Chapter 19. Sampling Methods for Soil and Litter Fauna

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Chapter 19
Sampling Methods for Soil and Litter Fauna

Grizelle González, Maria Fernanda Barberena-Arias, Wei Huang, and Claudia M. Ospina-Sánchez

19.1 Importance and Challenges in the Study of Soil and Litter Fauna

Shaping aboveground biodiversity, regulating the functioning of terrestrial ecosystems, and influencing the ecological and evolutionary response of ecosystems to environmental change are some of the major roles of belowground communities revealed by recent advances in soil biodiversity research (Bardgett et al. 2005; Bardgett and Wardle 2010; Bardgett and van der Putten 2014). Soils host high biodiversity, with significant control over aboveground ecosystems. Belowground decomposers drive essential ecosystem functions, such as organic matter turnover and nutrient cycling (Wardle et al. 2004; Bardgett and Wardle 2010), and are therefore key determinants of soil fertility and nutrient uptake by plants (Coleman et al. 2004; Wardle et al. 2004; Bardgett and Wardle 2010; Culliney 2013; Menta and Remelli 2020). The soil is a key resource for several ecosystem and biosphere processes. These processes include plant production, cycling of organic matter and nutrients, storage of carbon and water, and release of nitrous oxides, carbon dioxide, and methane (Swift et al. 1979; Lavelle and Spain 2001).

Soil biology is challenged to understand the specific role of organisms, how they are organized in niches along soil resources, and how they respond to changing environments. Knowledge of soil species is difficult because of their small size and

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large biodiversity (Anderson 1975; André et al. 1994). Populations of small invertebrate animals spending most of their lifecycle in the soil are extremely large, reaching numbers as high as a quarter of a million per square meter (Fletcher 1976). It is estimated there are more than seven million unique terrestrial arthropods species living on Earth (Stork 2018). Yet this number could be extrapolated to ten million when animals of less than 200 μ are included (May 1990). This great range in species estimates can be attributed to the lack of knowledge of small arthropods (May 1988, 1994; André et al. 1994; Eisenhauer et al. 2017).

High numbers of individuals and species belonging to all terrestrial phyla can be found in soil and litter environments (e.g., Rotifera, Annelida, Mollusca, Tardigrada, Nematoda, and Arthropoda). Usually Arthropoda and Nematoda have the highest diversity. Indeed, in terms of species richness, arthropods may represent as much as 85% of the soil fauna (Culliney 2013). Representatives of all the arthropodan subphyla and of all their terrestrial classes can be found in the soil: Cheliceromorpha (scorpions, pseudoscorpions, spiders, harvestmen, and mites), Crustacea (amphipods and woodlice), Myriapoda (centipedes and millipedes), and Hexapoda (which include Collembola, Protura, and Diplura) and 20 out of the 26 orders of ectognathous hexapoda (true insects) (Greenslade 1985). Thus, the term microarthropod refers to an artificial grouping of the small arthropods that range in size from about 100 μm to a few millimeters and include the mites (Acari), Collembola, Symphyla, Protura, Diplura, Pauropoda, small centipedes and millipedes, and small insects from several orders (Coleman et al. 1999). Microarthropods include representatives of most trophic groups within the belowground food web (Walter et al. 1987; Moore et al. 1988).

Given the difficulty in the identification of all the species present in soil, many studies use functional groups divisions and abundance or species richness parameters to understand their role in ecosystems (Bengtsson 1998; Wolters 2001). Groups of soil fauna separated by size are also useful to understand their role in soil ecosystems. Classification by body size has widespread repercussions on sampling and study of the different groups. Most authors (e.g., Wallwork 1970) differentiate three size classes: micro-, meso-, and macrofauna based on body width with microfauna (<100 μm) and mesofauna (100 μm–2 mm) that participate as regulators in microorganisms' activities and macrofauna (>2 mm), which create microhabitats for other soil biota by reworking the soil (Wallwork 1970; Brussaard 1998). Other classifications include their role in ecosystem processes as they interact with microorganisms and function as micro-predators, litter transformers, and ecosystem engineers (Lavelle 1996). Microarthropods (definition from Coleman et al. 1999) can be identified to species level in addition to their characterization in functional groups (Potapov et al. 2016). Identification to species level makes comparisons among species within functional groups possible and can help determine how different species respond to environmental factors (Ponge and Salmon 2013; Potapov et al. 2016; Coyle et al. 2017).

Diversity estimates of soil and litter fauna that can be generalized are difficult to achieve because their belowground habitats are complex and vary in physicochemical characteristics that give rise to methodological challenges (Moldenke 1994). Results are affected by the mechanisms used to recover organisms from environmental samples. Thus, the type of sampling system used for any ecological investigation must be carefully planned as an integral part of the whole study (Macfadyen 1962); aas no one method can extract all soil and litter arthropods with equal efficiency from all types of soil and, it is most unlikely that any of the extraction methods currently available will extract any group or species of arthropod from soil and litter with complete efficiency (Fletcher 1976).

Rhea-Fournier and González (2017) discuss the plethora of challenges faced in soil research and argue that no singular method or tool is a panacea to the difficulties that may arise. Similarly, in this book chapter, we review numerous methodologies in the faunal toolbox of techniques and approaches to evaluate and quantify the influences of soil and litter fauna although qualitative techniques are also mentioned. In the following sections, we review passive and dynamic methods in the study of soil and litter fauna and provide methodological considerations for ecological studies of soil fauna with emphasis on the study of decomposition processes.

19.2 Methods in the Study of Soil and Litter Fauna

It is nearly impossible to count all the animals per unit area of soil in a site, so populations must be estimated from the numbers recovered from small samples of soil (Fletcher 1976). These samples can be extracted from samples of litter, cores, or cubes of soil of known surface area or volume. The numbers of animals recovered from the samples can then be converted into populations per unit area of soil at a known depth (usually 25 or 30 cm) providing the number and size of samples taken is adequate. Soil fauna can be extracted from a soil and litter sample either through passive (also known as mechanical) methods or via the use of dynamic (behavior) modification techniques. Mechanical methods are generally labor-intensive and work best on compact clay soils with low organic matter content, where meso- and macro-arthropods are most efficiently extracted (Moldenke 1994). 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 (Moldenke 1994). Most authors consider that dynamic methods for extracting arthropods from soil are more efficient on loose-textured soils with large air spaces that contain appreciable amounts of decaying plant material (Fletcher 1976). Dynamic methods have the advantage that animals are recovered undamaged and are suitable for taxonomic studies (see Fig. 19.1), whereas mechanical methods, although tending to damage specimens, yield data on resting stages of arthropods, particularly eggs and pupae (Fletcher 1976; Moldenke 1994).

