Chapter 4

A Quantitative Study of Litter and Soil Invertebrates Utilizing the Berlese Funnel

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Introduction

Why should anyone study the organisms of litter and soil? Is there any reason to be interested in the animals that inhabit the lawn we walk on or that live in the organic debris of the forest floor? For at least two reasons, the answer is "yes." Firstly, if there were no decomposer community, grass clippings remaining on the lawn and leaves shed in the forest would accumulate indefinitely. Eventually, that accumulation would drastically alter the environment. A little reflection reveals, also, that growing things would soon run out of nutrients without the recycling of resources. The debris-covered surfaces of the earth, both terrestrial and aquatic, would change dramatically without decomposer microorganisms, like bacteria and fungi, and invertebrate animals.

Secondly, arthropods are the dominant animal group throughout the world. They are nowhere more readily seen in diverse forms and high numbers than in litter and soil. Although insects and arachnids usually dominate the scene, seven classes of arthropods may be seen in a single sample. And, their abundance bespeaks their importance. Arthropods, generally, are viewed as regulators of decomposition, accelerating or delaying nutrient release from decomposing organic matter (Mattson, 1977). Studies of litter-soil organisms, therefore, permit easy, direct approaches to biological processes integral to life in virtually any natural situation. Such studies should be included in the students' repertory of critical experience.

The primary objective of this exercise is to acquaint students, at several proficiency levels, with invertebrates that inhabit the litter-soil zone. Briefly, the exercise entails collecting the organic matter from a measured surface area of soil, utilizing the Berlese Funnel method for extraction of animals from the sample, and studying these organisms for identification to major groups and other observations. In the process of preparation of the exercise, the instructor who has not had previous exposure to invertebrates in this way may be exposed to new vistas of information and recognize fresh research possibilities.

Perhaps the most logical place for this exercise is in the laboratory portion of an introductory biology course in a segment that deals with animals. Litter-soil organisms are of such a diversity of size, number, and kind that several kinds of information (identification of common groups, size and number relationships) can be addressed in one unit. Sophomore/junior invertebrate zoology courses can extend the basic recognition and density determinations of specimens to observations of morphological adaptation to the environment, and correlation to life history stages with habitat and seasons. Ecology classes may attempt to determine the roles played by dominant organisms,

formulate a detritovore food web, and observe the relationship between decomposition by fungi and bacteria and the processes of humification by invertebrates.

These tasks may be facilitated by a second laboratory study of live animals in an undistributed sample of litter. Inspection is done by placing a sample, 10 cm × 10 cm in surface area, on the stage of a dissecting microscope. While focusing on the three-dimensional scene at the uppermost surface of the sample, the student removes elements and progressively works down to the lower layers. Depending on one's care in maintaining moisture and temperature conditions in the sample, organisms may be seen in their own "living room," so to speak. The student can be assigned to respond to the impressions he or she gets with as little as a short written series of observations or as much as time and individual preparation will allow.

Students can identify and tally the contents of a typical Berlese sample in about 2 hours. If two students work together, and this is advisable if the number of microscopes and samples involved is of consequence, the sample can be analyzed, specimens measured and sorted into a numbers pyramid, and an observation statement composed in a 3-hour period. Depending somewhat on the thickness (depth) and organismal diversity of the live sample, inspection and the writing of an observational statement takes about 1 hour. A careful job on a sample containing many organisms in several strata can take an entire laboratory period.

Several factors influence how long it takes to obtain a sample for use. Assuming that all the apparatus is available and ready to use, the sample preparation consists of obtaining it from the forest floor or wherever, placing it in the funnel, processing for 3–5 days and transferring the sorted organisms from the Berlese collecting bottle to a petri dish for student use. One needs to plan far enough ahead to get these operations done in time for class use. It is convenient to have several funnels available for bulk processing; I use six sets of four funnels available for samples. Multiple samples for a laboratory section can be obtained in relatively short order.

Strictly speaking, the funnel design utilized in this exercise is more properly called the Tullgren funnel. Berlese used a funnel-shaped water jacket which, when the water was heated, caused animals to move downward into the collecting jar. Tullgren used an electric light bulb in a metal cylinder above the funnel to move animals down and out of the sample. The term "Berlese" is used here primarily because it is the more familiar of the two terms that refer to a funnel configuration for extraction.

Student Materials

The Student Outline that follows contains two descriptive sections and a record sheet which are given to students beforehand. Students are requested to read them carefully before the laboratory period. The record sheet is to be completed: on the front with a tally of organisms found in the sample assigned, and a numbers pyramid constructed on the back utilizing the summary totals according to body lengths of animals tallied.

An additional handout entitled "Representative Litter-Soil Invertebrates" (see Appendix A) is made available to students when they arrive for the laboratory. This 10-page collection of representative invertebrates is not included in this chapter because of difficulties in obtaining copyright permission for certain drawings. Readers may contact me at Alma College, or at home at (517) 463-3717, to obtain a master copy of these drawings. The numbers (1 through 27) on the Tally Sheet (page 79) refer to the taxonomic categories so arranged and numbered in the "Representative Litter-Soil Invertebrates" collection of drawings.

Student Outline

Litter and Soil Invertebrates

Litter is the term which refers to the non-living organic carpet (dead leaves, grass, twigs, etc.) covering the soil. It accumulates to varying depths depending on many factors, such as moisture, pH, soil make-up, temperature, the nature of the living vegetative cover (forest, meadow, field), and whatever management use man and other animals have for it (cultivation, mowing, grazing).

