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Chapter 24

Arthropods

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All literature reviews on the subject of soil arthropods basically come to the same generalizable conclusions: (i) soil is a relatively difficult medium from which to extract arthropods; (ii) the efficiency of any one extraction method varies between common soil types, because they differ significantly in chemical composition and microstructure; (iii) attempts to quantify *absolute* census counts or resident biomass for any volume of soil are unlikely to be profitable, due to biases in the extraction efficiency for each individual species (which varies by season, soil type, and horizon) which are prohibitively time-consuming to attempt to quantify; (iv) taxonomic identification is frequently not possible to specific precision, and even in the rare event when possible, the correct name often does not access ecological data sufficient to unambiguously assign it a defined functional role(s) in the soil being studied. If you are experienced in soil arthropod studies, you already are aware of these difficulties; if not, it's important to list them up front. Soil fauna is challenging to study, but is very rewarding when the complexity of the synergistic interactions between the fauna and the microbes are revealed.

Soil arthropods function in soil ecosystems in numerous ways: chemical transformation; structural architecture; mixing and transport. The importance of arthropods is not expressed in terms of percent of community respiration; arthropod contribution is usually $<< 10\%$. Arthropods, however, are now recognized as the catalytic regulators of microbial activity (reviews: Crossley, 1977; Seastedt, 1984; Visser, 1985; Fitter et al., 1985; Edwards et al., 1988; Shaw et al., 1991). In many ways the beneficial impact of arthropods is correlated with their physical activity, for instance shredding litter, burrowing in coarse woody debris, aeration of the soil and transport of inocula. Many studies evaluate the effect of arthropods upon a biological or physical process, such as mineralization or leaching. However, Anderson et al. (1985) have shown that the most critical effects of arthropods may be in mediating chemical transformations in the soil that are usually not directly monitored. Arthropods affect soil microbial

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communities as the direct result of feeding (or being fed upon by other predaceous arthropods). These effects are likely to be density dependent, with normal population levels stimulating microbial activity and plant growth and either decreased or epidemic population counts depressing growth response (Finlay, 1985). Again, it is the interplay of the arthropods on the microbes, as monitored ultimately by plant growth, that is often of most interest.

24–1 PRINCIPLES

Basically arthropods can be extracted from a sample of soil either through physical methods or behavior modification. Physical methods are generally labor-intensive and work best on soils with low organic matter content; meso- (0.5–2.5 cm) and macro-arthropods (> 2.5 cm) are most efficiently extracted. Inducing the biota to leave the soil on their own is labor-frugal and works best on soils with high organic matter content; microarthropods are efficiently extracted as well as larger taxa. There is no a priori best solution to the question of how to extract the biota. I would strongly suggest preliminary experimentation with the substrate in question prior to implementation of a research design. The efficiency of extraction varies for each species (in unpredictable ways) depending upon the physical and chemical characteristics of the medium. Obviously, if the research requires an accurate measure of immobile stages (i.e., eggs, pupae, and cysts), then a physical means must be utilized.

Since no one claims that any method is 100% efficient, there is no means to assess *absolute* efficiency. Several workers have compared the relative efficiencies of different methods on specific organisms in specific soil types. Some researchers have released a known number of specific organisms into the soil and then recovered them. Efficiency studies are summarized by Edwards and Fletcher (1970, 1971) and repeated largely unchanged in Edwards (1990). Universal advice is to experiment with different types of extraction techniques relevant to your particular taxon of interest, and with the range of soil types in the study area.

24–2 METHODS

24–2.1 Evaluation of Biota in the Field

24–2.1.1 Destructive Sampling of Soil for Biota

There are no universally applicable techniques that yield robust estimates of soil macroarthropod populations. Large arthropods (and earthworms) are, almost by definition, not present in densities that can be adequately sampled by typical coring devices. The usual method is to designate specific areas (perhaps 1 m^2), place a large light-colored sheet (or

denim) on the ground surface adjacent, and progressively use a spade to excavate the sample site. The contents of the spade can either be placed directly on the sheet and gingerly broken up by hand, or can be run through an archaeological sieve first. Field assistants search the substrate for the specific pest species, beneficial predator or worm in question.

24–2.1.1 Limitations

Prior knowledge of the range of depths that the target species inhabits is necessary; sample depth of more than a meter is required for worms under many conditions. This technique is very labor intensive, highly subject to individual bias, and efficient only for the largest most mobile taxa. It is seldom used to quantify more than one type of macroinvertebrate at a time, since search image efficiency becomes limiting.

A hand sorting variant of this method to characterize the community in forests with a deep litter layer is to sample a relatively large area (0.5–1.0 m^2), place it in a plastic bag, return to the laboratory and expose it in a shallow light-colored tub. A technician can pick through it thoroughly by eye aided by a large suspended magnifying lens; efficiency is probably directly dependent upon the activity of the species. Intermittent misting of the sample with formaldehyde (take prudent safety precautions) from an atomizer increases specimen visibility markedly, since the quiescent fauna are stimulated to activity.

24–2.1.2 Pitfall Trapping

The most widely used method for determining the relative abundances of larger arthropods is pitfall trapping (Greenslade, 1964; Southwood, 1978; Franke et al., 1988; Doube & Giller, 1990). A container (with or without preservatives) is sunk into the ground to a depth placing the lip at the surface of the soil (Fig. 24–1). In theory, epigeic species walking across the ground happen upon the traps by accident, fall in, and can't climb back out. As such, this trapping method measures relative activity of resident taxa (NOT density), draws captives from differing amounts of surrounding territory (dependent upon individual species mobilities) and is correlated with species-specific motor skills. As a passive collecting device it has no equal for simplicity and inexpensiveness; interpretation of the quantitative results is difficult (Gist & Crossley, 1973; Price & Shepard, 1980). Efficiency can be greatly increased in some habitats by erecting radially arranged barriers (1–2 m long) extending outwards from the trap that induce some of the fauna that encounter the barrier to funnel toward the trap along the wall surface.

However, the basic nature of the trap is seldom passive. Invertebrates are usually specifically attracted to the trap by the odor of the preservative or the odors emanating from the cut roots and fungal hyphae at the site of insertion. Trap catches are usually most numerous on the first several days of sampling, due to the freshness of the disturbance. All sorts of baits (e.g., meat, fruit, dung, and fungi) can be added to the traps to increase the

