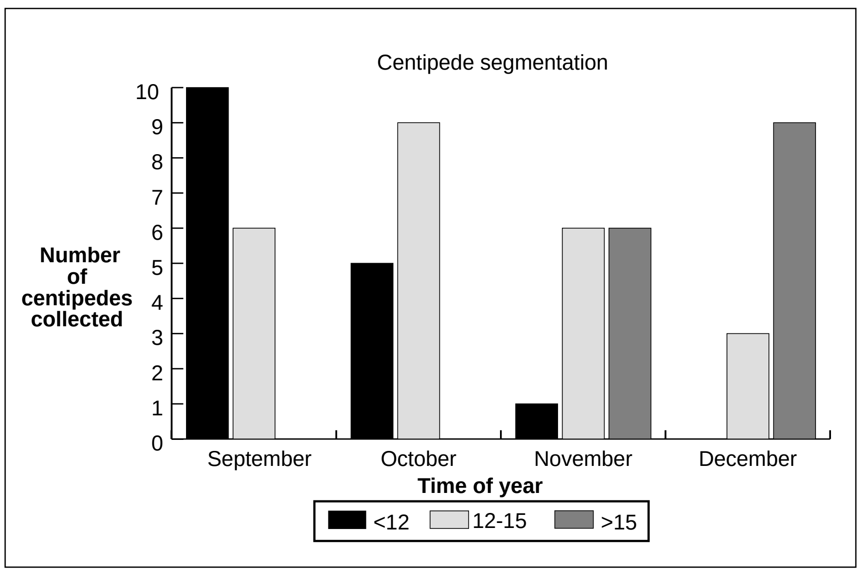
Collect soil-surface samples at various times during the year. Follow the sample procedure in the Core Experiment. Isolate the centipedes and determine the number of individuals of different ages by assuming that number of segments is correlated with age.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Hypothetical seasonal changes in the segmentation of a centipede population.

Month of collection	**Number of centipedes in each size class**		
	<12 Segments	**12 to 15 Segments**	**>15 Segments**
September	10	6	0
October	5	9	0
November	1	6	6
December	0	3	9



Graph C. Centipede segmentation over a four-month period.

Analyze these data for each size category by graphing the number of centipedes collected versus time of year. Advanced students can use Chi-Square Goodness of Fit Test to determine whether their results differ from a uniform distribution.

TEST QUESTION

If all members of one population within the community are similar in size and age, what does that tell you about the population?

Interpretation

Accept the hypothesis only if data show consistent decreases in the number of small individuals and consistent increases in the number of large individuals. The hypothesis should be rejected if all size or age categories are present during every sampling period.

Answer to Test Question

All members of the population belong to the same generation.

VARIATION 4
The Survival of Soil-Dwelling Organisms Following a Controlled Burn of Coastal Bermuda Grass

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from a Bermuda grass pasture before and after a controlled burn. They will evaluate the species diversity in both samples.

SAFETY PROCEDURE

The area should not be burned by the students. Fire departments and the park service do controlled burns. Any burn should be controlled carefully and should have been planned by one of these groups, not conducted solely for this exercise.

HYPOTHESIS GENERATION

Question

What effect does fire have on the organisms in the soil interface?

Sample Hypothesis

Fire will kill all the soil-surface organisms.

Rationale

Organisms cannot withstand the high temperatures and destruction of the fire's flames.

Sample Experimental Procedure

Collect soil-surface samples before and after a controlled burn of coastal Bermuda grass. Follow the sample procedure for the Core Experiment.

TEACHING TIP

Students can take additional samples over the next several months to measure the rates of recolonization for various taxa.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Number of species present in soil-surface samples before and after a controlled burn of coastal Bermuda grass.