Soil is a complex system involving the interactions of soil, air, and water. The inorganic soil particles, depending on their size and arrangement and the availability of air and water, determine what different groups of living organisms, both plants and animals, are successful living there. The smallest animals, for example, protozoa, rotifers and small nematodes, inhabit not soil proper but, rather, live in films of water enveloping soil particles and their aggregates of particles. Physiologically, they are aquatic animals. Mites, collembola (springtails), and others that are larger than protozoa occupy a different habitat in soil; they are active when the spaces between soil particles are filled with air saturated with water vapour. Soil, for them, is a system of caves in which they live, utilizing moist air for gaseous exchange rather than water. Larger organisms actually dig passages and enter into contact with both solid soil particles and water films. For these invertebrates (earthworms, insect larvae, millipedes) the soil serves as a true habitat.

The interface where litter and soil meet is an ecotone of a sort. If animals in that interface are active there is a certain amount of mixing between the organic matter and the inorganic soil. The zone of mixing is thicker if there are larger, active animals than if bacteria and fungi are the main organisms that decompose the litter there. Normally, in forests, meadows, lawns, roadsides and places where fresh plant material is produced seasonally, there are active animals, mostly invertebrates, that utilize dead plant matter as their main source of food.

These dead plant eaters are called detritus feeders or detritovores, as compared with herbivores and carnivores. Detritovores chew up plant matter, extract out of it a relatively small amount of the nutrition that is there, and pass it through their guts to be released as feces. This process happens repeatedly in a succession of different kinds of animals until the nutrition is gone—recycled—into the new protoplasm of living plants and animals.

In this exercise you will examine and learn to recognize the invertebrates found in litter and the litter-soil interface. These resident invertebrates have already been collected for you.

The method used for separating these animals from litter and soil is the Berlese Method. The sample was placed in a funnel-shaped cone about 40 cm in length. The wide end of the cone was upright and covered with a hood containing a light bulb. The narrow end of the cone was down and inserted into a bottle containing 70% alcohol. With the light bulb burning, the light and heat that were produced created gradients of three different physical conditions. All three gradients caused animals to move downward and, if they could make it all the way, into the alcohol where they were preserved. In such a set up, what are the three gradients produced by lighting the bulb? Observe the group of four Berlese funnels on demonstration in the laboratory for ideas. Obviously not all kinds of animals found in the samples would be able to move through the sample to the alcohol. These animals will not be represented in the sample you examine. What sorts of animals do you think would not be represented (refer to the second paragraph of this exercise)?

The samples available in the laboratory have been obtained from a variety of habitats: deciduous forest, conifer forest, sod from fence rows, lawns, meadows, campus, etc. The sample you will examine is labelled so you know its source.

Each sample came from the litter: litter-soil interface material 10 cm × 20 cm in surface area. Hence, you can, and should, calculate how many of each of the different kinds of invertebrates you identify might be found in a 1-m2 sample. How do you do that? (10 cm × 20 cm is what part of 100 cm × 100 cm?)

Procedure

Your laboratory instructor will give you directions on what to do. As a preview, you will work in pairs. You and your partner will count the same sample, you should work together to identify the various kinds of animals. You will be given a tally sheet on which to record the numbers of organisms counted and the numbers converted to 1 m2. Hand this in when you are finished.

To aid in identification, a series of sheets showing outlines of the animal types normally encountered will be available to you. You will be expected to make a tally of the various animals in your sample according to size (length). A piece of millimeter graph paper will be provided for this task. Finally, with these numbers of animals sorted according to length, construct a "number pyramid" of your sample. Carefully read the supplementary instructions which follow.

Hand in the completed tally sheet, with the number pyramid on the back. On a separate sheet, each member of the pair should, independently, answer the questions posed and compose a one- or two-paragraph statement on your reaction to or interpretation of the number and kinds of animals found in your sample. Such things as the role played by various groups in the environment sampled, the relationship between size and number of organisms, and energy flow might be discussed.

Materials

Dissecting microscope (per two students)
Litter-soil invertebrates, sample in plastic petri dish
Millimeter graph paper, 5 cm × 10 cm piece
Forceps
Dissecting needle
Litter-soil invertebrates tally sheet
Representative litter-soil invertebrates, collection of drawings
Berlese funnels (on demonstration)

Supplementary Instructions

(Please read before coming to the laboratory)

In this relatively simple exercise you are faced with several challenges. First, you need to be able to follow directions and organize your efforts as efficiently as possible or else you will not finish your task in the time allotted. Second, your powers of observation and interpretation may be taxed. Not only are you faced with identification, that is, recognition, of a lot of strange animal groups, you also are asked to make decisions about size categories and observations on roles that some of the major groups play in this litter-soil environment.

When you first begin viewing your sample in the petri dish it is suggested that you scan the entire dish with the purpose of identifying the major (dominant) groups. Learn to distinguish between mites (#7), collembola (#13), and psocids (#21). Protura (#14) and Thysanura (#15) look somewhat alike. Check the outlines of numbers 9, 10, 11, and 12 for similarities. Utilize the distinguishing characters mentioned on the tally sheet and on the "Representatives" collection of diagrams. Do not hesitate to ask for assistance in both how to proceed and animal identifications.

When you first begin your tally, it is suggested that you concentrate on the larger specimens: earthworms, millipedes, centipedes, larger spiders, beetles, ants, etc. Using forceps simply remove

them, as counted, to the lid of the petri dish. Return them to the main sample when the tallying task is completed. Add enough alcohol to the lid so these specimens do not dry out.