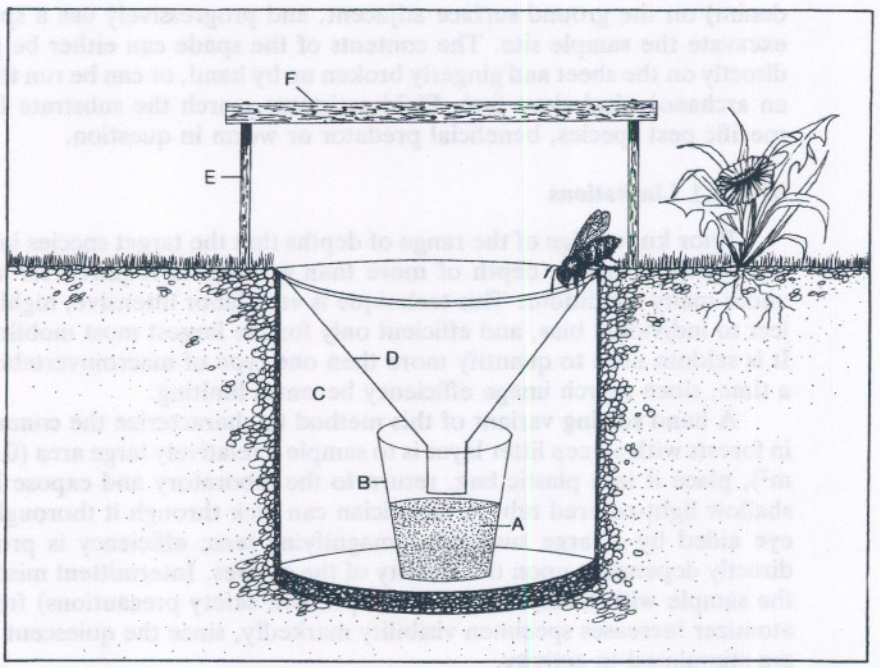

Fig. 24–1. Pitfall trap. Epigeic species will enter trap space under the rain roof (F), walk or slip down the funnel (D) and be preserved in the polypropylene or ethylene glycol (antifreeze = A) contained in the 6 to 8 oz. plastic cup (B). The entire trapping apparatus is housed in a 1 gallon plastic can (C), sunk into the ground so that its top is flush with the ground surface. The soil immediately adjacent to the gallon can (C) must be firmly compacted to prohibit subsidence and maximize accessibility to the arthropod fauna. The rain-roof (F) can be made of composition-board or aluminum sheeting—the former is easily supported by ten-penny nails (E), the latter requires reflexing the corners up over the nail heads. The trap may preserve a variety of arthropod types, including winged species that are either truly soil-associated (e.g., soil-nesting bee, as pictured) or simply attracted to scents emanating from the fluid. Traps can be run for specific portions of a day, or continuously for several weeks.

sampling range (and the degree to which they attract flying insects). Trap design can be modified to accommodate battery-powered fraction collectors to quantify periods of maximal activity. Traps can also be sunk sequentially below the soil surface to assess differential species behavior by depth (Loreau, 1987).

24–2.1.2.1 Limitations

Three major concerns in installing a pitfall grid are:

1. Minimizing damage to the resident populations of small vertebrates (mammals, reptiles, and amphibians). To this end it is critical to install a slippery funnel with a basal diameter small enough (about 2 cm, depending upon local fauna) to exclude most vertebrates.

2. Inactivation of the preservative by rain/surface flow dilution. Rain can be excluded by a square roof made of aluminum sheeting or particle-board and suspended by nails from the corners. Surface flow is more difficult to predict; avoid concave surfaces and trench around the trap to direct the water flow.

3. Vandalism by larger vertebrates (human and non-human) is unavoidable. Animals either remove traps to drink the preservative they contain or the meals they have already caught. A statistician should be consulted *prior* to installing a grid to ensure the design will be robust if occasional samples are lost. Though it is possible to estimate absolute densities from the ratio of captures within a grid versus on the periphery, the usual use of pitfalling is in estimating differing relative abundances at different sites.

24–2.2 Sampling Soil Cores (review: Kubiena, 1938; Spence, 1985)

24–2.2.1 Passive Extraction of Biota from Soil Cores

Most samples are taken by soil coring devices familiar to soil scientists. I mention the pictured device (Fig. 24–2) since we use it frequently, and it is tied to a specific extraction method mentioned below. A double-cylinder hammer-driven core sampler developed to assay bulk density (Blake & Hartge, 1986) is relatively efficient at sampling arthropods because of its: (i) relatively wide core diameter (about 7.5 cm); and (ii) design features to minimize compaction during sampling. The inner sleeve of the corer is normally aluminum, but since it is not directly driven by the hammer it can just as easily be made of PVC plastic. Each sample within the plastic collar can be covered with flexible screening, placed directly into a plastic bag, stored in a cooler, taken to the lab and subsequently placed in a high-gradient extractor without further handling.

24–2.2.2 Faunal Distribution in Soil Cores by Microscopic Methods

Soil is a diverse medium structurally and chemically. A major limitation affecting soil fauna studies is knowledge of the microenvironments inhabited by the different species. A number of techniques have been developed (but seldom used) to quantify the structure of soils on a scale relevant to arthropods (Anderson, 1978). The methods seek to accomplish three objectives: preserve the soil micro-architecture in situ, transport the sample to the laboratory unaltered, and section it for examination under the microscope. In the field, soils can either be quick frozen with water followed by liquid nitrogen (Froelich & Miles, 1986), or embedded in agar (Haarlov & Weis-Fogh, 1953) or gelatine (Anderson & Healey, 1970). Frozen soils are embedded in the laboratory; protein embedded samples are hardened in formalin prior to sectioning.

By far the most elegant, all-purpose, permanent technique is to embed the samples in epoxy (Rusek, 1985). The limitations are that the samples must be embedded in the laboratory, cured in an oven, and sectioned by

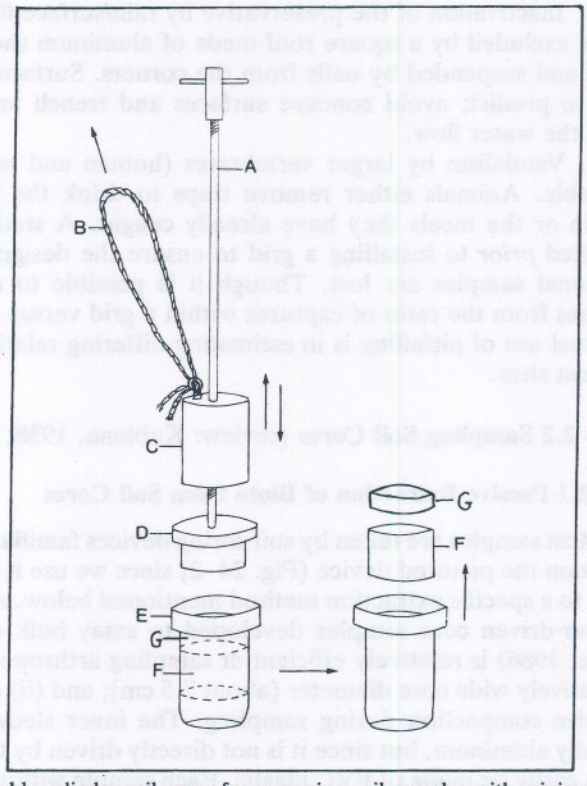

Fig. 24–2. Double-cylinder soil corer for removing soil samples with minimal compaction. The detachable core-cutter (E) is driven into the ground by the heavy weight (C) by pulling C up along the handle (A) with the rope attachment (B) and releasing it to fall onto the nested end-plate (D). When the core-cutter (E) is driven to the desired depth (bottom of wider ring flush with soil surface), the handle assembly (A–D) is removed, and the core-cutter removed from the ground. Holding the core-cutter (E) horizontally, the core itself within its sheath (rings F+G) is gently pushed out of the center and onto a plastic sheet. The lowest portion of the soil column and the uppermost portion adjacent to the nested end-plate (contained in the narrow sleeve [G]) are cut off with a pen-knife, leaving a noncompacted volumetric sample in F. A flexible screen is placed over the *top* of F and secured with a rubber band; the sample is inverted, placed in a ziplock plastic bag, and transported to the laboratory for extraction.

high-technological geological saws. (The saws must be switched from oil-lubrication to water-lubrication during the process, thus limiting access to other concurrent users of a shared facility.)

24–2.2.3 Extraction of Soil Arthropods through Behavior Modification (reviews: Kevan, 1955; Evans et al., 1961; Murphy, 1962; Phillipson, 1970; Dunger & Fiedler, 1989; Edwards, 1990)

24–2.2.3.1 Dry Funnels. The most commonly used extraction procedure for the majority of soil arthropods involves a funnel apparatus origi-

nally proposed by Berlese, and subsequently modified by many other workers (A. Tullgren [1918], most prominently). In this method the sample is placed in a metal funnel supported on a wire mesh; an environmental stimulant is applied above the sample and a bottle with a preservative fluid catches the biota as they burrow through the sample and drop through the screening.