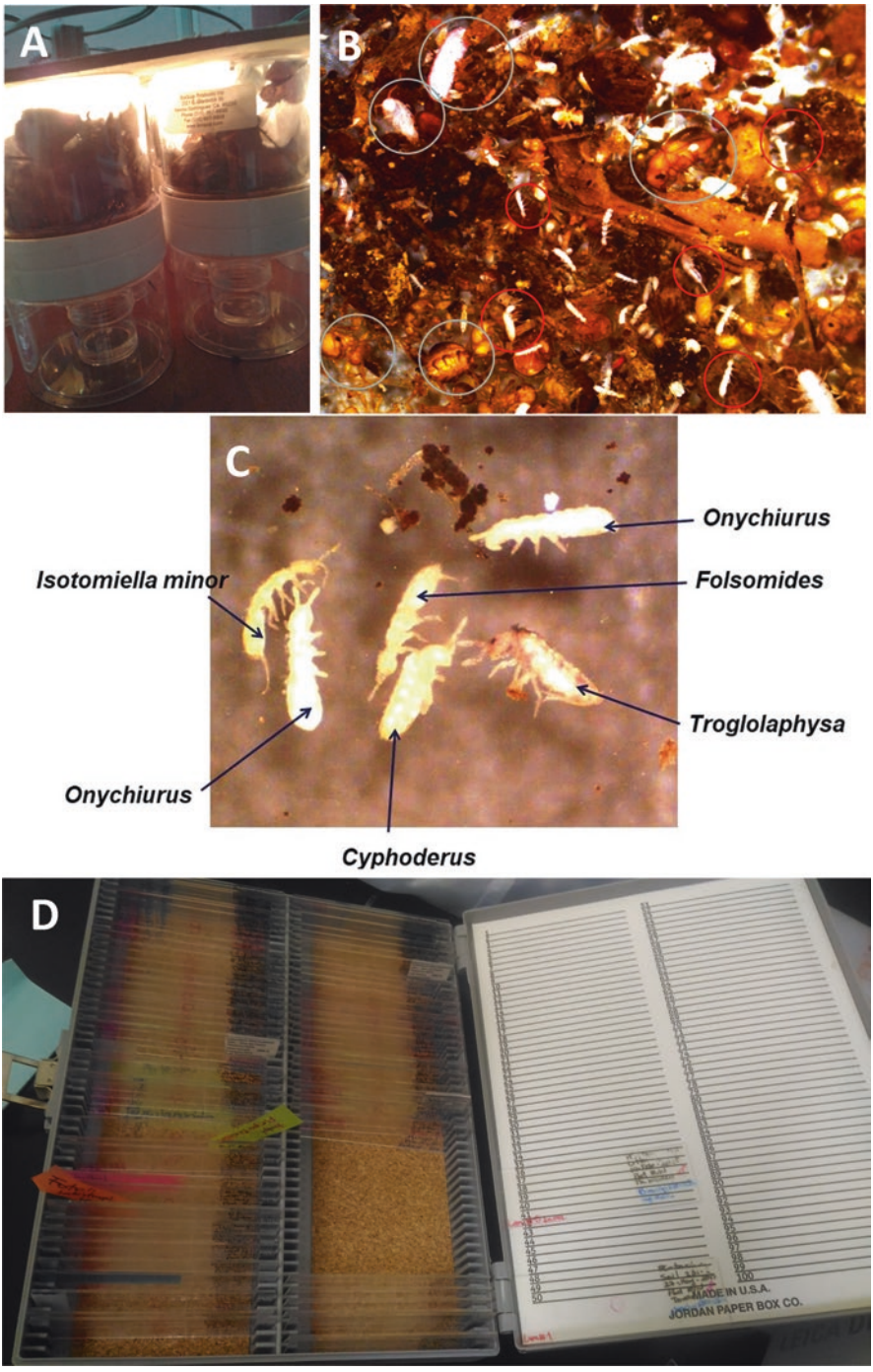

Fig. 19.1 Collembola species identification: separation. (**a**) After field collections, the sample is exposed to a light source in the Berlese-Tullgren funnels; after 7–10 days the sample is dry, and the arthropods are in 70% alcohol inside the collecting vial. (**b**) The collembolans (red circles) are separated from the other arthropods (blue circles). (**c**) The morphospecies of Collembola are separated under the stereoscopy. (**d**) Some Collembola specimens are prepared in slides for further identification. Photos by Claudia M. Ospina-Sánchez

19.2.1 Passive (and Mechanical) Methods on the Study of Soil and Litter Fauna

Observational Studies The ability to individually observe and identify organisms is an important research component for effective conservation and management programs (González et al. 2006). Methods of marking or tagging individual organisms are especially important in genetics and selective breeding studies, where individual identification is essential (Arce et al. 2003). Yet visual identification is often difficult. Paints, dyes, and stains are widely used in studies of insect population dynamics (e.g., Coppedge et al. 1979; Haagsma and Rust 1993). Fluorescent markers have several advantages over liquid paints, and they are becoming the best technique for color-marking insects and aquatic fauna (e.g., Doupé et al. 2003; Welker et al. 1975), but are not as often used in the study of soil invertebrates (González et al. 2006). Fluorescent markers can be a useful tool for carrying out short-term mark and recount studies of earthworms so we can better understand the worm's basic biology, demography, and ethology (González et al. 2006). The technique allows for rapid and inexpensive marking of large numbers of individuals (Turchin 1998). It is also environmentally safe, cost-effective, and easy to use.

There are other observational studies of earthworms that focused on their activities or incidence of casts. Rhea-Fournier and González (2017) described how passive methods for soil within the sphere of earthworm influence (also known as drilosphere) exist. The burrows of soil-dwelling earthworms open to the soil surface, providing visual evidence allowing investigators to sample burrow soil (Parkin and Berry 1999; Gorres et al. 2001). Alternatively, the presence of surface-casting species provides investigators with a visual cue to compare drilosphere and non-drilosphere soil by sampling the casts themselves (Aira et al. 2003; Hong et al. 2011) or the underlying soil (Bohlen et al. 2004). Other visual indicators can be exploited in northern temperate forests where patches of soil invaded with non-native earthworms contrast greatly with earthworm-free patches where thick organic horizons remain (Burtelow et al. 1998; Suárez et al. 2006). A quite different passive approach employed by Lavelle et al. (1992) is utilizing the difference in size of soil aggregates and the casts of geophagous earthworms by passing soil through a 2 mm mesh sieve to exclude casts. This technique works by looking specifically at casts; however, the effect of earthworms extends beyond the casts themselves. For direct study, fresh casts can be obtained by lightly squeezing on the posterior end of a collected or raised worm (Barois and Lavelle 1986; Lavelle et al. 1992).

There are other techniques that allow for the repetitive, nondestructive observation of the soil biota. Root boxes can facilitate photographic documentation of soil arthropod activities (Rygiewicz et al. 1988; Unestam and Stenstrom 1989). Density estimates of microarthropods observed with minirhizotrons can be underestimated depending on time efforts of the observation periods, levels of lights, and optical resolution (Lussenhop and Fogel 1993). Yet, time-lapse video can be used to sample microarthropod density in rhizotrons, where better visualization of all the biota can

be achieved by using long working length microscope objectives and stains (Lussenhop and Fogel 1993).