Each sample utilized in this exercise has been inspected, but not necessarily tallied completely. It is known, therefore, which of the less common groups (for example pseudoscorpions, #6; Symphyla, #11; Pauropoda, #12; Protura, #14) are present in your sample. This is done so as to better evaluate how observant you are in your inspection and tally of the sample.

The petri dish bottoms containing your sample have been ruled with parallel line scratches in the plastic for the purpose of providing you with guide lines so you may keep track of portions of the sample that have been tallied. It is suggested that you begin at the top-most tier and proceed from side to side toward the bottom of the dish, much as you would mow a circular lawn by starting at one place on the perimeter and cutting back and forth until you reached the opposite side. You will need to decide how (when) to count those animals that touch a line.

To measure organisms and thereby place them in the size categories indicated on the tally sheet, place the piece of millimeter graph paper beneath the petri dish. The length recorded should be the maximum (that is, stretched out) length of the animal.

When handling the sample in the petri dish, that is, moving it from laboratory table surface to microscope stage and back, be sure to carry it horizontally. Spilled fluid almost certainly will carry animals out of the sample.

The most difficult identification decisions to make will probably involve the two most numerous groups: mites (eight legs, except certain immature forms with six legs) and collembola (six legs, and usually a “spring” tail or furcula). There are many different body forms of mites and several different body forms of collembola. Refer to numbers 7 and 13 on the “Representatives” sheets.

Usually there are collembola and mites that are hydrophobic, that is, are not wetted by the 70% alcohol preservative. In other words, they float. Look for them on the surface and be sure to include them in your tally and numbers pyramid.

Insect larvae are not always easy to identify to the ordinal level. If you cannot decide with some confidence on the correct Order, then assign the animal in question to item #26 on the tally sheet, simply “Larvae”.

Your sample probably will have a few to many animals whose body lengths are so short as to be near the limits of visibility on your microscope. Be sure to inspect the sample early in your tallying operation, under the maximum magnification of your binocular microscope, so that you become aware of these small forms.

If the sample you are inspecting has inorganic particles and/or organic bits of leaves, twigs, feces, etc., be sure to sort through this material looking for animals. Also, observe these bits, while under magnification, to get a close-up look at the environment from which the animals in your sample were collected. These observations can be drawn upon when you compose the one- to two-paragraph assignment on your impressions.

As you work with your sample the alcohol preservative level may decrease to the point where some specimens are not completely covered. If this becomes the case, request additional preservative from a laboratory assistant. Details on animals not completely immersed cannot be observed as well as those that are covered.

Litter-Soil Invertebrates Tally Sheet

Student name(s):
Date and lab number: Sample number/label:

Phylum Class (Identifying Characteristics) Order/Family	Length of body (in mm)					
	< 1	1–5	6–10	11–15	> 15	Total
1. Nematoda (unsegmented, pointed ends)						
Annelida						
2. Enchytraeidae (white, pot worms)						
Lumbricidae (red earth worms)						
3. Mollusca, Gastropoda (snails, slugs)						
Arthropoda (jointed legs)						
Arachnida (4 pair of legs)						
4. Araneae = (Arenida) (spiders)						
5. Opiliones = (Phalangida) (harvestmen)						
6. Pseudoscorpionida						
7. Acarina (mites)						
Crustacea						
8. Isopoda (sow bugs, pill bugs)						
9. Chilopoda (centipedes) (1 pr legs/segment)						
10. Diplopoda (millipedes) (2 pr legs/segment)						
11. Symphyla (10–12 pr legs, segmented antenna)						
12. Pauropoda (branched antenna, 9 pr legs)						
Insecta = (Hexapoda) (3 pr of legs)						
13. Collembola (springtails) (furcula)						
14. Protura (0.6–1.5 mm, no eyes or antennae)						
15. Thysanura (Diplura) (2–3 caudal filaments)						
16. Diptera (flies) (1 pr of wings)						
17. Coleoptera (beetles) (elytra)						
18. Hemiptera (true bugs) (triangle area)						
19. Homoptera (aphids, leafhoppers)						
20. Hymenoptera (bees, wasps, ants)						
21. Psocoptera (plant, book lice)						
22. Dermaptera (earwigs)						
23. Lepidoptera (moths, butterflies)						
24. Orthoptera (grasshoppers, crickets)						
25. Thysanoptera (thrips)						
26. Larvae						
27. Other						
Total number of organisms						

* The numbers 1 to 25 (above) refer to the groups that will be similarly numbered on the diagrams of representative soil-litter invertebrates.

Notes for the Instructor

Preliminary Preparations

Berlese funnel set-ups range in expense from elaborate custom-built multiple units, with provision for control of room and funnel temperature and rheostatic control of wattage output in each funnel, to make-shift funnels fashioned from cardboard or aluminum foil and light from a table lamp. For the funnel to successfully extract animals from the sample the three gradients mentioned in the Student Outline (light-dark, warm-cool, humid-dry) must be produced. The time and expense one expends to invest in fabricating the apparatus to produce these gradients is up to the instructor. Intermediate in expense and effort involved is the set of four funnels I use (Figure 4.1). This set-up is portable and may be dismantled and stored compactly. Six such sets will accommodate a class of 24 students so that each group has the opportunity to do all steps in the analysis: obtain sample, process it in the funnel, and analyze it via microscopic examination. Since 3–5 days are needed for processing, the sample is obtained during part of one laboratory period and analysis is completed in the second period.