The kinetic stimulant usually applied is either heat combined with light (electric light bulb), or a repellant chemical (formalin, tear gas). Within extreme limits, it is widely observed that extraction efficiency increases with decreasing Wattage (25–75 W) and with decreasing sample volumes. However, since the number of funnels is usually limited, longer extraction times can result in prolonged storage of samples which may produce population artifacts. Extraction efficiency is increased by alternating heating and cooling periods. If samples resting on the mesh screening are more than 10 cm in thickness, layers of the upper already-dried soil can be removed sequentially to promote extraction from the remaining substrate.

Efficiency in very thick samples is reduced, because non-even distribution of soil components permits fauna to migrate to foci of pleasing microclimate (i.e., around a large root or coarse woody debris) within the soil column, which subsequently become surrounded by inhospitable conditions forcing the fauna to perish in situ. Large sample volumes function much better for qualitative (presence/absence) determination, than for robust quantitative estimates of population density. Such qualitative studies can be enhanced by pre-filtering the sample through a mesh-size that removes larger stones, litter and roots; vigorous agitation within a double-bottomed burlap/denim bag with a screen mesh sewn in over a bottom zippered chamber, breaks up well-decayed pieces of wood in the sample (exposing the inhabitants). This preagitation technique is used in the field when the samples are being collected, significantly reducing volumes and weights to be transported and housed in coolers.

There is no standard size or shape to the funnel apparatus itself. Mesoarthropods are generally extracted in funnels averaging 30 cm in diameter and 45 cm in depth. Microarthropods are usually extracted in funnels 5 to 12.5 cm in diameter by 7.5 to 20 cm high. Although macroarthropods are successfully extracted from funnels (if the mesh size permits), their low numbers are such that funnel extraction is an inappropriate means of determining population densities. (Macroarthropods are generally estimated through a different technique—see "pitfall trapping" above).

24–2.2.3.1.1 Mesoarthropod Extractor

If used under nonextreme conditions, metal Berlese funnels can last for several decades (even with heavy use). However, start-up costs for the necessary welding and socket wiring can exceed $150/unit (Fig. 24–3). To reduce costs of fabrication, yet maintain all-metal construction, I suggest using a standard galvanized water pail (leaving at least 3–4 cm of the rim of the base to which three 5-mm diam. dowels can be welded) and

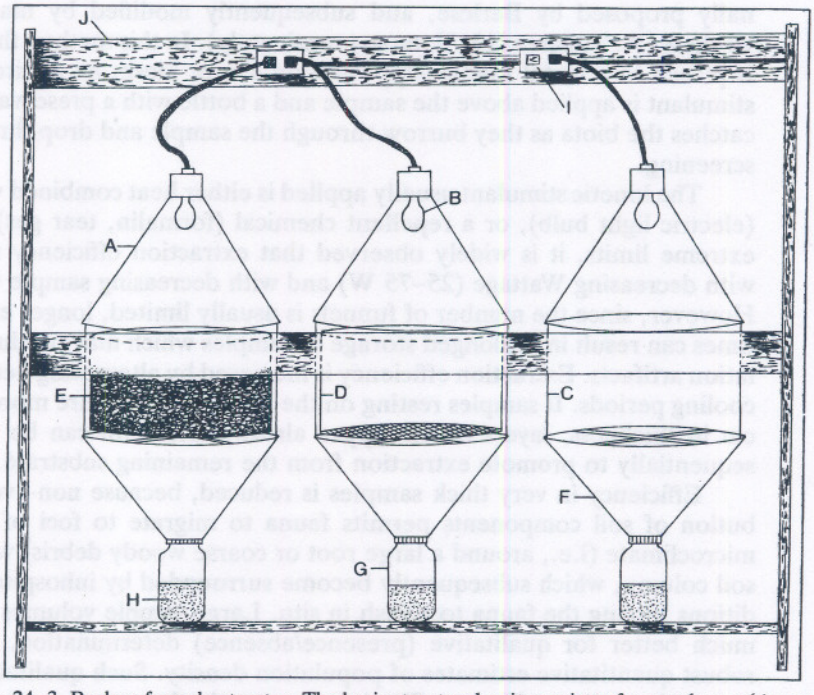

Fig. 24–3. Berlese funnel extractor. The basic structural unit consists of a metal cone (A; must be vented to release evaporated water!) with electric light bulb (B) resting upon a metal combination cylinder (C) and funnel (F). A convenient number of these metal units (i.e., 10–15) are attached to a wooden frame (J) and provided with individual electric outlets (I). The sample (E) is housed in a metal cylinder with ¼ in. mesh floor (D), supported on cross-pieces welded at the junction between C and F. The sample (E) is placed in the sample housing (D) over a dry empty collecting bottle; the throughfall soil is removed, replaced into the sample and a new collecting jar (G) with ethylene or propylene glycol as preservative (H) is placed underneath.

aluminum sheets (cut to size by shears and stapled together; used 1 by 1.6 m aluminum sheets can be purchased from the recycling department of nearly every newspaper company for a few cents each). Galvanized stove pipe with a disc of hardware mesh and a plastic funnel secured by duct tape also works efficiently. To avoid the expense of soldering (and resoldering) collecting jar tops to the base of the funnel, use a non-evaporative fixative (e.g., 50:50 antifreeze ethylene glycol/water). WARNING: DO NOT attempt to use cardboard canisters covered with aluminum foil to house the samples; it is only a matter of time before a fire occurs.

24–2.2.3.1.2 Microarthropod Extractor

Macfadyen (1953) developed a sophisticated apparatus designed to control heating and drying of the sample surface while independently controlling cooling and humidification of the sample base. There have been a number of modifications of this "high-gradient design" which significantly

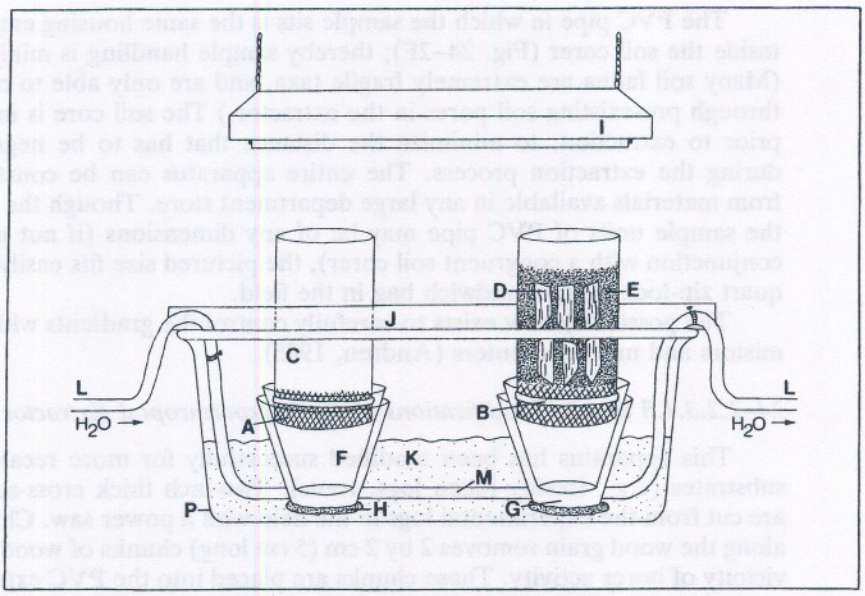

Fig. 24–4. Inexpensive high-gradient extractor design. The basic design consists of a series of samples (D + E), enclosed in sections of PVC pipe (C), held above collecting cups (F + G) which are glued (H) to the bottom of a plastic tray (P). (Gluing is not permanent; to avoid periodic regluing the cups can be affixed to ¼ in. hardware mesh with rubber washers and short bolts.) The water (K) which fills the plastic tray bathes copper tubing (M) which is connected by hoses (L), either to other such units or to a container equipped with a sump pump to which ice is periodically added. Ice water is continually circulated, cooling in turn both the copper tube (M) and the water bath (K). The heat stimulus is applied by light (I; 250 Watt heat lamp) from above (insulated by white plastic beadboard [J]), which forces arthropods in the sample (D) to travel through the soil or packing material (E), through the mesh (A; secured by rubber band B) into the collecting cup (F). The collecting cup is provided with a solution of cycloheximide (0.2 g/L) to prevent specimen decay. The specimen cup (F) is removed from its retainer cup (G) and the sample decanted with a squirt bottle into a storage vial. To prevent damage from prolonged usage and hydraulic leaks at the hose clamps, the plastic tray should have an overflow hole installed below the level of the top of the retainer cups and the pan housing the sump pump should be connected to a toilet float valve, which will automatically add water in case of a leak. (Basic idea developed in conjunction with B.L. Fichter, Dep. of Entomology, and K Cromack, Dep. of Forest Science, Oregon State University.)