Nicholas and Parkinson (1967) have reviewed in detail several techniques that have been developed to quantify the structure of soils on a scale relevant to arthropods based on the preparation of undisturbed soil sections. These 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 (Moldenke 1994). In the field, soils can either be quickly frozen with water followed by liquid nitrogen (Froehlich and Miles 1986) or embedded in agar, gelatin, or epoxy (Haarlov and Weis-Fogh 1953; Anderson and Healey 1970; Rusek 1985). However, all these techniques suffer from one or another serious drawback and, thus, their application has been very restricted. For example, the success of the agar-gelatin technique depends on obtaining thin sections of good quality that could be inspected under intense illumination with a binocular microscope. However, this is possible only if the soil is rich in organic matter, for gelatin is unable to bind mineral particles effectively (Pande 1975).

Observation using dissection stereoscopes and microscopy remains an essential tool for the separation and identification of individual animal specimens in the study of soil biology (see Box 19.1 for case on the study of collembolans and Figs. 19.1 and 19.2).

Pitfall Traps Pitfall traps rely on animal movement and are used to collect ground active organisms that fall into the collecting pan or trap. This trap can consist of a vial buried into the ground and carefully aligned with the ground surface to prevent any protruding edge to become a potential barrier for the free movement of organisms. Pitfall traps usually have an inserted cone-shaped device to prevent the escape

Box 19.1 Species Identification, the Case of Collembola Separation and Identification in the Tropics

1. *Separation*. After field collections, the samples are installed in Berlese-Tullgren funnels and exposed to a light source for 7–10 days until the sample is dried (Fig. 19.1a). Samples extracted via Berlese-Tullgren funnels produce a rich number of animals. Thus, it is necessary to separate the Collembola from the other arthropods (Fig. 19.1b). Although a few conspicuous species can be identified under the dissection stereoscope, most of the specimens would need further preparation to be identified using microscopy. This process begins with the separation of specimens of Collembola into morphospecies using a dissecting microscope (Fig. 19.1c). Two or three specimens of each morphospecies are cleared using Nesbitt solution and fixed in slides using Mac André II solution (Mari Mutt 1976). To harden the solution, the slides are dried in a slide warmer at 45–50 °C for at least 7 days. At the final step, each specimen in the slide is labeled with its collecting data (Fig. 19.1d).

2. *Description*. Collembolans are not well known in the tropics; therefore, it is common to find new species. The first characteristic to note is the coloration pattern that must be seen under the dissecting microscope (Fig. 19.2a). For the specimens that cannot be identified using primary literature, it is necessary to look for revisions of the group and the original descriptions of the species and genera. Then one can determine if the specimens belong to a new species. The principal criterion used for this determination is morphological differentiation (Gisin 1967; Soto-Adames 2002; Yoshii 1989), in combination with differences in chaetotaxy (Carapelli et al. 1995; Jordana and Baquero 2005; Soto-Adames 2002). These characteristics must be seen under the microscope (Fig. 19.2b). For new species description, it is necessary to draw all the morphological characteristics, made with the aid of a drawing tube (Fig. 19.2c–d). As the final step of description, all drawings are digitized and, with species descriptions, submitted for review as a new species in a specialized peer review journal (Fig. 19.2e–f).

of fallen organisms and a covering ceiling to prevent the trap to be flooded with rainfall (see Fig. 19.3). In addition, traps can be partially filled with a killing solution. For collembolans, for example, the use of pitfall tramps in 1.5 ml microtubes (more commonly used for molecular biology laboratory work) is common. The traps are filled with 1–1.2 ml of ethanol and baited with a small amount of limburger cheese and then placed wherever there was suitable substrate over 2 days (Soto-Adames and Taylor 2010).

Pitfall trap efficiency, as any other ecological method, varies because of several factors such as location of the traps and the intensity, pattern, and timing of sampling (Southwood and Henderson 2000). In addition, there has been an enormous amount of information about the efficiency and variation of pitfall traps (Brown and Matthews 2016) including reports of variation in pitfall trap size and in the materials from which they are made, such as glass, plastic, or metallic (Luff 1975). Luff (1975) reports glass vials to be more effective than plastic and metallic because there was almost no escape in the glass vials. In addition, the size of organisms can affect their escape as larger organisms escape more from small traps and small organisms escape more from large traps. There have been also reports of limitations in accurately reflecting the species composition and sex ratio that come from more extensive and intensive samplings (Topping and Sunderland 1992). As a consequence, there has been a call to standardize the use of these traps by taking into account potential sources of variation such as level of activity of organisms (e.g., due to mate or oviposition site searching) (Brown and Matthews 2016), the choice of materials, and size of the pitfall traps, among others.

Physical Removal Passive methods of soil and litter fauna sampling include hand sorting, washing and sifting, and flotation techniques (Coleman et al. 1999). Coleman et al. (1999) give an overview of sorting soils by hand for earthworm