Common name of grouped species	Number of species present	
	Before burn	After burn
Annelid worms	1	1
Spiders	3	0
Mites	5	3
Centipedes	1	0
Millipedes	2	1
Springtails	5	1
Grasshoppers & crickets	1	0
Thrips	1	1
Beetles and weevils	3	2
Cockroaches	1	1
Ants, bees, and wasps	3	2

Interpretation

The hypothesis should be accepted if the kinds of species differ before and after the fire. No individuals from any species will be present. Reject the hypothesis if some of every species group survive. Some taxonomic groups may be more heat-tolerant than others. Some may be less susceptible because of their position in the soil strata.

Answer to Test Question

60%

To analyze these data, calculate the total number of species collected before and after the burn. This number should be expressed as a percentage of the original density. Advanced students can use a diversity index to compare the samples. (See Core Experiment for instructions for calculating a diversity index.)

TEST QUESTION

Using the following information, what percent of mites survived after burning?

Table 7. Number of species present in humus samples before and after a controlled burn of coastal Bermuda grass.

Common name of grouped species	**Number of species present**	
	Before burn	**After burn**
Annelid worms	1	1
Spiders	3	0
Mites	5	3
Centipedes	1	0
Millipedes	2	1
Springtails	3	1

VARIATION 5

The Arthropod Fauna in Different Types of Compost

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from two compost piles, one containing mostly grass and the other containing mostly leaves. They will identify and count all representatives of major taxa.

HYPOTHESIS GENERATION

Question

Is there a difference in the number of organisms found between compost piles with leaves and ones with grass growing?

Sample Hypothesis

The relative abundance of animal species will be lower in samples from the compost pile containing mostly grass than in the compost pile of leaves.

Rationale

There will be fewer organisms in the compost pile with grass, as there will be less decaying matter as an energy source.

Sample Experimental Procedure

Collect samples of several types of compost. These types may include compost from leaves, grass clippings, vegetable scraps, or a mixture of leaves, clippings and vegetables. Follow the sample procedure for the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 8. Number of individuals in samples collected from several types of compost.

Common name of grouped species	Number of individuals present	
	Leaf-rich compost	Grass-rich compost
Annelid worms	4	6
Spiders	6	8
Mites	18	4
Sowbugs	10	8
Centipedes	3	7
Millipedes	6	6
Symphylans	15	9
Springtails	57	43
Thrips	12	5
Ants	10	4
Beetles	7	3

Analyze these data by ranking the identified arthropods by abundance. Determine which groups are most and least abundant and draw pie charts showing the relative abundance of each group. Advanced students can also use a diversity index to compare samples.

Interpretation

Accept the hypothesis if there is a difference in the rank order of the groups between compost piles. Reject the hypothesis if the ranking is similar for all types of compost.

TEST QUESTION

Which variable is LEAST likely to affect the abundance of insects in a compost pile?

A. Moisture content
B. Distance to woods
C. Temperature
D. Predators

Answer to Test Question

B

VARIATION 6
The Density of Small Annelids in Soils with Different Moisture Contents

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will collect samples from sites with different percent moisture content and process in a Berlese-Tullgren. They will count all collected enchytraeid worms.

TEACHING TIP

Let students discover on their own that soils with 30 to 85% moisture will have fewer worms than soils with 0 to 20% moisture (Wallwork, 1976).

HYPOTHESIS GENERATION

Question

How does moisture content of soil affect worm population?

Sample Hypothesis

There will be more worms in moist soil than in dry soil.

TEACHER'S NOTES

Interpretation

If the number of worms increases with increasing soil moisture, accept the hypothesis. If the number of worms does not increase with increasing soil moisture, reject the hypothesis.

Rationale

Worms need moisture. Without it, they dehydrate and die. Therefore, worms should be found in higher density in moist soils.

