A critical review of the microcosm method in soil animal ecology

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The microcosm method is widely used in research on soil animal ecology because it allows for controlled manipulations of fauna, climate, and soil properties. However, differences in biotic and abiotic conditions between the microcosm and the natural soil ecosystem it is intended to represent may bias the experimental results. On the basis of literature, potential influences of the following factors were discussed: (1) Microcosm volume and duration of the incubation period. (2) Preparation of the microcosm substrate, with emphasis on the choice between homogenised substrate and intact soil cores. (3) Incubation conditions (moisture, temperature and drainage). (4) Biotic diversity in the microcosm soil, with emphasis on (a) the general problem of low biodiversity in microcosms, (b) the unnatural situation in microcosms without plants, (c) differences in microflora composition between "faunal" and "non-faunal" treatments, and (d) relative densities of the organisms present. Possible ways of dealing with a few of these problems were also discussed.

Key words: Microcosm, soil fauna, methods

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INTRODUCTION

Studies of soil animal ecology often require that the animals of interest live in more or less natural surroundings while environmental factors of importance to their behaviour, function, and survival are manipulated. Since this is difficult in the field, a widely used approach is to construct microcosms, i.e. artificial soil ecosystems in the laboratory. In these systems, parameters such as temperature, moisture, substrate, and the input and output of organisms and chemical substances, can be controlled. This makes it possible not only to measure the inputs and outputs of the system more easily, but also to vary one factor at a time while the others are kept stable. An additional advantage is that ecosystem responses to toxic chemical substances can be studied without releasing these substances into nature.

However, it is very probable that the results from microcosm experiments are affected by the necessarily somewhat unnatural biotic and abiotic conditions in a microcosm soil. In addition, varying one ecological factor between treatments could lead to unintended changes in other parameters. It is important to be aware of such problems when constructing, running, and interpreting microcosm experiments, and the purpose of this review is to discuss the most important of these potential side effects.

Some microcosm studies deal with a very small number of known species (e.g. Elliott et al. 1980, Anderson et al. 1981), in which no simulation of a natural ecosystem is attempted. This means that it is difficult to extrapolate the results to a field situation, but also that there is relatively little danger that the artificiality will lead to a misinterpretation of the results. Such studies are outside the scope of this review. However, when studying general ecosystem effects of factors such as temperature, moisture, chemical substances, or the presence or absence of various organisms, it is often necessary to use microcosms containing a large and partly unknown number of species, particularly among the microflora (e.g. Anderson et al. 1983, Setälä et al. 1988, Bengtsson et al. 1988, Hågvar 1988, Persson 1989, Huhta & Setälä 1990, Setälä et al. 1991, Setälä & Huhta 1991, Teuben 1991, Sulkava et al. 1996, Huhta et al. 1998b). In such experiments, where the microcosm is often intended to resemble a natural soil ecosystem as closely as possible, there is a large number of potential interactions between different organisms, as well as between organisms and abiotic factors. The greater the number of unknown variables in the experiment, the more probable it is that the artificial conditions will bias the results in a manner not provided for. For this reason, the following discussion focuses mainly on microcosm use in research on soil animal ecology in systems involving a large diversity of interactions between soil organisms.

MICROCOSM SIZE AND DURATION OF INCUBATION

Different soil organisms have different activity patterns, and may require different amounts of space or time to observe their activities. This could influence experimental results when microcosms are used. Several authors have discussed points relating to this:

As pointed out by Elliott et al. (1986), "we must understand how organisms deal with their environment at the level of resolution at which they experience it. Even if we average over a square meter or centimeter, we may not obtain a clear understanding of the factors that directly affect the microorganisms: a size scale of millimeters and micrometers should be considered.

Soil organisms can indirectly affect landscape formation. For example, the presence of earthworms can cause greater infiltration which results in less erosion and which, over a long enough period of time, will have a considerable effect on the landscape."