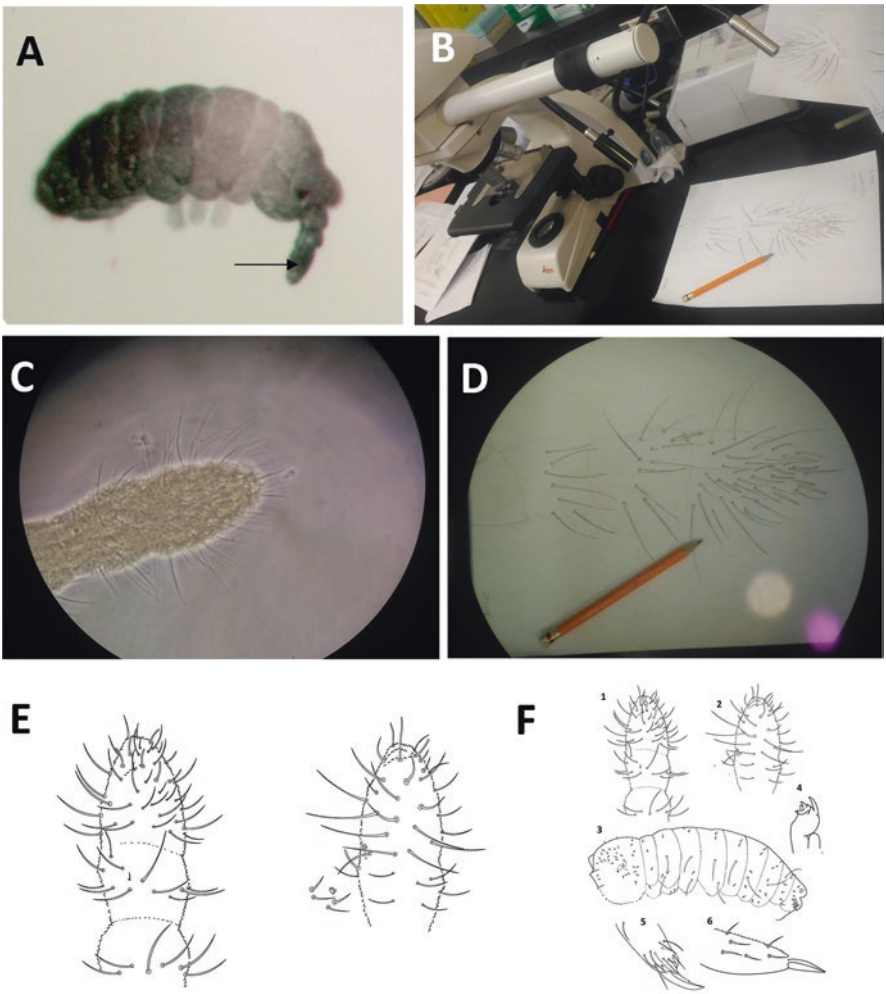

Fig. 19.2 Collembola species identification: description. (**a**) Specimen of *Brachystomella cyrillae* sp. n. under stereoscopy; the arrow indicates the antenna. (**b**) The new species needs a full description including morphological schemes under the microscopy using a lucid camera. (**c**) Detail of the antenna of *B. cyrillae* sp. n. at 100×. (**d**) The same antenna drawing under the lucid camera. (**e**) The figures are inked and digitalized. (**f**) All the figures are included in the new species description. (Photos and drawings by Claudia M. Ospina-Sánchez)

sampling. Hand sorting typically samples a 25 × 25 cm area to a known depth. While small sample areas increase the fraction of fragmented worms and can be inefficient where population densities are low, larger areas can decrease efficiency purely due to the time required to process the amount of soil. Wet sieving or washing can be applied in addition to detect smaller species and cocoons or instead of hand sorting in grassland systems where fibrous roots are very dense (Coleman

Fig. 19.3 Pitfall traps installed in a tropical rain forest consisting of a funnel connected to a vial buried into the ground and carefully aligned with the ground surface and a covering ceiling to prevent the trap to be flooded with rainfall. (Photo by María M. Rivera)

et al. 1999). Hand sorting or sieving can be undesirable in field experiments as it destroys the pedology and soil texture of the site (thus porosity and hydrology) and likely disturbs many other classes of soil biota (Rhea-Fournier and González 2017). While time- and labor-consuming, control (or ambient) earthworm treatments could also be sorted without earthworm removal to duplicate the disturbance; however, the resulting soil would not reflect any natural system, even rarely agroecosystems (Rhea-Fournier and González 2017). Zhang et al. (2010) applied a 1 cm sieve to sort out native earthworm cocoons, stating this mesh size was too large to disrupt soil aggregates, replacing the soil into field-installed mesocosms in a rubber plantation and then inoculating the mesocosm with worms.

There are mechanical methods which separate the arthropods from soil by physical means or depend on the oleophilic and hydrophobic properties of the arthropod cuticle. Physical extraction techniques are usually based on dispersing soil samples in a solvent with the subsequent flotation of soil invertebrates based on density differences (salt or sugar solutions) or on the affinity of the arthropod cuticle for organic solvents (Moldenke 1994). Detailed descriptions of the heptane flotation method are provided by Walter et al. (1987) and Geurs et al. (1991). The heptane flotation technique is based on the principle that the waxy cuticle of the arthropods has chemical affinity for the heptane and not the water. Walter et al. (1987) prove

this method is an efficient extractant of arthropods for dryland soils and soils with little organic material and for studies where both dead and live animals in all life stages are to be collected. However, the heptane flotation technique has multiple disadvantages because soft-bodied animals could get damaged during the processing of the sample and it is time-consuming (limiting the number of replicate samples that can be processed in a given time) and expensive (requires the availability of a fume hood and proper organic waste disposal facilities) (Moldenke 1994).

An elutriation technique using upward currents of water to separate soil and animals was used by Törne (1962) to separate Pauropoda and small Collembola from light mineral soils with low contents of organic matter. Müller and Naglitsch (1957) and Müller (1962) put soil samples in saturated sodium chloride solution and centrifuged them to collect the arthropods. The supernatant liquid was then decanted and the residual animals counted and identified and the procedure repeated until no more animals were recovered (Fletcher 1976).

19.2.2 Dynamic/Behavior Methods in the Study of Soil and Litter Fauna

Those methods where animals leave the sample in response to either physical or chemical repellents or attractants are referred to as "behavior methods" (Macfadyen 1957) or "dynamic methods" (Murphy 1962). Behavior or dynamic methods of extracting arthropods from soil are divided into two main types, dry funnel methods and wet funnel methods (Fletcher 1976). Simple dry and wet funnel extractions are usually not labor-intensive. They require no expensive equipment and can yield a wide variety of fauna (Moldenke 1994). The efficiency of both dry and wet funnel extractions is dependent upon the volume of the sample (usually 5–20 g); the greater the surface-to-volume ratio, the more efficient is the extraction. Since the extraction is dependent upon behavior induced in the invertebrate, extraction efficiency is species-specific as well as site-specific (Moldenke 1994). The stimuli responsible for the removal of microarthropods from samples during the extraction process are usually associated with the moisture content of the sample in relation to its water-retaining capacity (Fletcher 1976).

Dry Funnels Funnels can be used to collect smaller and more slowly moving organisms from soil and litter samples when compared with pitfall traps. Funnels have a mesh where the sample is placed and left to dry out and a bottom collecting vial partially filled with killing solution. As the sample dries, an environmental gradient is created within the sample to which organisms differentially respond. The dry funnel apparatus described by Berlese (1905) was one of the first behavior or dynamic methods. The Berlese extractor employed the direct application of heat to a littler sample via a heated water jacket. The surrounding flow of warm water established a temperature gradient throughout the sample, driving the arthropods into a collection vial. Later, Tullgren (1918) introduced a modification to the Berlese

via an electric light bulb as a source of heat to dry out sample material and collect arthropods from it. Macfadyen (1953) further modified the funnel setup to create a high gradient to maximize extraction. In this high-gradient design, samples are placed in bulb-heated funnels that are immersed in a water bath to which ice is periodically added to cool the water, therefore creating a large temperature difference that stimulates the downward movement of organisms to fall in the collecting vial (Moldenke 1994). Around the same time as the development of the Berlese funnel, the Winkler-Moczarski eclector was manufactured (Holdhaus 1910). The Winkler funnels were lightweight and did not rely on electricity, which allowed for the extraction of arthropods from substrate, using a basic setup that comprised a series of thin mesh bags hanging vertically within a funnel casing. The Winkler funnels are collapsible and versatile to take to remote sites, and the sample drying accounts partially for recovery of organisms, as samples need to be periodically mixed to promote the movement of organisms and their fall into the collecting vial (Besuchet et al. 1987).