reduce cost but maintain the increased levels of microarthropod extraction. The modification I employ costs about $250 for a total of 72 separate extractors (Fig. 24–4). A unit of nine extractors each can be cooled independently with simple periodic addition of ice (and an overflow valve), or several units can be hooked together in sets of 8 to 10 unit trays with ice water recycled by a 1 horsepower pump through a coil of copper tubing in an ice chest periodically refilled with block ice. Eight dish-pan units fill a laboratory/greenhouse bench and generally is all a single 20-amp circuit breaker will support.

The PVC pipe in which the sample sits is the same housing employed inside the soil corer (Fig. 24–2F); thereby sample handling is minimized. (Many soil fauna are extremely fragile taxa, and are only able to migrate through pre-existing soil pores in the extractor.) The soil core is inverted prior to extraction, to minimize the distance that has to be negotiated during the extraction process. The entire apparatus can be constructed from materials available in any large department store. Though the size of the sample units of PVC pipe may be of any dimensions (if not used in conjunction with a congruent soil corer), the pictured size fits easily into a quart zip-lock plastic sandwich bag in the field.

The possibility now exists to carefully control the gradients with thermistors and microcomputers (Andren, 1985).

24–2.2.3.1.3 Special Modifications of the Microarthropod Extractor

This apparatus has been modified successfully for more recalcitrant substrates (e.g., freshly fallen logs, roots). Two-inch thick cross-sections are cut from the experimental logs in the field with a power saw. Chiseling along the wood grain removes 2 by 2 cm (5 cm long) chunks of wood in the vicinity of borer activity. These chunks are placed into the PVC extractor, packed with moistened sterilized sawdust and extracted as above. Very large numbers of commensal arthropods and nematode worms can be discovered in this manner. It is particularly important to place antibiotics in the collecting cup when extracting from wood, since numerous dissolved sugars drip into the cup (originating largely from the fresh sawdust).

Since dry funnels require healthy invertebrates, it is crucial to minimize the length of time between field collecting and extracting. Norton and Kethley (1988) describe a portable nylon apparatus suitable for airplane travel and overnight extraction.

24–2.2.3.2 Extraction of Soil Arthropods with Wet Funnels

Hydrophilic invertebrates are not effectively separated by Berlese or high-gradient funnels, since the medium dehydrates rapidly and most forms are capable of becoming cryptobiotic. Immersing in water a sample wrapped in cheesecloth (holds soil particles in, allows egress of biota) stimulates nematodes, tardigrades, copepods, planaria and enchytraeids to locomote. If the sample is placed in a funnel over a mesh, the emerging fauna will sink to the bottom of the water column. A clamp at the bottom can be released and the fauna decanted to a plate for counting or a vial for storage (see chapter 22 by Ingham in this book). Applying heat with a light bulb to the surface may speed the process (O'Conner, 1955); the average Baermann extraction is run for 2 to 4 d.

Milne et al. (1958) reversed the process by placing the base of the soil core in a water bath and gradually raising the temperature until the insect larvae all emerged from the heated sample onto the top of the soil surface.

Simple wet-funnel extraction, like Berlese/dry funnel extraction, is not labor-intensive, it requires no expensive equipment, and it yields a wide

variety of fauna. As with dry funnel extraction, efficiency is dependent upon the volume of the sample (usually 5–20 g); the greater the surface to volume ratio, the more efficient is extraction. Since extraction is dependent upon behavior induced in the invertebrate, extraction efficiency is species-specific and context-specific.

24–2.3 Extraction of Soil Arthropods by Physical Methods

24–2.3.1 Flotation in a Salt Solution

Most soil invertebrates are characterized by a specific gravity slightly greater than water (1.0), but none higher than 1.1 (Edwards, 1967). Therefore, soil immersed and agitated in a dense salt solution yields all the fauna to the surface. Unfortunately, most of the organic debris in the soil also floats to the surface, and because of its bulk it can obscure the fauna from enumeration. Efficiency can be increased by prewashing the soil sample through a series of sieves; this method is particularly effective if you are censusing for one particular organism of known size distribution. Ladell (1936) demonstrated that a sample can: (i) be washed through a series of sieves and collected in a modified beaker; (ii) the beaker is filled from the bottom with salt solution, and as it fills it is agitated by bubbling compressed gas injected along with the salt; specimens in the beaker are decanted into a collecting tube and washed; (iii) specimens (plus organic debris) are agitated with a mixture of heptane (benzene) and water, let settle, and the specimens pipetted from the organic layer, washed and preserved in 70% ethanol for analysis.

Edwards et al. (1970) adapted and mechanized the system to reduce handling time, increase efficiency of extraction and minimize operator bias. Four samples in wire mesh are simultaneously rotated and subjected to a spray of water. The resultant washed and screened sample is transferred to a container and vigorously agitated in a mixture of zinc sulfate solution (specific gravity 1.4) and 1:1 xylene + carbon tetrachloride (specific gravity 1.2); use enough of each solution to insure adequate vertical separation in the beaker. After separation of the liquids, the solvents can be bled off from a stopcock in the bottom or more liquid can be added, and then the topmost layer containing all the invertebrates decanted through a lateral vent. The mechanism works because the organic debris floats on the surface of the salt solution, whereas the specimens float on top of the organic layer. Both these methods are labor intensive.

24–2.3.2 Extraction of Microarthropods by Elutriation

This process is also driven by the difference in specific gravity of soil fauna and the soil particles. Heavy soil particles are allowed to sink in an upwards flow of water which carries the invertebrates with it to the top (Oostenbrink, 1960, 1970; Seinhurst, 1956, 1962). Historically, elutriation was used primarily for nematode extraction, nevertheless it has been adapted for arthropods (von Torne, 1962; Bieri & Delucchi, 1980).

These methods work best if the soil aggregates are broken down and the arthropods exposed to the liquid before being placed in the current; dispersing agents such as sodium citrate, sodium oxalate or Calgon are used to presoak the samples. This procedure is labor intensive, and the apparatus relatively costly to manufacture, but the major limitation is that the soil sample must be relatively small.

24–2.3.3 Extraction of Microarthropods by Centrifugation

(review: Goodey, 1957; Muller, 1962)

Though standard practice for nematode extraction, centrifugation is seldom used for arthropods though it works on the same principle. A sample is washed through a series of decreasing mesh diameter sieves. The appropriate filtrate is centrifuged in a dense solution (usually sucrose) which concentrates the fauna at the top. The fauna-containing layer is decanted, washed and preserved for analysis. Though considerably more labor intensive and time-consuming than other methods, in theory it should be excellent for sampling even non-mobile life stages such as eggs and cysts effectively.