Another possible effect that could be difficult to observe in a small area was suggested by Satchell (1974): "Perhaps the most important consequence of the disintegration of litter by arthropod feeding is the flattening of the topography of the litter layer so that material, initially subject to the fluctuating and relatively low humidity of the ambient atmosphere, is transported into the higher and more stable humidity near the soil surface."

An aspect of the time problem was commented upon by Beyers & Odum (1993, p. 14): "--- there are animals with large territories which have large, although infrequent actions on a small-sized system. However, when a small ecosystem is enclosed in a container, the action of the larger realm may be excluded. Thus, influences acting over a longer period tend to be excluded."

Problems like those indicated above may be more or less inevitable whether the researcher uses microcosms, field studies, or other methods, since any time or space scale will probably be better suited to highlight some ecosystem interactions than others. In addition, there may be extra scale effects in microcosm studies, depending on the chosen substrate volume, surface area, and incubation period:

Some ecological interactions may occur after others in a time sequence, leading to erroneous conclusions if the experiment is terminated too soon. According to Setälä (1995), who discussed the interactions between soil fauna, ectomycorrhizal fungi and plant growth, "there is a time lag until the improved nutrient mineralization mediated by soil fauna will be reflected in enhanced primary production, which in turn induces a positive feedback by nourishing the detrital food web with litter and root exudates of greater amount and better quality [---]. Thus, a momentary negative influence of mycorrhizal grazers on the fungal biomass can become positive for the mycorrhizal fungus with time through an indirect mutualistic relationship between soil fauna and plants."

According to Seastedt (1984), microarthropods could perhaps have a greater importance for decomposition in recalcitrant materials than in more easily decomposable substrates. Short-term studies, in which most of the respiration activity comes from the decomposition of easily degradable substrates, could therefore underestimate the microarthropod effect on litter decomposition. Seastedt (1984) also suggested that although some short-term studies that show a positive microarthropod effect on litter mass loss may not show a similar arthropod effect on mineralisation of elements such as nitrogen, this could be different in the long run. This is because high initial C:N ratios of the litter cause rapid immobilisation of mineral N by the microflora in the early stages of decomposition, whereas decreasing C:N ratios in the substrate with time, after much of the carbon has been lost through respiration, will facilitate net nitrogen mineralisation (cf. Seastedt 1984).

When partially or totally sterilised soil is reinoculated with fungi and bacteria, there may be an initial period of high metabolic activity in the microcosms before the system stabilises. This could be caused partly by rapid decomposition of the animals and microflora killed by the sterilisation, or (if the substrate was frozen) of the contents of plant cells made accessible because of cell wall rupture during freezing (cf. Swift et al. 1979 p. 256). In the microcosm experiment conducted by Setälä et al. (1988), for instance, the respiration rate in the microcosms diminished considerably during the first eight weeks after microflora reinoculation of the substrate, and then stabilised. In Sulkava & Huhta (1998), this initial phase lasted about two weeks. On the other hand, a long incubation time could also be a problem, as observed by Setälä & Huhta (1990), who found that the initial positive effect of soil animals on the litter decomposition rate became negative after about a year of incubation. The reason could be that readily decomposable substrates had been used up faster in some experimental treatments than in others, or because of structural changes in the substrate such as accumulation of mesofaunal faeces (Setälä & Huhta 1990). The exact duration of such incubation phases (i.e. initial "flush", stabilisation, exhaustion), will presumably vary with the type of substrate and organisms present. Since new litter is often not added during the course of microcosm experiments it is relevant to mention the comment by Verhoef (1996), quoted in the discussion of substrate treatment below, that the effect of soil animals on litter decomposition depends on the degree of breakdown of the litter.