Berlese-Tullgren funnels (as modernly known) use light to intensify the environmental gradient as the sample dries out and thus accelerate the recovery of organisms (see Fig. 19.1). Heat, coming from a light bulb that is placed on top of the sample avoiding direct contact, is often applied to samples to force the outward movement of fauna (Coleman et al. 2004), but it is expensive and logistically laborious in remote field conditions in comparison to extractions without light. Heat increases sample temperature and speeds drying (Coleman et al. 2004), but it may also burn organisms before their collection, decreasing estimates of their abundance (Walter et al. 1987). While extraction with light is based on photophobia and dehydration, extraction without light is based only on photophobia. It is useful for microarthropods to be extracted from soils using cores placed on a high-gradient-type extractor (Crossley Jr and Blair 1991). Although grab litter or soil samples from different habitat types are useful for initial qualitative surveys, intact soil cores of known diameter and depth are important to report population numbers on a per unit area or unit volume basis. Coleman et al. (2004) recommend a core diameter of 5 cm, preferably collected with a split corer containing an inner sleeve to keep the cores intact (see Fig. 19.4).

Tuf (2015) compared pitfalls and Berlese-Tullgren funnels efficiency to assess Chilopoda diversity and found more chilopods recovered from Berlese-Tullgren than from pitfalls, but some ecological groups such as large lithobiomorphs were unique to pitfalls. Several authors have compared the efficiency of Winkler and Berlese funnels, for example, Owens and Carlton (2015) compared the diversity of Coleoptera and found similar species richness, including similar richness of rare species, although the total richness using Berlese was higher than using Winkler in all but one collection site (see Fig. 19.1).

In assessing the efficiency of Winkler and Berlese-Tullgren funnels to extract arthropods from litter samples, Sakchoowong et al. (2007) compared data at different taxonomic resolution levels, i.e., at the broad arthropod orders/classes level, at the Coleoptera family level, and at the Staphylinidae (Coleoptera), subfamily Pselaphinae genera level. Across all resolution levels, they reported higher total

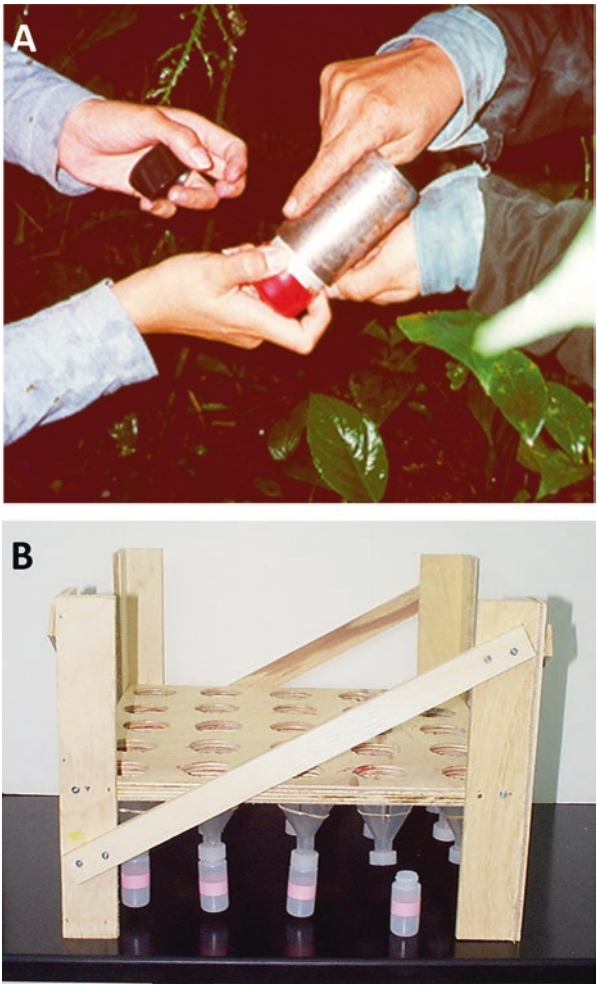

Fig. 19.4 (**a**) Soil cores taken with a double-cylinder hammer sampler are efficient at sampling arthropods due to minimal compaction during sampling. The inner sleeve of the corer is usually aluminum, and placing plastic caps at the ends of inner corer after collection facilitates transportation of the sample from the field into the laboratory, where they can be placed surface side down on a (**b**) high-gradient extractor with light bulbs on top of the samples

arthropod abundance and richness in Tullgren extractions than in Winkler extractions, including the collection of some groups only in Tullgren extractions. For example, almost all Chilopoda, eight Coleoptera families (e.g., Salpingidae, Nitidulidae), and eight Pselaphinae genera (e.g., *Leptoplectus* sp., *Euplectodina* sp.) were only collected using Berlese-Tullgren. Nevertheless, there were similar patterns of dominance and proportions of arthropods common to both methodologies, at all resolution levels. For example, with both funnels Hymenoptera-Formicidae was the dominant group followed by Coleoptera, and arachnids and myriapods were

among the least abundant groups. Also, Staphylinidae (Coleoptera) and *Plagiophorus* sp1. (Staphylinidae, Pselaphinae) were consistently the most abundant groups at the family and genera resolution levels.

These authors compared Winkler with no use of light and Berlese-Tullgren with a 60 W light bulb, therefore modifying the environmental conditions within the samples by allowing Winkler samples to naturally dry out, while Berlese-Tullgren samples were artificially dried out. Over a week, Winkler samples' humidity decreased from 35.2% to 31.6%, while Berlese-Tullgren samples' humidity decreased from 37.5% to 3.6%. These data show that the use of light in Berlese-Tullgren results in a steeper environmental gradient within the sample that produced higher arthropod abundance and richness and the collection of some arthropod groups unique to extraction with light. Nevertheless, both extraction methodologies showed similar efficiency in mirroring the abundance and dominance pattern of arthropods.

Barberena-Arias et al. (2012) compared arthropod extraction efficiencies using light and with no light for litter and soil samples coming from habitats with contrasting temperature and rainfall, i.e., dry and wet forests. For litter arthropods, they used Berlese-Tullgren funnels and found that extraction with light resulted in higher litter arthropod abundance than extraction with no light. On the other hand, for soil arthropods, they used modified metallic funnels covered with wooden ceilings to place samples collected with a soil corer or manually with a shovel. They found that extraction with no light resulted in higher soil arthropod abundance than extractions with light for both manual and corer samples. These data show different patterns of extraction as litter arthropods were better extracted with the use of light, while soil arthropods were better extracted with no use of light.

Furthermore, litter samples that used light consistently recovered more arthropod groups than litter samples with no light (Table 19.1, data from Barberena-Arias et al. 2012). Soil samples collected with corers and that used light during extraction consistently returned fewer arthropod groups than corers with no light and shovel samples. In addition, some arthropod groups were unique to some methodologies, for example, Chilopoda and Ricinulei, both predators, were exclusively recovered with the use of light, while others such as Blattodea were recovered with no light. These data suggest that litter organisms are more resistant to dehydration, therefore requiring a steeper environmental gradient within the sample to exit, such as the one created with the use of artificial light. Soil samples collected manually returned higher number of arthropod groups including some unique groups (e.g., Opiliones) than corer samples suggesting that the use of corers may compact the sample making it difficult for organisms to exit.