Attention may be drawn to several aspects of design and construction. The funnel can be of any material but the internal surface should be smooth. It is better if it is painted black for purposes of facilitating the light-dark gradient and sealing the surface for handling the moisture of the sample. Three tabs positioned approximately two-thirds the distance from the small to the large end of the funnel provide support for a removable shelf of hardware cloth for supporting the sample. Use a 1/4" mesh hardware cloth disc and add on smaller meshes if the sample particles are particularly fine (see Figure 4.1). A small mesh size may limit the passage of larger animals; to accommodate this and a fine sample, use the 1/4" mesh disc and add smaller area discs or squares of finer mesh. Pile the fine material on these areas. Always leave at least one small area of larger mesh uncovered by sample so larger, more ambulatory animals like beetles and spiders can find a way downward.

The funnels in Figure 4.1 were made of galvanized metal by a local sheet-metal fabricator. The pattern and size for this funnel are seen in Figure 4.2 in which the squares are 1" on a side. The larger, upper opening of the funnel is 11 1/2" in diameter and supports the light-reflector unit whose diameter is 12 1/2". The lower opening is 1" and fits conveniently into a number of styles of collecting bottles. Overlap along the straight margins of the funnel is 1" and is riveted to make a tight seam.

The socket holding the 25- or 40-watt light bulb should have its own on-off switch for convenience in control of a set of funnels. The light-reflector unit may be composed of a socket with threads that fit into the reflector or into a circular bracket which attaches to the reflector via three adjustable thumbscrews (Figure 4.1). The latter is slightly preferred because it allows greater flexibility in bulb wattage by providing a small heat and air vent space in the bracket.

The frame support for the funnels shown in Figure 4.1 is of 1/2" plywood and 1" x 1" wood strips; the top and bottom shelves have wood strips attached at three sides by nails. The two end panels attach to the shelves by screws into the wood strips. Removal of the screws disengages the four pieces of the frame and allows compact storage. The end panels measure 28" × 16". The shelves are 16" × 50 1/2". Holes for the funnels in the top shelf are 9 3/4" in diameter. The centers for the four holes are located along a line 8" from the front edge and successively 6 1/2", 19", 31 1/2", and 44" from one end. The vertical stability of the funnels is increased if the holes in the top shelf are cut with a bevel which approximates the angle of the funnel which it receives. Stability of the entire frame is greatly enhanced by the attachment, with screws, of a plywood panel, 16" × 18" to the back margin of the two shelves. The bottom shelf is attached to the end panels so that the upper surface is 10 1/2" above the lower border of the panels.