24–2.3.4 Extraction of Microarthropods Based upon the Properties of the Cuticle

Although originally employed in the salt flotation method to increase separation of microarthropods from organic debris, the lipophilic nature of the cuticle can be used to extract arthropods directly from the soil. Aucamp & Ryke (1964) agitated aqueous soil samples in a container lined with grease. The arthropods adhered to the removable walls, which could be observed directly under the microscope.

Walter et al. (1987; Geurs et al., 1990) add heptane and then water to a sample previously fixed with ethanol and placed in a vacuum to remove air bubbles from plant debris. The sample is agitated and the invertebrates that float to the water/heptane interface, are decanted to a sieve, and preserved in alcohol. This approach is not useful in soils high in soluble organics because a tar-like layer forms at the organic/inorganic interface. As mentioned in the section on Berlese extraction, many taxa (often characteristic of more mesic soils) are not sufficiently lipophilic and probably would be overlooked by this method. The authors present evidence that for their soils, heptane extraction is significantly more efficient than high-gradient extraction.

24–3 PROCESSING THE EXTRACTED BIOTA SAMPLE

Relative to gathering samples in the field, extracting them in the lab (particularly with funnels), and identifying the material takes by far the most time. Since species-rich soils require sufficient replicate samples to

adequately estimate abundances, decreasing handling time per sample is the key.

A typical fully extracted Berlese sample of 0.3 m^2 (to a depth of 10 cm) of soil from Pacific Northwest forests will contain 500 to 10 000 individual mites and insects embedded in a soil layer many times their volume. Taking an aliquot, diluting it, and laboriously counting all the morphotypes present severely underestimates species richness, consumes a vast amount of time, and cannot in most cases be trusted quantitatively. The following procedure is one I personally employ (Fig. 24–5) because I have found it to increase the speed of processing more than 10-fold, and allows accurate quantification of the entire arthropod fauna.

Step 1: Transfer sample obtained from under the Berlese extractor (B = arthropods + debris) to 10 to 100 mL vial (A); if previously stored in 70% ethanol, dilute the alcohol (C) to $< 20\%$.

Step 2: Add several drops of mineral or vegetable oil (D) to vial with pipette (E).

Step 3: Cap (F) the vial and agitate to expose all the arthropods to the oil. Let set for half an hour and allow oil layer to separate on top (bringing with it nearly all the specimens out of the debris).

Step 4: With a pipette transfer oil layer (with specimens) to petri plate (4D), transfer alcohol and precipitate to another petri plate (4B). It is probably not possible (and certainly not practical) for the initial sorting and counting to identify all of the taxa to the species level. If one is interested in all the different types of taxa present in a sample, it is only necessary at this time to determine the morphospecies present in the non-organic bottom residue which will subsequently be discarded. All the other morphospecies will be preserved in oil in a labelled bulk well.

Step 5: With pipette (or fine forceps), transfer specimens from petri plates to plastic 12 to 15 hole well-plate (H); rough sort to major taxonomic groupings at this time (e.g., beetles, flies, mites, and springtails).

Step 6: Further sort each broad taxonomic category to finer units (e.g., separate major families of beetles); employ a 96-well plate.

Step 7: Sort family groups to individual morphospecies; archive most specimens from the oil layer in mineral oil on a well-plate.

Step 8: Remove the specimens from the alcohol and precipitate and archive them in 50:50 ethanol/water, with a drop of glycerine added. These specimens (and also the spiders removed from the oil layer) will dehydrate if stored in oil. Dehydrated specimens are often not possible to identify because of the distortion that has taken place; the drop of glycerine permits insect cuticles to maintain their plasticity even if the alcohol subsequently evaporates. Soaking accidentally dehydrated specimens in warm lactic acid usually returns them to useful condition.

Step 9: Send more than a single synoptic specimen of each "morphospecies" to the consulting taxonomist. Although the specialist may conclude that your "morphospecies" is more than one taxon, with your specimens sorted and archived in this manner, it is easy to reexamine your tentative identifications in the light of the new resolution afforded by the

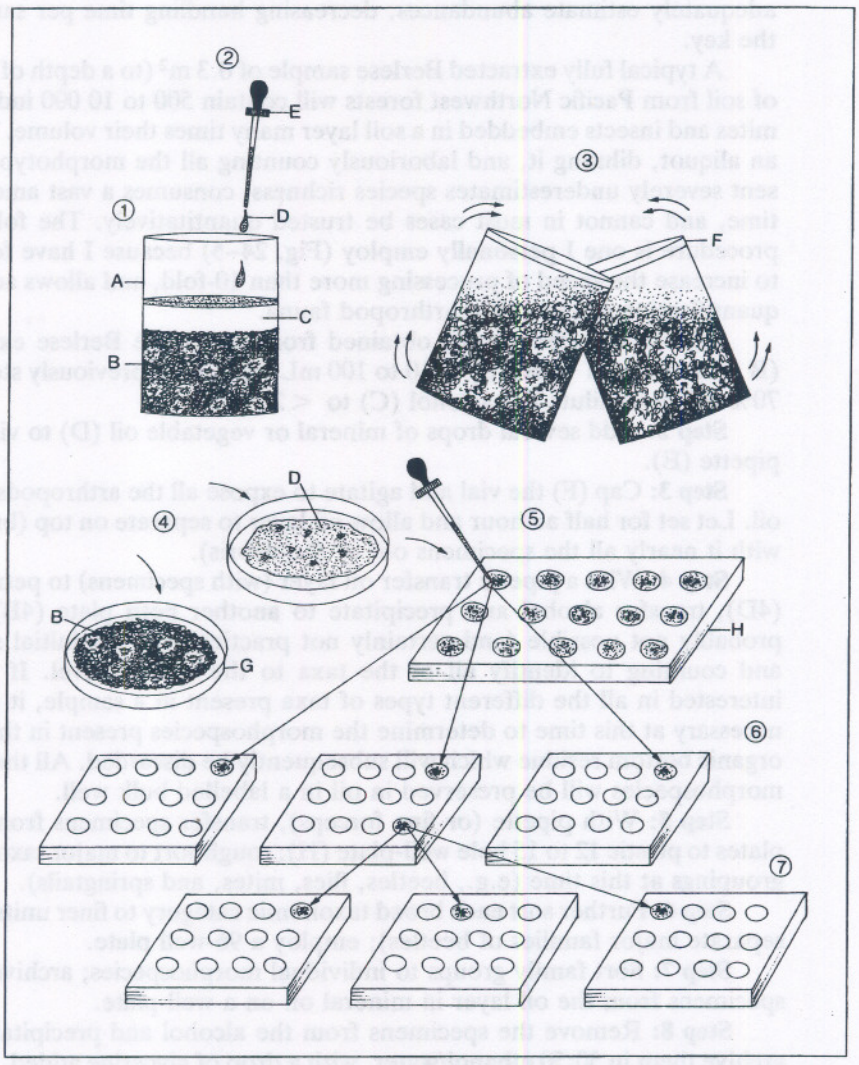

Fig. 24–5. Multi-species sample processing protocol. Step 1: Transfer sample to vial and dilute the alcohol. Step 2: Add several drops of mineral oil to vial with pipette. Step 3: Cap the vial and agitate; let set. Step 4: With pipette transfer oil layer (with specimens) to petri plate (4D), and transfer alcohol (with debris) to another petri plate (4B). Step 5: With pipette (or fine forceps), transfer specimens from petri plates to plastic 12 to 15 hole well-plate (H); rough sort to major taxonomic groupings at this time (e.g., beetles, flies, mites, and springtails). Step 6: Further sort a broad taxonomic category to finer units (e.g., separate major families of beetles); employ a 96-well plate. Step 7: Sort family groups to individual morphospecies. (Under certain circumstances, steps 5–7 may be replaced by transferring all the specimens to a 50-mm plastic petri plate, withdrawing as much oil as possible with a micropipette, and sorting with a probe/tweezers the fauna into separate clusters. Specimens can remain "on display" for at least a decade for easy teaching or reference purposes.)

taxonomist and correct your data accordingly. A diverse assemblage of taxa can be stored in oil for over a decade with no signs of contamination or decay.