Varying results come not only from variations in collection and extraction techniques but also in the time that samples are left to allow arthropods to respond to the gradient within the sample and exit. Delsinne and Arias-Penna (2012) found that increasing extraction time doubled the abundance of recovered arthropods as the sample moisture affected total numbers of recovered arthropods, but the proportion and dominance of species were correctly mirrored. Barberena-Arias et al. (2012) found that the time it took arthropods to exit the sample was higher for samples

Table 19.1 Average abundance (ind/m^2) for arthropod groups collected from litter and soil samples in dry and wet forests in Puerto Rico

Habitat type	Litter				Soil							
Forest type	Dry forest		Moist forest		Dry forest				Wet forest			
Extraction type	With light	No light	With light	No light	Corer with light	Corer no light	Manual with light	Manual no light	Corer with light	Corer no light	Manual with light	Manual no light
O. Acari	1565	453	550	231	270	224	269	301	150	189	100	129
O. Araneae	133	200	225	135			300	100	100	130	113	100
O. Pseudoscorpiones	310	263	180	282	167	114	100	300	100	100	100	100
O. Coleoptera	138	163	160	133	100	100	167	130	100	100	100	100
O. Collembola	1000	455	376	384	180	165	203	212	140	132	157	248
O. Diplura	100	186	100	175		100	100		100	114	100	133
O. Diptera	338	246	294	207	675	100	100	100	100	100	182	122
O. Homoptera	200	150	188	325		125	100	100	100	120	100	100
O. Hymenoptera	1771	300	325	543	117	700	120	263	125	171	167	360
O. Protura	100	100	157	240		114	100	117	100	174	100	100
O. Thysanoptera	217	100	333	100	100		150					
O. Isopoda	100	150	120	173	100	100				117		100
O. Schizomida	100		100	100								
O. Embioptera	100		100									
O. Orthoptera	100			100								
O. Hemiptera	308	155	100			100		150		100		
O. Solifugae	300			100			100				100	
O. Psocoptera	120	100			100	100				100		
O. Opiliones	114	100					100					100
C. Chilopoda	200											
C. Diplopoda		200					100					
O. Blattodea		100										
O. Lepidoptera		100										

O. Ricinulei			100									
O. Isoptera			200						200		200	
O. Neuroptera				100								
O. Palpigradi							100					
O. Thysanura							100					
Average abundance	366	196	212	208	201	170	138	177	120	127	127	141
Number of groups	20	18	17	16	9	12	16	10	11	13	12	12
Total abundance	7313	3520	3609	3328	1809	2043	2208	1773	1315	1647	1518	1692

Arthropod extraction efficiency was compared with and without light and for soil samples collected with a corer or shovel. Letters in front of animal group denominate order (O.) or class (C.) levels of taxonomic classification

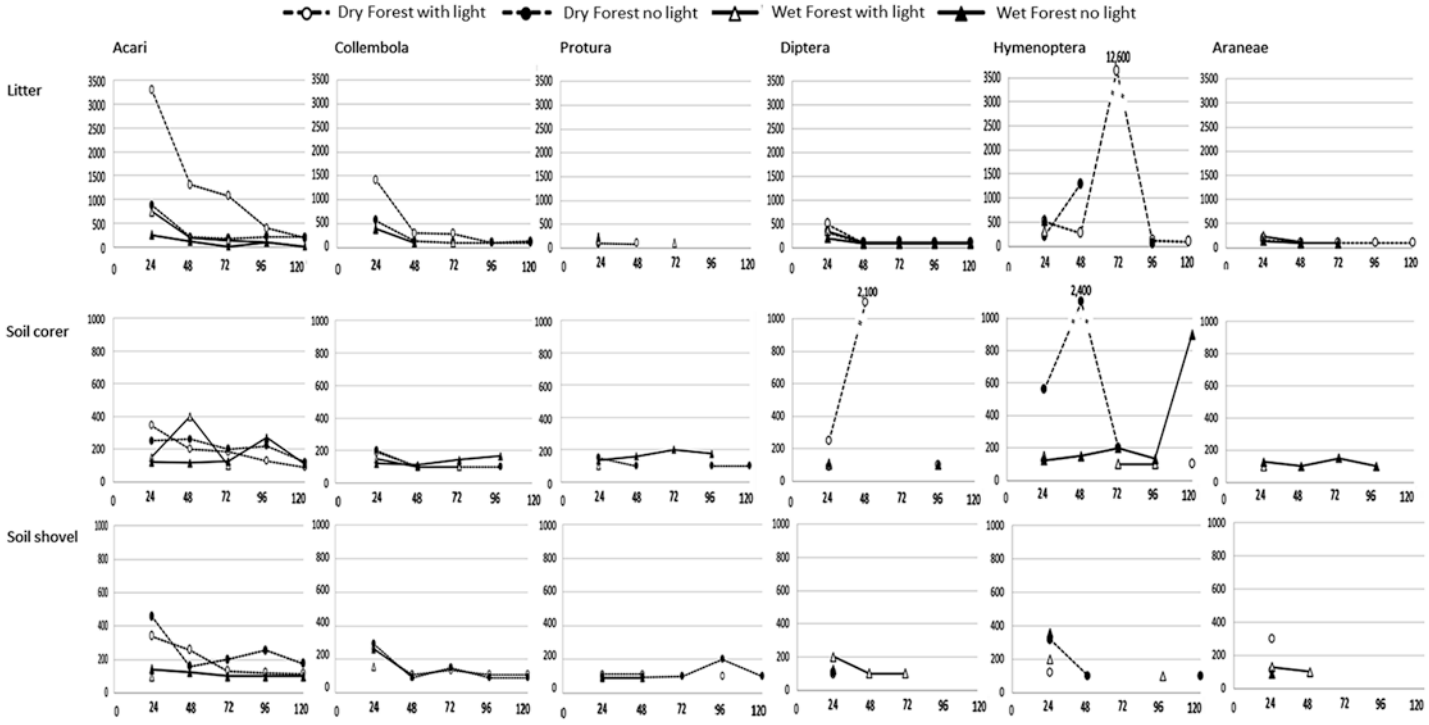

Fig. 19.5 Examples of extraction variation through time. Figures show average abundance (ind/m^2) through extraction time (24, 48, 72, 96, 120 h) for arthropods recovered from litter and soil samples from dry and wet forests in Puerto Rico. Please note that Y-axis scales vary among collection methodologies, also broken lines show outlier abundances, and single points show arthropods recovered only at that time

coming from dry forests than for samples coming from wet forests as 90% of wet forest arthropods exited the samples in the first 48 h, while it took 144 h for 90% of dry forest arthropods to exit the sample. Furthermore, different groups of arthropods showed peak extractions at different times (Fig. 19.5, data from Barberena-Arias et al. 2012). For example, in litter samples, Acari and Collembola progressively exited the sample within the first 24–48 h, but the majority of Hymenoptera left the sample after 72 h. In soil corers, the majority of Diptera and Hymenoptera left the sample at 48 h, while Protura peakextraction occurred in manual no light samples at 96 h.