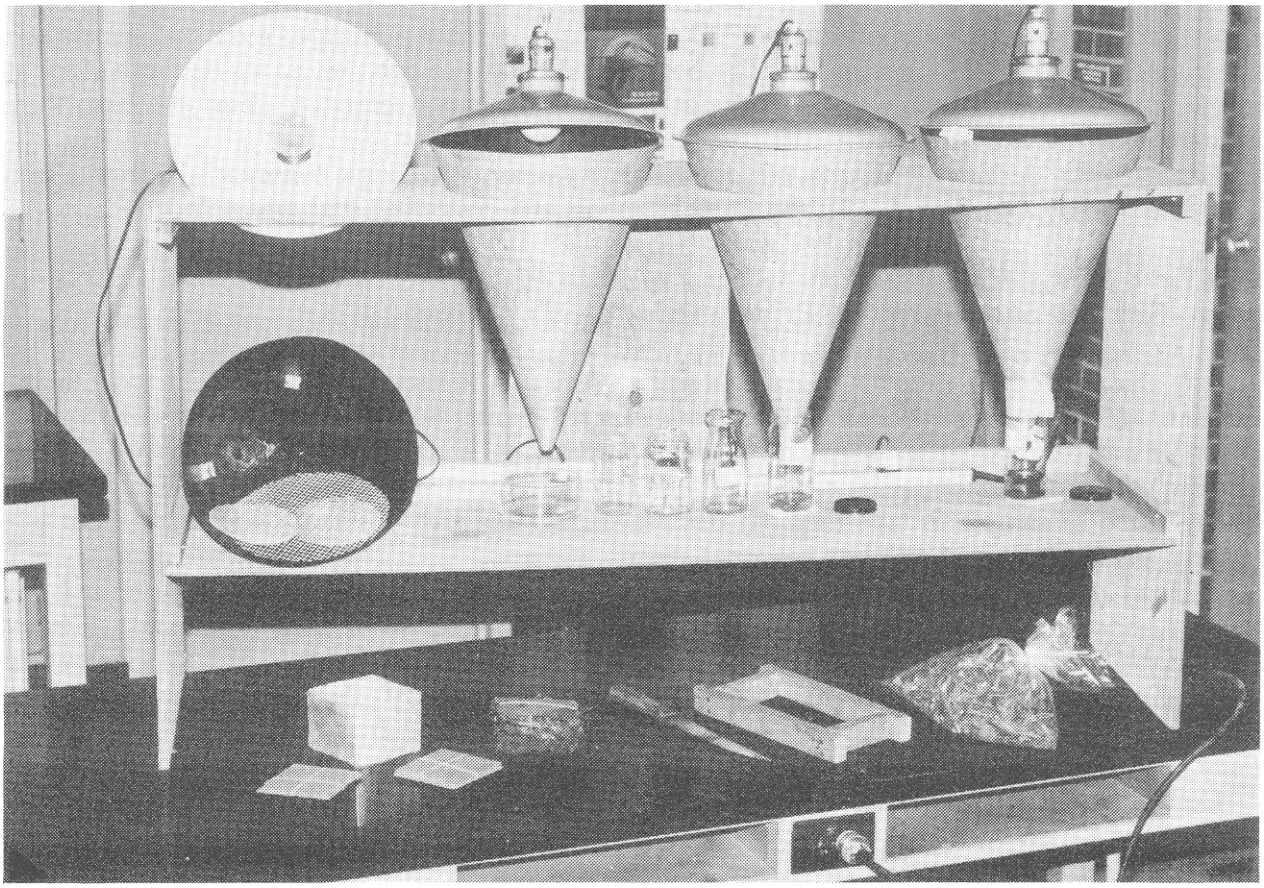

Figure 4.1. Set of four Berlese funnels illustrating the Berlese Method of extraction of invertebrates from litter and soil samples. Other equipment are also shown. See text for details.

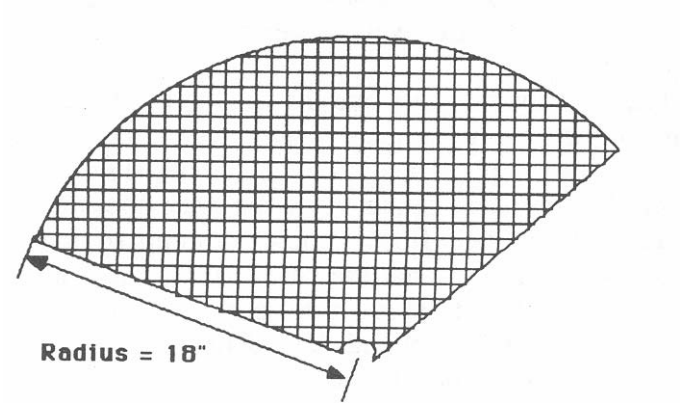

Figure 4.2. Pattern for Berlese funnels seen in Figure 4.1.

The four-funnel units just described may be stacked one above the other for use and in storage; to do so, add a wood strip approximately 16" × 4" × 1/2" to the top outside of the end panels so the strip extends 2" above the upper edge. The bottom end panels of one unit fit inside these strips atop the end panels of the bottom unit. A four-outlet power bar is convenient to distribute electricity to the four light-reflector units.

A satisfactory Berlese funnel unit, minus the light-reflector, is available commercially from Carolina Biological Supply Co. (#65-4148); the funnel support and collecting vial are included.

Sample Site and Sample-Taking Procedure

Any site with organic matter covering a soil surface can be sampled. Those with accumulations of organic debris and a minimum of disturbances yield greater numbers and diversity of organisms than barer or disturbed sites. The sod of fence rows, lawns, and pastures are productive. Even the very dry, sparse material beneath cacti in deserts have produced an impressive assemblage of forms. The best kind of sample to take for both Berlese sorting and live observation is forest floor where the litter accumulates and exhibits seasonal stratification.

Somewhat surprisingly, sample taking is not limited to any particular season of the year. In general, moisture and warm temperatures promote growth and reproduction of litter organisms. However, amazingly high populations and level of activity continue into the fall and cold of winter in temperate zones. If there is a snow cover that falls before hard freezes occur the litter temperature remains at or above freezing and organisms continue activity. Sometimes the richest samples are found in winter.

Collecting the sample is easy: (1) Gather what is desired into a plastic bag, transport to the funnel unit, and place it on the hardware-cloth floor of the funnel. (2) Turn on the light, place the collection bottle containing alcohol under the funnel and you are in business.

Several considerations about the make-up of the sample may be helpful, however. Funnel extraction works best on organisms that are ambulatory and are not extremely dependent on water. Most nematodes and other small soil invertebrates are simply not able to make the trek from their location in the sample down the funnel wall and into the alcohol of the collecting bottle. Except for nematodes, most other types of invertebrates characteristic of litter and the upper soil-litter interface will be observable in the Berlese funnel extraction sample. Even mites and collembola shorter than 1 mm in length are represented. A detailed discussion of funnel extraction efficiency, sample handling techniques, and collection biases applied to specific groups of invertebrates may be found in Tamura (1976).

The kind of sample I recommend for study is a quantitative one that includes all the litter and only the soil that is mixed with decaying organic matter in the litter-soil interface. Use a wood frame (see Figure 4.1) to define a certain surface area, for example, 10 cm × 20 cm. Using a serrated knife, cut through the litter and into the soil along this frame in much the same way one would cut a rectangular piece out of the center of a sheet cake. Using your fingers and a minimum of disturbance, remove the sample to a plastic bag. Place soil crumbs, etc., that are part of the sample in the bag. Enclose a label with the sample, for identification, and tie to prevent desiccation. The sample may be stored in a refrigerator or even at room temperature for several days if necessary.

When pouring the sample onto the hardware cloth of the funnel, fine particles invariably fall through the hardware cloth. Use a finger bowl or other suitable container under the small opening of the funnel to collect that material. Place another container under the funnel and return this fallen-through material to the sample. Repeat this procedure. Make sure part of the sample has not lodged just above the lower orifice of the funnel. When all the sample is perched on the hardware cloth, rap the funnel lightly to dislodge pieces just ready to fall and return them to the

rest of the sample. This is done to help insure a relatively clean sample in the alcohol. As processing progresses, drying occurs and bits of soil drop into the alcohol. All of this droppage obscures organisms during microscopic inspection and is undesirable, but often unavoidable. In the process of spreading the sample on the hardware cloth try to leave a small area of screen uncovered so large invertebrates have an access through the mesh. For the first day or so of processing, the light-reflector unit can be perched somewhat ajar on the funnel to prevent overheating of organisms in the upper portion of the sample in the funnel (note the right-most funnel in Figure 4.1).