24–3.1 Comments

1. With vision unimpeded by soil particles it is easy to count the different "morphospecies" separately. Technicians with limited skill in identification affirm it is much easier to distinguish the different "morphospecies" when they are all clustered together in the drop of oil, than when they are spread out through a diversionary field-of-view.

2. Only invertebrates with hydrophilic exoskeletons will remain in the debris on the bottom of the vial. These specimens will include primarily insect larvae (usually Diptera), worms (oligochaetes and enchytraeids), molluscs (snails and slugs), isopods, larger millipedes, and occasional species in groups normally extracted with the oil (i.e., *Nanhermannia*—oribatid mite; *Zercon*—gamasid mite). So few types of fauna possess hydrophilic exoskeletons (they are usually the large-bodied species), that after processing a few samples, the researcher will develop sufficient skill to scan the debris at the bottom of the ethanol/water layer in a few seconds in search of specimens.

3. Though it is far quicker to cut your sample containers with oil at step 2, and store the majority of specimens in oil, it is also possible to cut with Epsom salt ($MgSO_4 \cdot H_2O$) and store the specimens in glycerine (except the soft-bodied forms that must be in alcohol or ethylene glycol). Glycerine is easier to remove from the synoptic specimens sent to the taxonomist.

4. The taxa most in need of authoritative identification are the non-rare ones—simply take examples of these out of alcohol-preserved samples that have never been cut with oil. Cutting with Epsom salt has the benefit of separating all the fauna from the mineral soil, but it includes a lot of organic debris in the sample as well. The same is true of centrifuging in sugar solutions. Cutting by specific gravity either with salt or sugar requires thorough washing of the specimens before preservation—the smallest amount of salt can crystallize and the smallest amount of sugar can result in bacterial growth in the sample. The organic debris (leaves, stems, and fungal hyphae) that rises with specimens in the extraction has to be removed by tweezers; even if only a little bit remains, fungal growth will be significant.

24–4 BIOTA IDENTIFICATION

(review: Behan et al., 1985; Dindal, 1990)

With probably more than 75% of the terrestrial biota associated with the soil for at least one major phase of its life cycle (Southwood, 1978),

identification of soil fauna is a formidable task. In North America the most comprehensive reference text is Dindal (1990); this book is a compilation of identification keys, ecology, and bibliographic references by all of the leading specialists on different soil taxa. This is where any research group should start. Insect keys can be supplemented by the most widely used references in North America (Borer et al., 1976; Stehr, 1987, 1991), Krantz (1986) for mites, and oribatids may be supplemented with Moldenke & Fichter (1988; [copies available from the author]). Differing taxa can be identified to different levels of resolution with the Dindal book. A fundamental problem is that even with this book, and with the assistance of taxonomic specialists, identification past the level of genus is usually impossible for the most abundant taxa. Most of the species in these groups have never been given scientific names in North America. Taxonomists are a severely limiting quantity. If a research project requires identifications to be made, arrangements must be made before research is begun and the taxonomist(s) must be a participant in the design of the proposed research.

24–5 PRESERVATION AND ARCHIVING

(reviews: Martin, 1977; Steyskal et al., 1986)

24–5.1 Preservation

There are numerous fixatives and storing solutions available, each has its own specific advantages/disadvantages relative to certain taxa. Ethanol (70–80%) is by far the most frequent fixative and long-term preservative. Isopropyl (rubbing) alcohol or a 5% solution of 40% formalin are acceptable alternatives. When BOTH fixing and storing in ethanol, it is critical to: (i) decant and renew the solution prior to long-term storage (more than 1–2 wk); and (ii) add glycerine to the storage solution (several drops per 100 mL). A major expense in curating samples is the cost of vials and caps. Regardless of how they are advertised, no caps/stoppers/corks are evaporation proof. Neoprene stoppers are the most efficient (but far more costly than the vials themselves). The gelatine in the preservative protects the insect tissues, if the alcohol fully evaporates while archived.

Earthworms must be relaxed before killing and fixing. Place worms in a solution of 1 part $MgSO_4$ (saturated):3 to 4 parts of water for 1 to 2 h. Kill and fix by dipping in Bouin's solution for 2 to 20 s, blotting on paper and immersing (overnight) until stiff in FAA (90 mL 50% ethanol + 5 mL glacial acetic acid + 5 mL formalin solution). Place in 70% ethanol for permanent storage.

Bouin's and Carnoy's fixatives are widely used when the ultimate aim is sectioned microscope analysis of the samples. Bouin's fixative is made from 75 mL of picric acid solution (add 1 g picric acid crystals to 75 mL water), 25 mL of 40% formalin and 5 mL glacial acetic acid. Fix for at least 1 h, wash in water and dehydrate through an alcohol concentration series. Carnoy's fixative is made from 60 mL absolute ethanol, 10 mL of glacial

acetic acid and 30 mL of chloroform ($CHCl_3$). Fix for 15 min to 2 h and wash in 95% ethanol. After fixation, specimens can be stored in 70% alcohol until embedded.

24–6 ARCHIVING

Ultimately most specimens will end up pinned, stored in alcohol or put on slides when they are examined by a taxonomist. It is VERY important to be consistent with conventional methods in labeling the specimens, since most will be incorporated into existing museum collections (see chapter 7 on preservation in Borer et al., 1976). Be sure the labels on pinned insects do not exceed 2 × 1 cm; place additional information on separate labels stacked on the pin. Be sure to use alcohol-insoluble ink on labels placed in bottles (most xeroxed labels will NOT last unless they are on high quality bond paper). Use only the right-hand side of a slide for collection information (see chapter 34 on slide making techniques in Krantz, 1986).

Keep most of your specimens in alcohol (or glycerine) for easy storage. Slide making is time-consumptive, and often crucial characters are not easy to observe—it is very helpful to have a supply of specimens in alcohol that can be placed on temporary hanging-drop slides for rapid analysis. Before examination with a dissecting stereomicroscope, specimens usually require clearing in either lactic acid (most specimens can be stored in lactic acid indefinitely); glycerol (50 mL)-water (50 mL distilled)-acetic acid (3 mL glacial); or lactophenol (50 g phenol-25 mL water + 50 mL lactic acid). The latter two clearing agents are too strong to store specimens in; rinse and store in ethanol.

Quick observation slides use a hanging-drop. The well on the slide is half-filled with lactic acid, the specimen inserted, and a square cover slip placed on top so that it projects over part of the well. The specimen caught in the meniscus can be manipulated with a teasing needle to examine all of its surfaces.

24–6.1 Assigning Morphospecies to Functional Ecological Groupings

(reviews: Kevan, 1968; Wallwork, 1970; Edwards & Lofty, 1977; Bal, 1982; Eisenbeis & Wichard, 1987)

This is no easy task—the diversity of meaningful ecological roles is large; and the information base available for most soil fauna is extremely limited (even in Europe, where more by far is known). However, two generalizations are universally affirmed: the more precise the taxonomic identification, the more likely that useful functional groupings can be accomplished; equally true, the more precise the identification the more time and effort required to achieve it (= more cost). Certain broad taxonomic groupings contain more precise functional information than others. For example, Chilopoda are all invertebrate predators and Collembola are mostly fungivores. In contrast, Coleoptera are too diverse to be labeled

with a specific ecological function. The aim should NOT be to take all identifications to the same taxonomic resolution (e.g., order), but to take identifications to a level (different for each group) that is ecologically meaningful. How can you accomplish this? There are really no reference texts that are, at one and the same time, detailed enough for some groups but not overly complex and confusing for others.