Sample Experimental Procedure

Choose three sample collection sites that appear to have consistently different moisture content, such as a frequently watered lawn area and a lawn not well watered. Collect two samples from each site. One sample from each site will be treated according to the procedure from the Core Experiment. The second sample from each site will be examined for its moisture content. The moisture content can be determined by first massing the sample, and then drying it in an oven at 100˚C overnight before massing again. Use the following equation to calculate the moisture content:

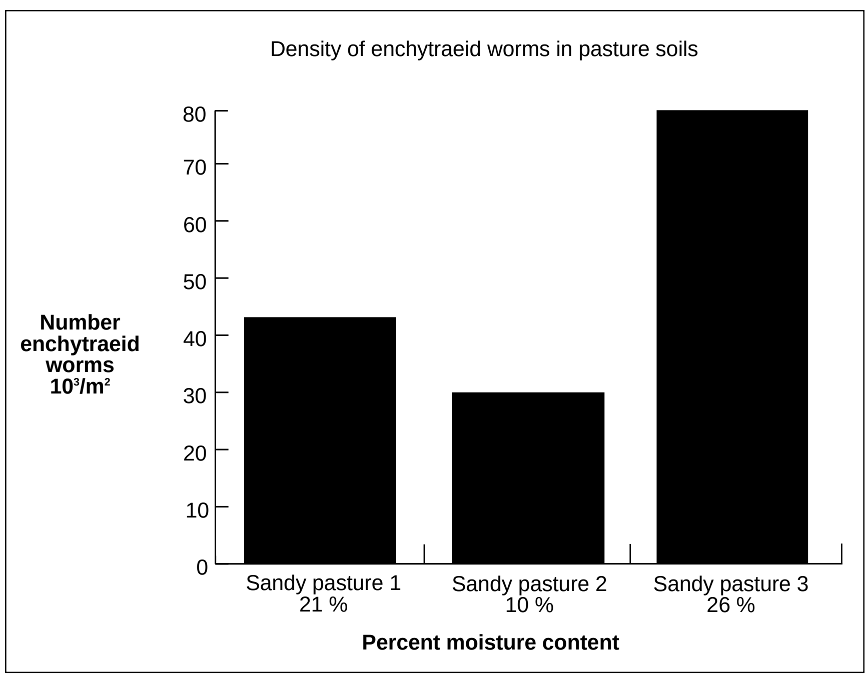
$$\frac{\textit{Mass of Dried Sample}}{\textit{Mass of Undried Sample}} \; x\ 100\% = \%\ \textit{Moisture Content}$$

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 9. Density of enchytraeid worms in pasture soils. The worm density data are summarized from the literature in Wallwork (1970). The moisture content values are hypothetical.

Site	Number of enchytraeid worms ($10^3/m^2$)	Percent moisture content
Sandy pasture 1	44	21
Sandy pasture 2	30	10
Sandy pasture 3	74	26



Graph D. Enchytraeid worm density.

TEST QUESTION

How would you use soil moisture data to find the best site for collecting fishing worms if large fishing worms are influenced by soil moisture the same way enchytraeid worms are influenced?

VARIATION 7

The Soil pH and Diversity of Soil Organisms

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from sites with different hydrogen ion concentrations. They will identify, classify, and count all organisms collected.

MATERIALS NEEDED

- 1 vial Hydrion paper (pH 1 to 12)

HYPOTHESIS GENERATION

Question

How does soil pH affect species diversity?

Sample Hypothesis

Species diversity will decrease as the pH diverges from the neutral pH 7.

Rationale

Organisms survive within specific pH ranges. A neutral pH is optimum. Variance from this would be injurious to the organism.

Sample Experimental Procedure

Test the pH of soil from sites that you expect might have a different pH. Test the soil pH by mixing a match-head sized chunk of soil with about the same volume of water to make a paste. Use Hydrion paper to determine the approximate pH. Collect samples from soils of high, low, and neutral pH. Treat them according to the instructions for the Core Experiment.

Answer to Test Question

One would select soils with high moisture content over sites with lower moisture content because more earthworms are in moist soil.