The container to receive the animal sample should have alcohol in an amount that can be contained in the bottom of a petri dish, about 40 ml. A larger volume does not allow for transferring the entire sample to one-half a petri dish for examination; a lesser volume frequently is too little considering that evaporation occurs at a rate not easily anticipated. If the litter-soil sample is generally moist to dry, 70% alcohol in the collecting bottle is the preferred concentration. When the sample is wet to saturated, much of that sample water finds its way into the alcohol, causing dilution. That dilution sometimes is so great the organisms do not preserve adequately. To help counter this dilution effect, begin with 95% alcohol instead of 70% in the collecting bottle.

Eight-ounce round, large mouth, screw-cap specimen jars are conveniently-sized containers for receiving the sample (see Figure 4.1). To prevent the occasional escape of agile organisms, a strip of masking tape may be put around the junction between the mouth of the jar and the funnel. Do not seal the masking tape on these surfaces with firm pressure because the tugging that is necessary to remove it may cause a lot of unwanted dirt and fine particles to fall into the sample. If the funnel does not extend tightly down into the mouth of the specimen jar, the latter can be easily propped up by a piece of foam rubber underneath it. See the two funnels with specimen jars in place in Figure 4.1.

If time is limited in the laboratory period for analysis of the sample the instructor or an assistant may, beforehand, transfer the sample from the specimen jar to the one-half petri dish. One should be aware that many mites and most collembola are hydrophobic and, characteristically, float on the surface of the alcohol. Therefore, the sample needs to be transferred carefully. Swirl the alcohol and contents gently and pour very deliberately into the petri dish bottom. A petri dish measuring 100 mm × 15 mm will provide adequate capacity for the sample and also has sufficiently low vertical clearance on the microscope stage.

Since floating organisms typically adhere to sides of the specimen jar, it is desirable to use a medicine dropper or small syringe to rinse the jar walls. Use alcohol from the sample (now in the petri dish) for rinsing. Rinse several times in such a manner that all organisms are transferred. Remove alcohol for rinsing from an area of the sample where few organisms occur to cut down on their transfer back to the specimen jar. If the quantitative nature of the exercise is not important not as much attention needs to be given to this detail.

The petri dish bottom containing the sample will be one-half to two-thirds full of alcohol and specimens and must be moved carefully to avoid spillage. Any alcohol spilled must be assumed to carry organisms out of the sample because of those that are floating.

Analysis of the Sample

Analysis of the sample is to be done by two students working together. This saves time and requires fewer microscopes and samples than if students work independently. Students working in pairs in an introductory course probably learn more in this exercise than they would if working separately. In upper-division courses, students would be better served if they worked

independently. They should collect, process, and handle their own sample and follow through in the analysis of it.

Students should be advised to read all instructions carefully. In order for them to do everything correctly they will have many details to observe. The critical laboratory instructor can use this exercise to observe the students' abilities to (1) follow directions, (2) exercise care in carrying out procedures, (3) be orderly and efficient, (4) complete a complex task, (5) understand processes involved, (6) make correct decisions, and (6) observe major and minor details.

If the sample to be analyzed by the students is already in the petri dish, the instructor, beforehand, can have manipulated the sample contents to any extent desired. I do just what the student directions indicate, that is, inspect the sample and make a checklist of the presence of some of the more obscure groups. I add two or three specimens of certain groups. During the laboratory period I have this partial inventory available so I can easily check the accuracy of tallies by the student pair before they complete the exercise and leave the laboratory. Students somehow find it easier to view the completion of the task more seriously if they know that you know how well they are doing.

Samples that are used repeatedly in courses with many sections eventually begin to show the signs of wear and tear. Hard, brittle specimens such as millipedes break into segments, soft ones such as annelid worms become punctured and malformed and the preserving fluid becomes cloudy. It is wise to have replacement specimens ready and make substitutions at appropriate times. Often, the fluid can be carefully pipetted away from the sample and replaced with fresh 70% ethanol. To be sure that small and rare forms are not inadvertently lost in fluid exchange, cover the intake port of a 10-ml syringe with filter paper and slowly aspirate fluid into the syringe through the filter. Should the filter become plugged, gently back flush and change to a new filter area. One can always check for the loss of specimens in both the pipetted fluid and the filter paper surface by microscopic examination. Extra samples should always be available because accidental spillage and droppage are to be expected by students, though not accepted too graciously by the instructor.

When the student has a sample handed to him or her, the activities and expectations which ensue have all the trappings of an exercise. This does not detract from the utility of the experience to the student, especially at an introductory level: directions are to be followed, data-taking is performed, and certain observations are expected. However, for the student to have a fuller conceptualization of what is happening, the student needs to participate in the entire process. This means that the student goes to the habitat, selects the exact site, takes the sample, loads it into the funnel, and follows through all the steps in preparation up to the juncture of placing the sample on the microscope stage, as well as the following steps in analysis already described in the Materials section. This is a much more fleshed out “trip” than beginning with only the prepared, manipulated, and recorded sample. Both scenarios have their place. The more complete one adds an aspect of three-dimensionality and wholeness and should be used in upper-division classes.