We are currently developing an illustrated computer program, for use on Macintosh pc's, which starts to address this need (Moldenke et al., 1990). The intent of the design of COMTESA (*CO*mputer *T*axonomy and *E*cology of *S*oil *A*nimals) is that it is useful to all levels of users from novice to experienced soil zoologist. COMTESA can be used by the novice to differentiate basic functional groups with ease. Knowledgeable scientists and their research staff can use COMTESA to differentiate to the species or functional group levels, and can use the supplementary modules to store and retrieve information and scientific references about organisms at any level of organization (order, family, genus, . . .). Print-based keys currently available for identification of arthropods are often designed to cover wide geographic regions, such that their level of resolution is poor for the scale at which most ecological studies are conducted. The modular approach of COMTESA copes with this problem since it is conceptually divided into two parts designed to deal with the different scales of resolution. Part I distinguishes about 150 different functional/taxonomic groups and has an ecological emphasis. For instance, the xylevorous and microphytophagous species of oribatid mites are distinguished from one another while predaceous spiders are divided into component hunting guilds. This part of the key should be useful across North America with only minor alterations to meet local needs. Part II consists of modules that provide identification to the generic and species level and are specific to site/region/ecosystem. The key is driven by clicking a mouse on the proper choice of diagrammatic images. Unfortunately, it will be many years before such a system is modified to work at the species level for a widespread series of ecosystems.

24–6.2 Transforming Census Data

(review: Edwards, 1967; Phillipson, 1970; Petersen & Luxton, 1982)

Since large-bodied species are usually infrequent in samples and small-bodied species usually the most abundant, enumeration data will produce community analyses very different from analyses based on biomass or respiratory consumption. Average weights for most taxa can be obtained by weighing individuals on an electrobalance. The smallest springtails, oribatids, and prostig mites (< 5 µg each) require pooled samples of about a dozen individuals. The most appropriate biomass transformation for earthworms with digestive systems filled with soil has been addressed by Bouche (1966).

For each class of soil organisms, a specific relationship between length (easily measured with an optical micrometer) and weight exists (Reichle, 1967; Jarosik, 1989). These average conversion factors can be used for most studies, with the caveat that unusually shaped or exceptionally large species should be weighed directly.

Respiratory equivalents for dry biomass of many taxa are available as well as direct measurements of respiratory rates (Berthet, 1967) and feeding rates (O'Conner, 1963; Coleman, 1968) under controlled lab conditions. As the authors point out, these studies are limited and extrapolation to other field conditions would not be recommended (Healey, 1970; Persson & Lohm, 1977). The direct and indirect effect (regulatory effect of a fungivore's activity on its resource's metabolism) is testable in microcosms (Anderson & Ineson, 1982). Total respiratory rates of: (i) soil with endemic microflora and (ii) soil with endemic microflora PLUS differing population levels of particular fungivores, could be determined analogously to Anderson's ANREG model experimentation for N mineralization (Anderson et al., 1985). An exemplary study of individual species contribution to community respiratory rates is that of Luxton (1981).

24–7 REARING

Efficient laboratory rearing is the key to associating unidentifiable immature stages, quantifying respiration and nutrient mineralization rates, and determining the functional roles played and microhabitats selected by arthropods. There have been several techniques developed, but they have been employed in too few instances to have made an impact upon the vast number of fundamental questions remaining.

The most fundamental apparatus to maintain and observe microarthropods is a container partially filled with a mixture of Plaster of Paris and powdered charcoal. Water is periodically added to the substrate to maintain high humidity and very fine mesh is glued over a port in the cap to permit transfer of gasses. Small groups of oribatids (Evans et al., 1961; Sengbusch, 1974; Arlian & Wooley, 1970; Krantz, 1986) or gamasids can be kept in a 3 cm tall by 3 cm diam. container. Periodic feeding and especially removal of wastes is necessary to keep the culture healthy; the main cause of death in most rearing attempts is overgrowth by fungi.

Simple microcosms have been proposed by Anderson and Ineson (1982; Fig. 24–6) and refined by Taylor and Parkinson (1988), which permit measurement of both respiration and nutrient leaching. A soil sample (with or without specific arthropods) is placed in a cylinder over a nylon mesh and inserted into a slightly wider cylinder to rest on top of inert beads and a leaching port. The air-tight lid can either be provided with a rubber injection septum, a trough holding gas-absorbant chemicals, or a conductivity cell for measurement of CO_2 production. The latter reduces volumetric errors in the titration process and allows continuous monitoring of

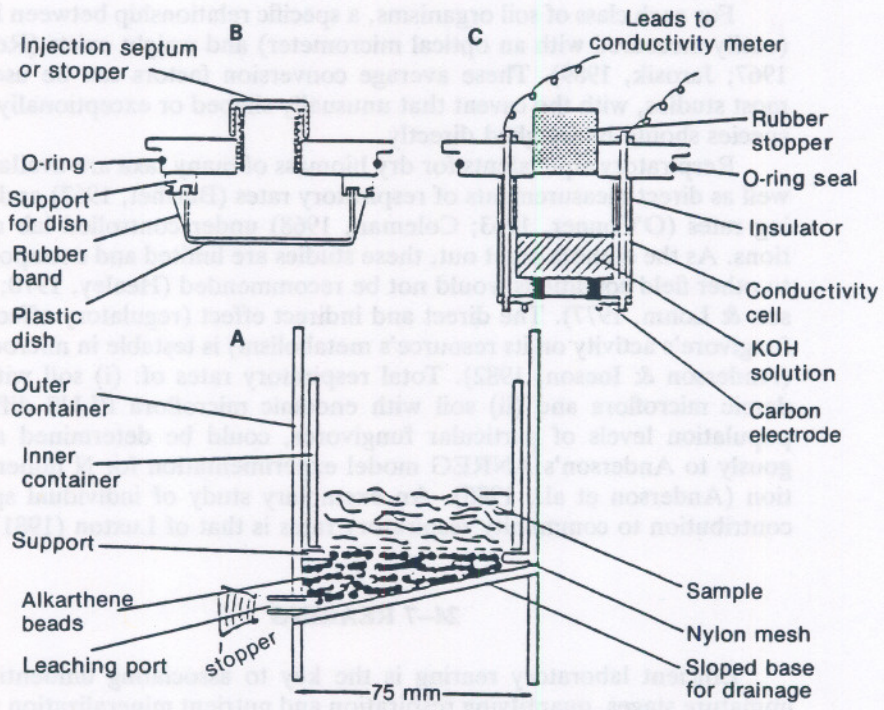

Fig. 24–6. Simple microcosm design of Anderson and Ineson (with permission from 1982), permitting measurement of both leachates and gaseous exchange from soil. (A) Base section showing inner sample container and drainage system for leachate sampling. (B) Lid to hold absorbants for gas assays. (C) Lid fitted with conductivity cell for measuring CO_2 evolution.

respiratory activity. Sensitivity must be adjusted to the rate of respiration; calibration is explained in Anderson and Ineson (1982). Leaching is achieved by flooding the substrate and drawing off the soluble nutrients through a leaching port. The leaching port may also be used to introduce inhibitors, pesticides, or nutrient amendments.

The key to unraveling the functional roles of soil arthropods in nature is to investigate their interactions with the normal component of microflora—"in order to express the ecological niche of the animals rather than their ability to adapt to artificial conditions" (Hagvar, 1988). Hagvar defaunated undisturbed forest soil samples, allowed the reestablishment of a natural microflora in the field, then investigated the interactions of mites with microflora under a set of controlled laboratory conditions.