TEACHING TIP

Acidic sites are likely to be found under pines or where acid rain is a problem. Basic sites are likely to be found in areas used by animals, lime-treated soils, or near lime outcroppings.

VARIATION 8

Interpretation

Each group of organisms tends to have a pH that is specific to their survival.

Answer to Test Question

Answers will vary with student creativity. They could search for bodies of water with different acidity or take water from one site and observe changes after altering the pH.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 10. Number of soil organisms from soils of different pH values. The organism values are approximated from data summarized from the literature in Wallwork (1970). The pH values are hypothetical.

Common name of grouped species	Number of individuals collected per m^2 from:		
	Limestone grassland (pH 8.2)	Moor (pH 7.8)	Grassland (pH 6.9)
Ticks and mites	45,300	43,000	78,800
Collembola	77,900	38,900	55,900
Enchytraeid worms	18,500	195,000	123,000
Lumbricid worms	389	0	11

Calculate Simpson's Diversity Index. See the Core Experiment for directions.

TEST QUESTION

How does water pH affect diversity?

VARIATION 8
Carnivores and Herbivores in the Schoolyard

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will determine the number of herbivores and carnivores in the schoolyard. Based on these findings, they will determine whether herbivores' or carnivores' survival is more adapted to this environment.

HYPOTHESIS GENERATION

Question

Are more herbivores or carnivores found in schoolyard soil surface?

Sample Hypothesis

More herbivores than carnivores will be found in a soil-surface sample collected from school grounds.

Rationale

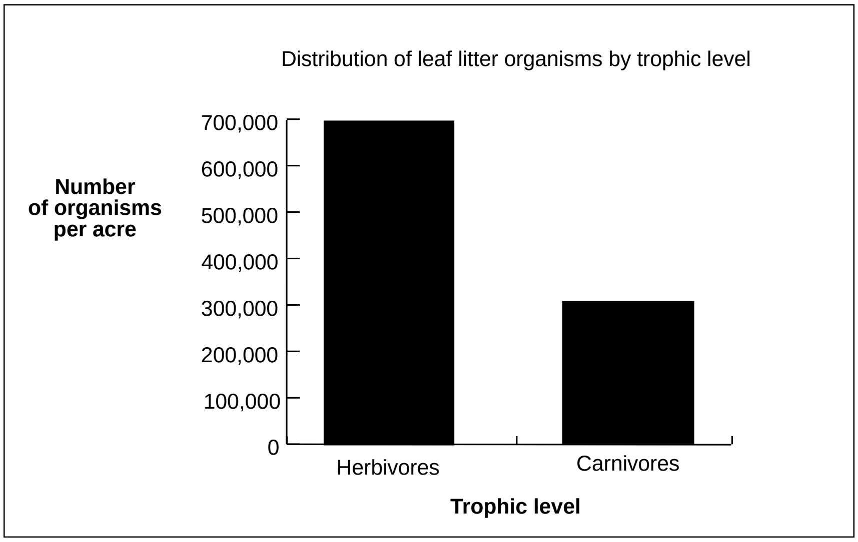
Students should derive their own rationale for the hypothesis.

Sample Experimental Procedure

1. Collect soil-surface samples from the school ground.
2. Follow the sample procedure from the Core Experiment.
3. Determine how many of these organisms were herbivores or carnivores.