*Wet Funnels*Wet funnels are used to separate organisms from samples and are particularly effective to recover hydrophilic organisms (Moldenke 1994). This technique is recommended for the extraction of animals extremely sensible to desiccation, works well for the separation of nematodes and rotifers from soil, and can be used to separate nematode larvae from soil samples. Wet funnels are based on the mechanisms originally devised by Baermann (1917). The Baermann apparatus consists of a funnel connected to a clipped rubber pipe. Soil samples are placed inside cloth bags, securely tied up and fastened to an upper supporting rod. Water is added to the funnel ensuring that the sample is completely immersed in the water, and it is left to stand for at least 24 h, and the average extraction is run for 2 to 4 days. Nematode larvae will actively migrate from the sample to the water and then sink to the bottom. The bottom aliquot is then recovered to count and identify organisms (Demelash et al. 2016). Dinaburg (1942) reports that Cort et al. (1922) suggested water to be warmer than the soil to maximize the larval migration from the sample to the water. Warmer water uses the thermotropism of nematodes to separate larvae from the sample and maximize larval recovery (Demelash et al. 2016) for more accurate density estimates.

There are several variations to the wet funnel method that include cold-water extractions, hot-water extractions, sample heating extraction, and duration of the extraction (Didden et al. 1995). The cold-water extraction relies on hydrophilic behavior of organisms but fails to recover inactive life stages such as eggs or pupae (Edwards 1991). The wet funnel method was modified by applying heat with a light bulb to the surface, which speeds the separation process (O'Connor 1955). Milne et al. (1958) (in Moldenke 1994) reversed the temperature gradient by heating the soil sample and accelerating the migration of other invertebrates to the water including copepods, enchytraeids, and tardigrades. Didden et al. (1995) report longer extraction periods, and extraction without heating the sample nor the water to render the highest extraction efficiencies, for samples collected in Germany during the fall. In addition, variations in efficiency can come from different size of organisms and behavior (Edwards 1991) and different soils and environmental conditions to which organisms are adapted.

Chemical Repellents The use of chemical repellents or attractants are dynamic or behavioral methodologies to study soil fauna. One of the most common of such behavior methods involves liquid earthworm irritants applied to soils for extraction at the soil surface. When effective, these liquids can be considered vermifuges (Rhea-Fournier and González 2017). There are toxic and nontoxic options to expel

worms from the soil by means of irritation, and the efficiency of extraction of these vermifuges might depend on the climatic conditions, habitat characteristics, and the animal functional type. The application of dilute formalin to a known area of soil to expel earthworms has been widely used. Reviewing this method, Coleman et al. (1999) concluded that formalin is better for vertical burrowing (anecic) species, less useful for horizontal burrowing species, and ineffective for megascolecid species. In addition, climate restricts the formalin efficacy in cold (below 8 °C) or very wet or dry soils. Furthermore, as the flow path of formalin cannot be determined, it is difficult if not impossible to determine the volume of soil sampled with this method. A nontoxic alternative to formalin that acts largely in the same way is "mustard flour" or "hot mustard" with the active ingredient allyl isothiocyanate. Gunn (1992) concluded that mustard was an effective vermifuge, with better extracting efficiencies than formalin, potassium permanganate, and household detergent. Like mustard, cultivars of the genus *Allium* (onions) produce natural sulfur compounds in high densities that act as irritants to many animals including humans and earthworms. Steffen et al. (2013) tested the application of an onion solution as a vermifuge in both a sandy Ultisol and a clayey Oxisol compared to formalin. Results indicated that 175 g onion extract L^{-1} was the ideal concentration, with higher concentrations yielding less earthworms and lower concentrations being less efficient than formalin.

Other Environmental Stimuli The use of grunting and electroshocking can provide for environmental stimuli that would propel the extraction of invertebrates from the soil based on their reactive behavior. Grunting is a qualitative technique of worm collection used commonly by locals for generations in the Florida's Apalachicola National Forest. It involves driving a wooden stake into the ground and then rubbing a metal bar across the top, sending vibrations down the stake and into the ground. Earthworms emerge up to 12 m away and thousands can be collected in hours (Rhea-Fournier and González 2017). Another earthworm extraction method that shows great potential in limiting both physical soil disturbance and nontarget species effects is that of electroshock extraction. The electroshocking technique often involves stainless steel cylinders driven into the earth attached in a series by copper wires where a known electrical current and timing is applied. Findings by Blair et al. (1995) and Staddon et al. (2003) suggest that any nontarget effects of this method are limited or undetectable, making it ideal for earthworm exclusion experiments that do not aim to reduce other soil fauna. Electrical extraction is the least destructive and thus more desirable among other methods with no reliance on hazardous materials (Rhea-Fournier and González 2017).

19.3 Methodological Considerations for Ecological Studies of Soil and Litter Fauna

Soil organisms are important to many ecosystem processes, such as leaf-litter breakdown, soil formation, and nutrient cycling (D'Haese 2013). The chemical composition of plant residues and the nature of the decomposer community play an important role in decomposition and nutrient availability to plants (Tian and Brussaard 1993).

Faunal influences are strongest in the tropics (Heneghan et al. 1998; González and Seastedt 2001). The amount and quality of the litter layer may control the diversity and action of important soil organisms (Crossley Jr et al. 1992; Wall and Moore 1999; Wardle et al. 1999; González and Seastedt 2001). There are three main levels of litter decomposition control, which operate in the following order: climate> litter chemistry> soil organisms (Swift et al. 1979; Seastedt 1984; McClaugherty et al. 1985; Zak et al. 1990; Lavelle et al. 1993; Aerts 1997; Bengtsson 1998). Decomposition can be considered as a two-stage process. First, litter is broken down by detritivores to small pieces which can be chemically reduced. Second, through the activities of microorganisms (bacteria and fungi), these small pieces of organic matter are further reduced and mineralized into basic inorganic molecules, such as ammonium, phosphate, carbon dioxide, and water (González 2002). These can be taken up by plants or microorganisms, leached out of the system, or, in the case of gaseous breakdown products, released to the atmosphere (Swift et al. 1979; Golley 1983).

Chemical Use for Exclusion Experiments: Use of Naphthalene The addition of naphthalene, a polycyclic aromatic hydrocarbon ($C_{10}H_8$), to suppress soil fauna has been used for decades in the field (Williams and Wiegert 1971; Seastedt 1984). It is generally considered to have several advantages over other chemical biocides. It is volatile; it is water insoluble, which contributes to its immobility and persistence in the soil or litter; and it contains only carbon and hydrogen; therefore, it does not contribute exogenous nutrients (Blair et al. 1989).