How does an instructor do the extended scenario with a class of 20 students? Does this mean 20 Berlese funnel units are needed? I have accommodated this situation in two ways. Before I had a set of 24 units I divided the class into two groups and had one group work with litter and the other perform a different task. Then the groups switched tasks. Since the sample-taking and funnel-processing precedes sample analysis by a few days, two laboratory periods are involved. The group doing the different task worked at a dissection, identified a taxonomic group of organisms, or participated in other activities that required only limited, direct supervision. It is desirable to have the number of funnel units available exceed the number of students or samples needed. If different students take samples from a variety of sites, it is likely that some sites will yield an assemblage of organisms low in either numbers or diversity or the student may make some mistake in processing. The instructor easily accommodates such unforeseeable circumstances by processing one to three samples which can be used as substitutes for nonusable

ones taken by students. In this way an aspect of the unity of the activity is preserved, that is, the entire class is familiar with the location of the collection site. Data derived in this manner may have an increased degree of reliability.

The litter found in mesic deciduous and conifer forests is frequently deep enough to exhibit stratification caused by seasonal leaf fall. An interesting variation on Berlese sorting is to collect samples by horizontal strata rather than by the entire vertical profile. In temperate regions litter can be separated conveniently into three or four zones: (1) the upper or litter zone characterized by relatively whole, undecomposed leaves and organic debris dropped from the vegetation above ground; (2) the fermentation or fenestration zone in which fungi, bacteria, and organisms have begun digesting the softer parts of leaves, etc.; (3) the humus zone where leaves have lost their identity as leaves and organic matter appears crumbly from passing through the intestinal tracts of various invertebrates; and (4) the litter-soil interface created from the feeding and vertical movements of earthworms, insect larvae, and other larger invertebrates. Processing by stratum reveals another facet about the biology and function of the constituent organisms of a composite sample. Comparison of sites under experimental manipulation or environmental stress, such as testing effects of automobile exhaust on litter fauna (Giesy and Edgar, 1970), can be enhanced by stratal analysis.

The Living "Litter Sandwich"

An exciting companion activity to the quantitative Berlese extraction analysis is the microscopic inspection of an undisturbed chunk of that same litter-soil environment. At the time and place the Berlese sample is taken, retrieve a "piece" 10 cm × 10 cm that is specially cut and packaged for transport to the laboratory and microscopic examination. In a 3-hour laboratory period a class can travel to a nearby site (10-mile radius), collect both samples, return, place 10 cm × 20 cm samples in Berlese units, and still have an hour to observe the 10 cm × 10 cm sample in its fresh, live condition.

In the undisturbed sample many things can be observed; what is assigned and expected by the instructor needs to be gauged to the capability and background of the students. One needs only to read briefly in a number of sources to realize the aspects of biology and ecology exhibited by such a sample (Jackson and Raw, 1966; Mattson, 1976; Mason, 1977; Schaller, 1968; Swift et al., 1979). If the sample is well-stratified and undisturbed all the stages in humification can be observed in a continuum. One begins with the uppermost pieces and, by removal with forceps under suitable magnification and illumination, moves downward in space and, at the same time, backward in age of plant material. An uncompressed sample shows the nature of the material, the galleries and tunnels available to organisms, the colonies of bacteria and fungi at work and the resident invertebrates at home. If care is taken to prevent loss of water films on surfaces, one can appreciate and almost personally invade the habitat of the essentially aquatic inhabitants in litter; nematodes, in particular, can be seen. One of the most dramatic realizations is the increasing presence and eventual dominance of animal feces as one passes from the fermentation layer to the humus layer. Ultimately, humus is finely comminuted plant material that has passed, repeatedly, through the digestive tracts of invertebrates and remains shaped in configurations characteristic of the packager. Scatological studies, in miniature, may be made.

I use a metal (aluminum) frame, 10 cm × 10 cm by about 6 cm in height, to obtain this sample (see Figure 4.1). Place the frame on the site, after having first carefully palpated that surface area to assure that you probably will not be trying to cut through a submerged branch or rock, cut around the border of the frame with a long sharp knife, and then slip the frame downward into the cut. On the outside, clear away the litter from one of the sides of the frame. Locate the lower aspect of the litter soil interface with your fingers. After having defined this horizontal level, and

with the frame still unmoved, place a cardboard square, approximately 9 cm × 9 cm, at that location. Lift the sample upward, while holding the sample in the frame, resting on the cardboard. Place another cardboard square on the upper surface of the sample and carefully move the sample out of the frame and secure the cardboards in place with two rubber bands, all the while trying to keep the sample oriented more or less horizontally. Place the sample in a plastic bag and tie so the bag provides some support for the now exposed cut sides of the sample. If the sample is crumbly put rubber bands around the outside of the bag so as to provide support to the integrity of the sample organization. Now it can be carried in a knapsack or large pocket back to the laboratory. Care should be taken not to compress the sample or allow desiccation.

To examine, place the sample on a paper towel on the stage of a dissecting microscope (Figure 4.3). Add adequate illumination and begin at the litter surface. Depending on the assignment and information which the student has in hand, work down through the sample. Observe the following: changes in color; condition of surfaces of organic matter (intact and skeletonized leaves and fragments); evidence of bacterial and fungal activity; presence of water films and dew drops; kinds, numbers, and sizes of invertebrates; change in space size and configuration with change in stratum; and evidences of humification. Try to fill in the gaps as to what has happened over time to cause the lower, older layers to assume their present condition. Where do sand grains appear? What does this indicate? Have roots invaded any strata and, if so, what does this mean?