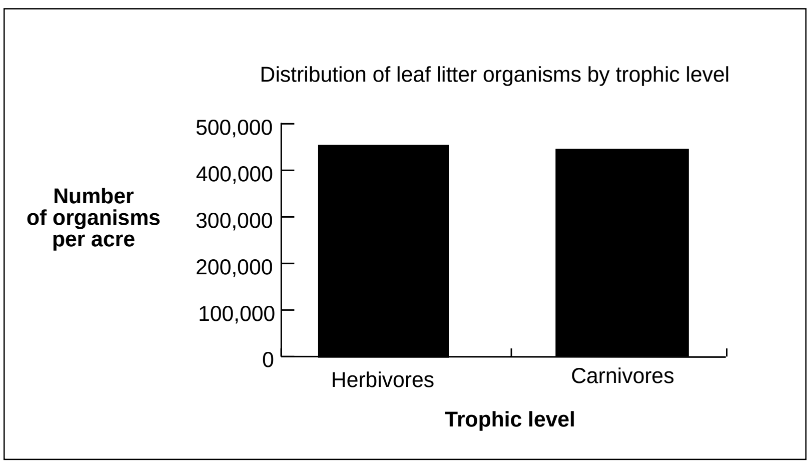
DATA ANALYSIS AND INTERPRETATION

Sample Data 1



Graph E. The number of soil organisms by diet collected per acre from leaf litter of a wet bottom area during spring.

Sample Data 2



Graph F. The number of soil organisms by diet collected per acre from leaf litter of a wet bottom area during spring.

TEST QUESTION

Examine the two graphs above. Account for the different ratios of herbivores to carnivores in the two communities.

Interpretation

More herbivores than carnivores were found in the sample of humus from the schoolyard, so the hypothesis is supported.

Interpretation

The same number of herbivores and carnivores were found in the sample of humus from the schoolyard, so the hypothesis is not supported.

Answer to Test Question

One would expect more herbivores than carnivores in a community because only 10% of the energy available to the herbivore is available to the carnivore that consumes it. This is based on the assumption that herbivores are smaller than carnivores. The second set of data, however, shows equal numbers of herbivores and carnivores—something unexpected. This may be explained by a larger biomass for individual species of herbivores than their carnivore predators. Also, the carnivores may consume other organisms such as decomposers, thus giving them an alternative food source. In any case, the carnivores will receive only 10% of the energy originally available to their prey.

VARIATION 9
Organism Size Distribution

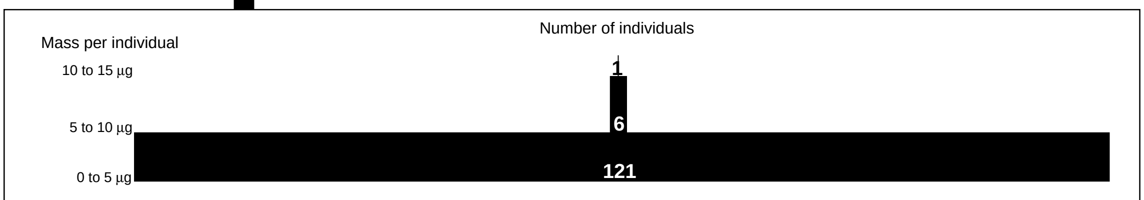
TEACHING TIPS
- Results are likely to be different in soils of different textures.
- A calibrated ocular micrometer will facilitate sizing.
- Biomass also can be used to assess size. Although biomass values, such as those shown in Table 11, are more common in the literature, they will be very difficult for students to collect.

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS

Students will collect and process Berlese-Tullgren samples from one site. They will count the number of organisms in each of several sizes of classes determined by body length.

Table 11. This pyramid of numbers, constructed from data presented in Engelmann (1961), shows the inverse relationship between body size and population density.



HYPOTHESIS GENERATION

Question
Is there a relationship between an organism's size and the number of individuals that will be found in a sample?

Sample Hypothesis
The number of individuals will increase as their size decreases.

Rationale
The smaller in size the organism, the greater the number of this species found in the sample.