Naphthalene additions significantly reduced soil invertebrate abundance and richness at global scale (Wall et al. 2008). A reduction in the total number of litter arthropods ranging between 86% and 99% by naphthalene was found in tropical and subalpine forests with contrasting soil moisture regimes (González and Seastedt 2001). Naphthalene treatment significantly inhibited densities of litter fauna 73–85% and the species richness of litter fauna 15–28% in a subalpine forest of center China (Liu et al. 2019). Naphthalene application suppressed arthropod abundances within litterbags by 60–65% in evergreen broadleaf forest, coniferous forest, dwarf forest, and alpine meadow of southeastern China (Wang et al. 2009). Naphthalene addition significantly reduced the abundance of microarthropod 45–52% in a tall grass prairie ecosystem (Cotrufo et al. 2014). The difference in the efficacy of naphthalene suppression may be due to the different soil fauna densities among these sites. The efficacy of naphthalene additions did not appear to be altered by time (Cotrufo et al. 2014; Liu et al. 2019), implying that soil microarthropods do not adapt to the presence of naphthalene. Surface additions of naphthalene are sufficient to reduce soil microarthropods down to a 20 cm depth (Cotrufo et al. 2014). While nematodes were not affected by the naphthalene additions (Coleman et al. 1994; Xiong et al. 2008; Cotrufo et al. 2014). It is likely an effect of the inability of naphthalene to be dissolved in water minimizing the exposure of nematodes to this biocide (Cotrufo et al. 2014).

Naphthalene represents a C source for soil microorganisms (Blair et al. 1989). Naphthalene may indirectly influence litter and soil processes through possible non-target effects on the microbial community (Newell et al. 1987). There is controversy

about the nontarget effects of naphthalene. Early microcosm studies suggested that naphthalene might directly affect microbial populations and activity and alter nitrogen dynamics (Newell et al. 1987; Blair et al. 1989). By contrast, a field incubation in tall grass prairie ecosystem reported that naphthalene addition has negligible direct effects on microbial abundance, community structure or activity, and C dynamics, although bacteria utilized naphthalene-derived C (Cotrufo et al. 2014). The discrepancy between these results could have emerged from fundamental differences between the two methods. The use of microcosms for studying the effects of naphthalene is not recommended as a model for understanding field experiments because the small volume of the microcosm exposes the soil biota to higher concentrations than under field conditions. During field studies it is not possible to differentiate whether the naphthalene directly affects microbial communities or indirectly through its effects on soil fauna. Nontarget effects of naphthalene may vary substantially in the field with variations in climatic conditions and soil types. A recent field experiment in the subalpine forests of western China showed that the naphthalene treatment had nontarget effects on the active bacterial community abundance (Lan et al. 2019).

*Mechanical Exclosures: Litterbags and Mesh of Different Sizes*Litterbags are often used to determine the effects of soil animals on litter decomposition rates using varied mesh sizes that exclude soil animals based on body size (Kampichler and Bruckner 2009). By choosing adequate mesh size, researchers can restrict or permit access of the three large groups of soil animals, as defined by their body width: microfauna (<0.1 mm), mesofauna (0.1–2 mm), and macrofauna (>2 mm) (Swift et al. 1979). There is general agreement that soil fauna abundance, biomass, and/or composition were significantly affected by mesh size (Brennan et al. 2009; Richardson et al. 2010), with lower abundance (Brennan et al. 2009; Yang et al. 2017) or biomass (Richardson et al. 2010) in the small mesh litterbags. But a study reported that the densities of major groups of litter arthropods did not significantly differ between small and large mesh size litterbags (González et al. 2003).

However, there are two main drawbacks to this technique. First, the faunal community surrounding the litterbags has not been excluded despite potentially large indirect effects of their activity on biotic and abiotic processes that control litter decomposition at the habitat scale (Bradford et al. 2002). It has been reported that increased soil-faunal functional complexity surrounding the litterbags inhibited litter decomposition by microfauna, bacteria, and fungi by ca 11.5% (Bradford et al. 2002). To predict reliably the effects of changed faunal community composition on litter decomposition requires experiments that exclude faunal groups from both the experimental litter and the habitat where the litter is placed. Second, larger mesh sizes will allow proportionally more litter fragments to exit the bags than smaller mesh sizes (Frouz et al. 2015). Loss of undecomposed fragmented litter was 10% higher from the coarse mesh (2 mm) than the fine mesh (50 μm) bags (Bradford et al. 2002). Additional approaches are required to determine the relative contribution of undecomposed fragments being lost from coarse mesh bags to litter mass loss from litterbags.

Concerns have been raised regarding microclimatic and leaching losses that can co-vary with mesh size and that might affect mass loss rates from litterbags independent of soil animal effects (Kampichler and Bruckner 2009). Enclosing the litter in bags may change the microclimatic conditions, which may then affect the activity of the soil biota (Bradford et al. 2002). However, Bokhorst and Wardle (2013) indicated that microclimatic differences between litterbags of different mesh sizes were minimal, and the effect of mesh size on litter mass because of UV radiation loss is likely to be minor in most cases. Fine mesh size physically protects confined litter against abrasive forces (e.g., wind, precipitations, water flow, etc.) (Lecerf 2017). Hence, leaching of soluble compounds could be different between litterbags of different mesh sizes. But previous study suggested that there was a small difference in leaching losses among different mesh sizes (Bokhorst and Wardle 2013). The effect of litter mass loss in the absence of soil animals is similar between mesh sizes, so method of excluding soil fauna through different mesh size litterbags is proven to be reliable methods for studying the soil fauna (Bokhorst and Wardle 2013).

19.4 Concluding Remarks

Now more than ever, understanding the role of soils and soil organisms in cycling carbon and other nutrients that shape our terrestrial ecosystems is important. Climate strongly affects the composition and activity of soil organisms, and they in turn affect the rates of turnover among carbon pools. The consequences of changing climate and land use affect natural processes as well as global society. Within this context, the study of soil ecology and health remains a relevant field of research. The study of soil biology as all other ecological sciences faces many challenges due to current acceleration of changing environments. One of the critical needs in many ecological fields is the identification of species (Anderson 1975; Eisenhauer et al. 2017). For soils, the relative importance of small organisms means that the number of species is very high, and thus the soil is the natural habitat for millions of species of bacteria, fungi, arachnids, arthropods, and worms that make up the broad functional groups that are widely studied in soil ecology (Andrén et al. 1995; Wardle 2006). A challenge faced in soil research is that no singular method or tool can be used to describe the abundance and diversity of life and functions that inhabit the litter and soil environments. We hope this review of methodologies for the study of soil and litter fauna will help elucidate the most appropriate technique to use based on resources at hand, organism of interest, and habitat characteristics.

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