The spatial scales in which arthropods act is poorly known. Basic elements such as how they locomote through the soil, how aggregated they

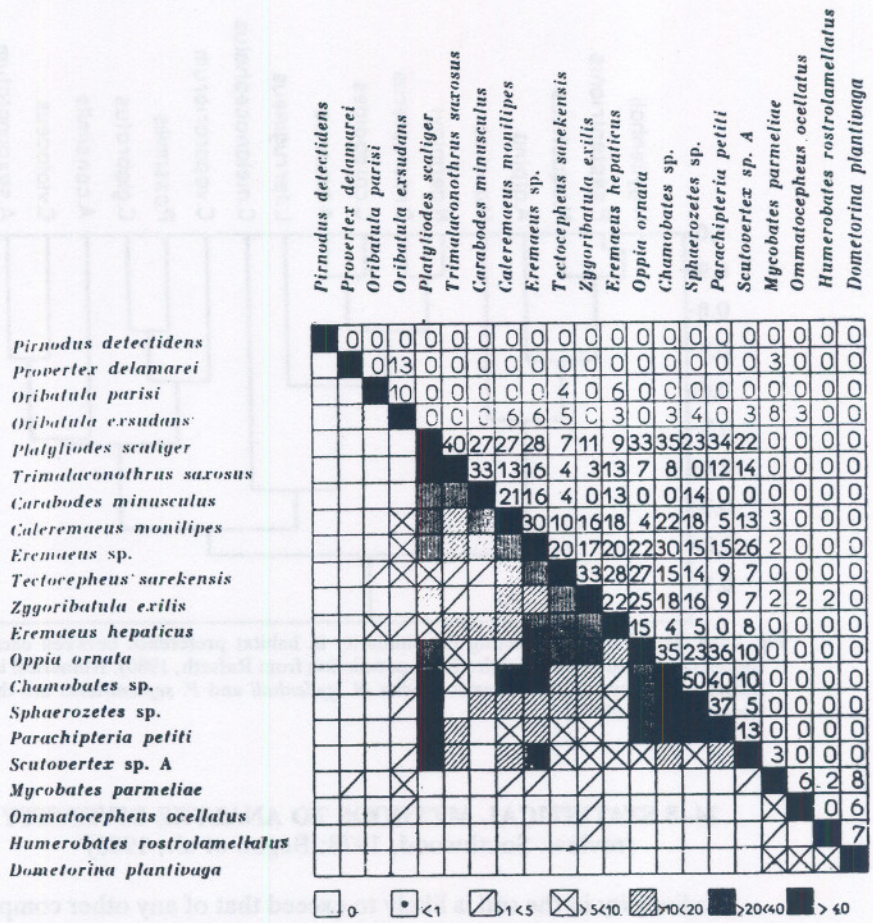

Fig. 24–7. Matrix table of oribatid mite biocoenosis (with permission from Trave, 1963). Representation to visualize the strength of species associations from samples with many replicates. In this example, *Trimalaconothrus* co-occurs with *Platyliodes* and *Sphaerozetes*, *Chamobates* and *Parachipteria* with one another more than 40% of the time; the great majority of the species co-occur in less than 10% of the sample replicates and are therefore excluded from this biocoenosis.

are and which microhabitats they select are unknown. The recent development of root boxes (analogous to rhizotrons, but somewhat less costly and easier to manipulate) should facilitate photographic documentation of soil arthropod activities (Rygiewicz et al., 1988; Unestam & Stenstrom, 1989). The design of Rygiewicz et al. (1988) is particularly promising, since it can differentiate activities within the rhizosphere associated with feeding upon roots vs. fungi.

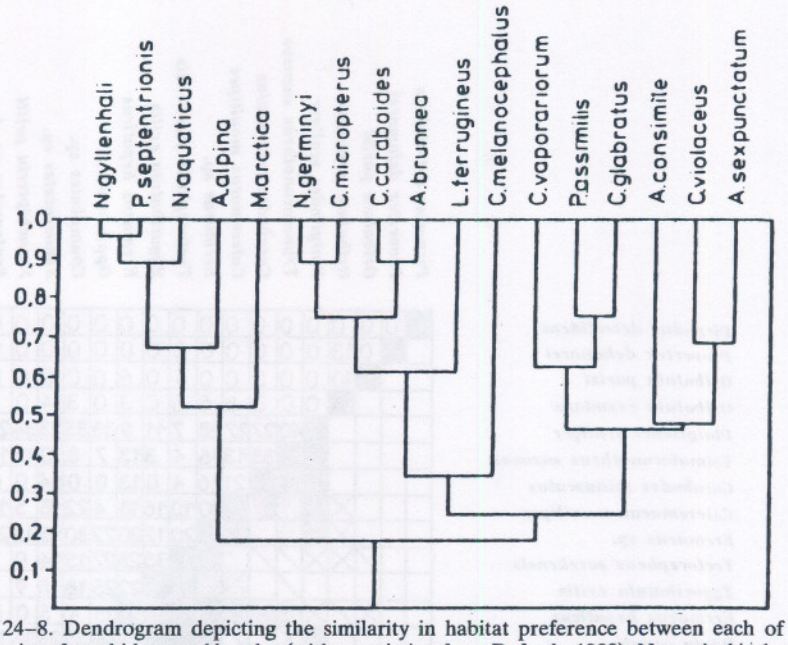

Fig. 24–8. Dendrogram depicting the similarity in habitat preference between each of 17 species of carabid ground beetles (with permission from Rafseth, 1980). Numerical index of habitat similarity on vertical axis; species *N. gyllenhali* and *P. septentrionis* are the most frequently associated.

24–8 STATISTICAL METHODS TO ANALYZE DIVERSITY

(review: Southwood, 1978; Begon et al., 1986)

Biodiversity in the soil is likely to exceed that of any other component of terrestrial ecosystems (Moldenke & Lattin, 1990). Not only is it difficult to quantify the interactions between species, but it is a practical concern simply to describe patterns of species occurrence. Moldenke (1990) tabulates the diversity likely to be found in a typical square meter of Northwest conifer forest soil. The question of whether there are basic patterns of co-occurrence, implying the existence of semi-independent microcommunities, is usually analyzed by constructing a matrix of co-occurrence frequencies (Trave, 1963; Fig. 24–7).

Degree of similarity in habitat preference can be represented as a dendrogram (Rafseth, 1980; Fig. 24–8). Similarly, the elegant feeding preference studies of Hartenstein (1962) could be transformed to matrix values reflecting feeding specialization and niche overlap (viz., Moldenke, 1975). The response of the diverse soil community to specific environmental variables can be quantified by techniques developed by phytosociologists. TWINSPAN and DECORANA are widely used examples of principal components analysis (Wauthy et al., 1989). TWINSPAN is a hierarchical analysis; Moldenke (unpublished data; McIver et al., 1991) used it to test

the relative importance of a series of environmental variables in determining the composition of ground-dwelling spider communities. DECORANA is a similar technique but does not impose a hierarchical dichotomy on the results. Response of complex community composition to single or multiple environmental changes are graphed such that each point represents the full range of species within a sample and the distance between points represents the degree of similarity between any two samples.

A specific benefit to these two community representation algorithms is that they calculate particular "indicator species" for each of the discriminations they perform. This permits reducing the diversity of fauna that has to be enumerated from several hundred per square meter to perhaps a dozen that can be easily learned by a technician; subsequent analysis for the effect of a given environmental insult (i.e., acid precipitation, management practice, and herbicide application) can thus be appreciably facilitated (Eyre et al., 1990).

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