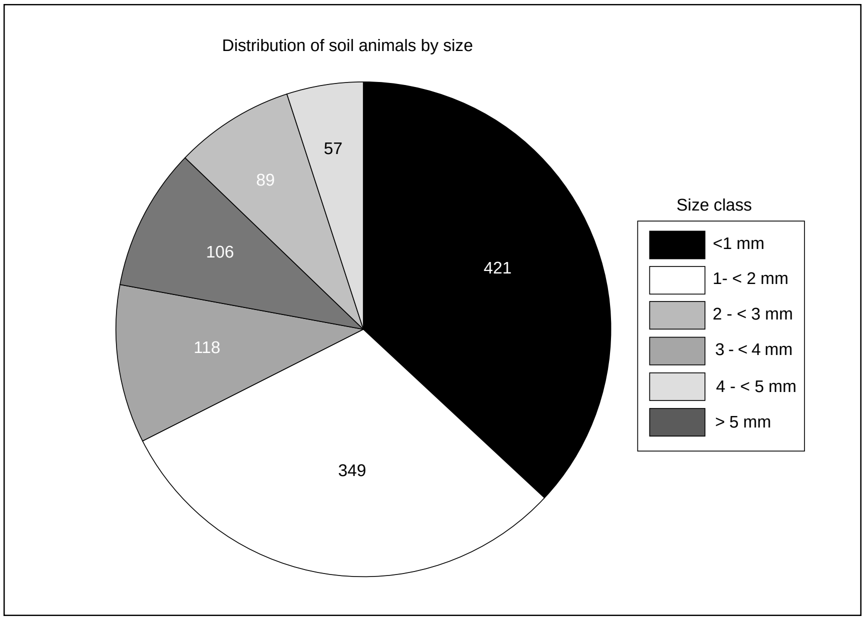
Sample Experimental Procedure
Collect a sample from forest or grassland soils and treat it according to the instructions for the Core Experiment. Establish size classes at millimeter intervals for organisms less than 5 mm long.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 12. Distribution of soil animals by size. These data are for cryptozoa from South Africa forest soils (Birch & Clark, 1953).

Size class	Number of organisms
<1 mm	421
1-<2 mm	349
2-<3 mm	118
3-<4 mm	106
4-<5 mm	89
>5 mm	57



Graph G. Distribution of soil animals by size.

TEST QUESTION

Design an experiment to determine whether the size distribution of soil organisms varies with soil texture.

Interpretation

Accept the hypothesis if the number of organisms decreases with increasing size. Reject the hypothesis if there are more organisms in the larger size classes.

Answer to Test Question

Answers will vary, but should include sampling from at least two sites, analyzing the texture of the soil and counting organisms by size class.

A Community Underfoot: Density and Diversity of Invertebrates in Soil or Ground Cover

Directions for Students

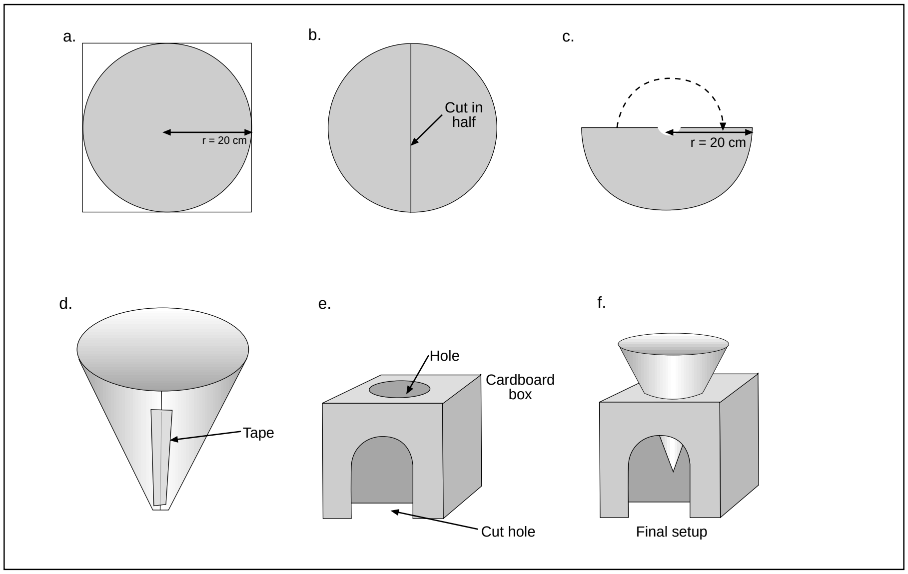


Figure 1. Extraction funnel assembly.