When the examination is completed the sample will probably be in shreds and needs to be discarded. Care should be taken to protect the microscope from dirt and dust. Depending on the instructions given, invertebrates may or may not have been listed, tallied, and/or preserved. I recommend retrieving representative forms and preserving them into 70% alcohol. Among other things, the student gains some impression of behavior and reaction to pursuit by the specimens and an appreciation of the difference in appearance between the living and dead condition. This is helpful when the quantitative sample is closely examined. It allows a better visual comparison between those forms observed alive and those extracted in the funnel. The number and kinds seen in the live 10 cm × 10 cm sample will be conspicuously fewer than one-half those extracted in the 10 cm × 20 cm sample. Why? Why the difference in the number and kinds in each sample?

The student is able to demonstrate powers of observation and knowledge in a final assignment that may be phrased something like, “Describe what you observed in the live sample.” A more difficult task might be the construction of a detritovore food web or the assignment of a relationship between external morphology and role of the animal in the habitat.

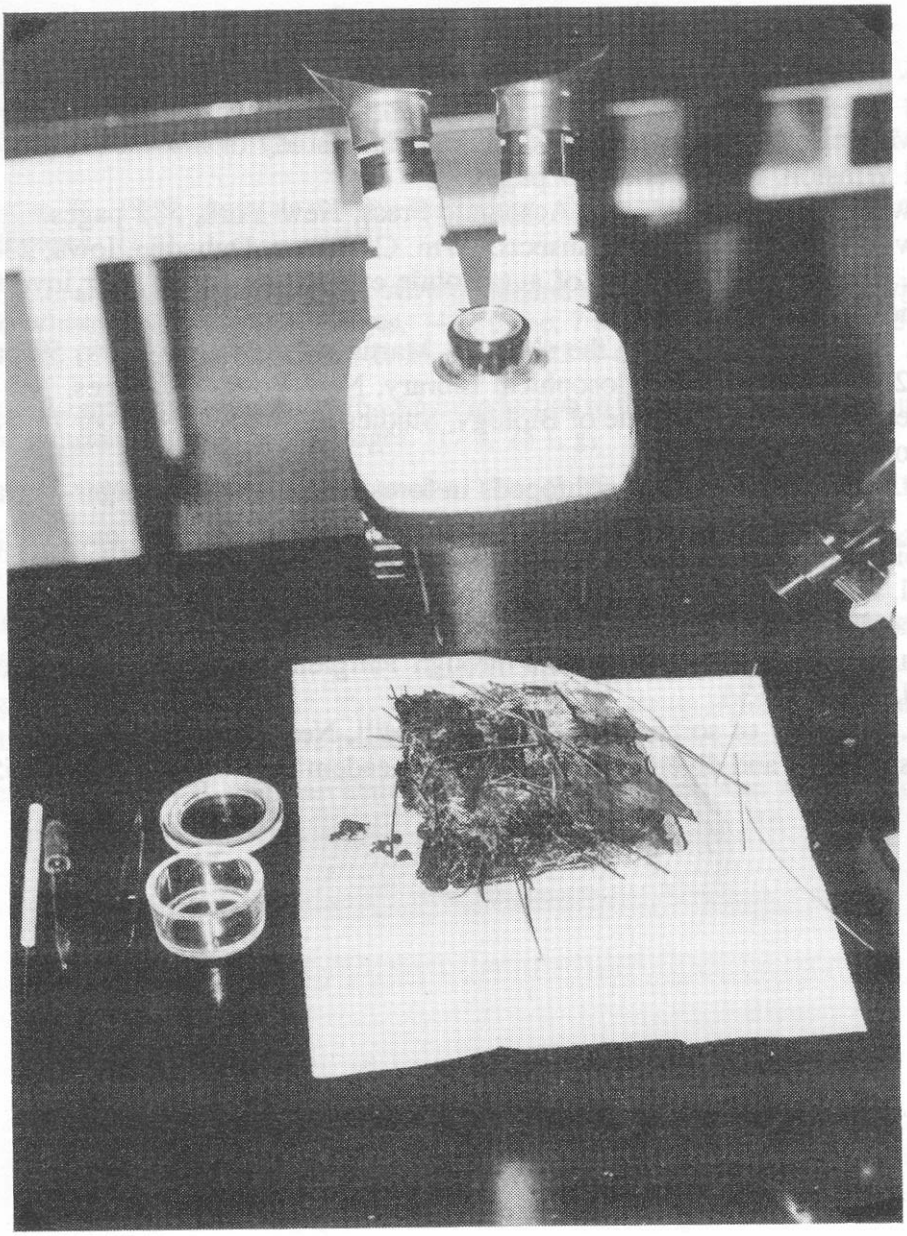

Figure 4.3. Stratified “litter sandwich” on the stage of a dissecting microscope.

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APPENDIX A
Representative Litter-Soil Invertebrates

Diagrams of representative litter-soil invertebrates (indicated in brackets) to be used by students in the laboratory can be obtained from the following sources:

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Borrer, D. J., and D. M. Delong. 1964. An introduction to the study of insects. Revised edition. Holt, Rinehart and Winston, New York, 819 pages. [4 h; 6 a–c; 7 a–d, q; 8 d; 11 a; 12 b; 13 a–f; 14 d; 15 a–c; 16 a–d; 17 a–d; 18 h, i; 19 a, b, k; 20 a–c; 21 a–j; 22; 23 a–d; 24 e, f; 25 a–e]

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