INTRODUCTION

Have you ever wondered what type of life exists beneath your feet? What do the organisms look like? How big are they? What do they eat? Do they benefit humans? You will explore the cryptic creatures of the below-ground world as you create and use a sampling device, learn to identify them, and construct simple trophic pyramids with your data.

OBJECTIVES

At the end of this lab, you should be able to:

- Construct a Berlese-Tullgren funnel to sample invertebrate fauna in soil or ground cover samples.
- Estimate the population density and diversity for different environmental conditions.
- Describe the effects of specific environmental variables on a target population or community structure.

SAFETY PROCEDURES

Wear gloves whenever handling soil samples and wash your hands at the beinning and end of the lab.

Do not use alcohol near an open flame, as it is flammable.

Do not drink the alcohol or inhale its fumes.

Have adequate ventilation in the classroom.

Reduce vapor levels by using a collecting jar that fits snugly with the funnel. Keep collecting jars and petri dishes covered.

Keep the 25-watt bulb at least 15 cm away from the larger Berlese-Tullgren funnel sample to prevent the ignition of any dry leaves.

Disturb the soil-surface as little as possible when collecting samples.

MATERIALS NEEDED

You will need the following for each group of two students in a class of 24:

- 3 2-L plastic soda bottles
- 1 pair scissors
- 3 10 x 10 x 5-cm soil-surface samples
- 1 pair of gloves
- 3 1-L (1-quart) sealable, plastic bags
- 3 10-cm^2 square 1/4 or 1/8-inch mesh hardware cloth or plastic needlepoint backing
- 3 500-mL glass jars with 7.5-cm diameter openings with tight lids
- 3 9-watt colorless light bulbs and 3 1-socket sections of a string of holiday lights or 3 25-watt shielded lights
- 1 stereoscope
- 1 eyedropper
- 1 forceps
- 1 9 x 14-cm (8.5 x 11-inch) sheet each of black and white construction paper
- 3 10-cm^2 square of aluminum foil or dark paper
- 5 sheets of newspaper
- 150.0 mL 70% isopropyl alcohol
- 1 garden trowel

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of soil organisms and ecology. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis.

Answer the following questions:
1. What is the question you are investigating?
2. Why is controlling variables important?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:
1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:
1. Construct a bar graph showing kinds of organisms collected and their abundance.
2. Do your data support or refute your hypothesis?
3. Using your specific data, explain your answer to Question #2.

4. Compare your data with that of other groups. Are these data the same or different? Why or why not?
5. Why is it necessary to have repeated sampling?
6. Will the number or kinds of organisms found in a sample always be the same?
7. What could cause the difference? Be specific.
8. What did you learn from this activity?
9. Make a statement that represents what you learned about animal diversity in the soil.

DESIGN OF VARIATIONS OF CORE EXPERIMENT

After collecting and analyzing these data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for experiments you could do next. Think of questions that occurred to you as you conducted the Core Experiment on organisms found in the soil surface. Design an experiment that is quantifiable and write your procedure in a numbered list of steps. Questions other students have studied include the following:

- How will cattle grazing affect density of mites in a pasture?
- Is millipede density affected by exposure to north- or south-facing slopes?
- Is the age of a generation of centipedes similar?
- What effect does fire have on the organisms in the soil interface?
- Is there a difference in the number of organisms found between compost piles with leaves and ones with grass growing?
- How does moisture content of soil affect worm population?
- How does soil pH affect species diversity?
- Are more herbivores or carnivores found in schoolyard soil surface?
- Is there a relationship between an organism's size and the number of individuals that will be found in a sample?