The microcosm soil will usually be enclosed in a plastic or glass container. Water transpired by plants in the microcosm could condense on the sides of the container, leading to moister conditions on the edges than in the middle (e.g. Setälä & Huhta 1991). Likewise, if intact soil cores are used in the microcosms, the necessary disturbance of the edges of the core when removing it from the field site may to some extent influence the nature of the core. The percentage of the substrate affected by this will depend on the surface area of the microcosm. In addition, increasing the volume of a microcosm substrate, or decreasing the surface/volume ratio, could increase the nitrification rate due to stimulation by the higher accumulation of CO_2 inside larger samples (Clark 1968).

Species diversities might also be affected by the microcosm volume. Diminishing species diversities with time is a general problem in microcosms (Hågvar 1995), and it seems likely that this decline may be more rapid in small than in large microcosms (cf. Hågvar 1995).

PREPARATION OF THE MICROCOSM SUBSTRATE

The substrate of the microcosms can, generally speaking, be added in at least two ways: Either (a) an intact soil core can be put in each microcosm box, or (b) the substrate can be mixed before being added to the microcosms in one or several layers.

Method (a) has the advantage of preserving the soil structure and a reasonably diverse assemblage of microhabitats, thus making the habitat for both microflora and animals more "natural". However, it may result in large differences between the replicates, so that a greater number of replicates may be needed to achieve the same level of significance in the results as for method (b).

Method (b) provides a roughly equal substrate in all microcosms, but the soil structure is destroyed. The homogenising will probably cause a reduction of microhabitat diversity in the microcosm soil, which in its turn could cause a reduction of both animal and microbial species richness. Mikola & Setälä (1998) and Setälä et al. (1991) attempted to compensate for this in their microcosms by mixing the main substrate (raw humus soil from a coniferous forest) with crushed birch leaves to make the environment more varied. If the substrate is dried and sieved as well as mixed, as it often must be to enable an equal amount to be added to each replicate, there may also be other changes in the substrate: Taylor & Parkinson (1988) pointed out that homogenisation of the microcosm substrate "must change properties of water and gas movement and retention, and microbial movement and growth through destruction of microstructure", and also that carbon and nitrogen mineralisation rates may change. Ausmus & O'Neill (1978) found that the CO_2 production rates in intact and homogenised soil cores were not significantly different over a sample period of 49 days, but that the leaching loss of dissolved organic carbon from intact soil cores was significantly lower than that from homogenised cores. Ausmus & O'Neill (1978) also found a higher variance in dissolved organic carbon loss between replicates in the homogenised soil than in the intact soil cores, and suggested that "disruption of bio-physico-chemical interactions within microsites" caused by the homogenisation, and variation in soil compaction between the homogenised replicates, might explain this variation. If homogenised soil is subject to high variance in dissolved organic carbon loss in general, this may be a good argument for using intact soil cores, since one main reason for homogenising the soil is to reduce the variation between replicates within a treatment. Ausmus & O'Neill (1978) concluded that intact soil cores in microcosms are an acceptable choice, and that CO_2 production alone is not sufficient as a measure of soil carbon dynamics.

If homogenising the soil changes the average pore size, this could perhaps change the relative abundances of animal species and thus the magnitude of some species interactions, since a large proportion of small pores could restrict movement for larger species and provide refuges from predation for smaller species (Elliott et al. 1980). If the microcosm contains several types of substrate material, the experimental results could depend on whether the substrates are mixed or present in separate patches, as shown by Sulkava & Huhta (1998), who measured litter decomposition with and without mesofauna in microcosms with two distinct types of substrate organisation. Each microcosm contained four types of plant litter, but this was grouped in two different ways: Either as four patches with one type of litter each, or as one large patch in which all four litters were mixed. They found that the decomposition rate in the mixed litter was lower than in the patchy litter when fauna was absent, but higher than in the patchy litter when fauna was present. This was attributed partly to effects of the distance between the different litters in the patchy treatment: "--- the distance that the animals must travel when moving from patch to patch (i.e. several centimeters), may be too great for a constant transport of material and nutrients between patches, and thus for efficient stimulation of decomposition. [---] The distance of millimeters between different litter particles in the mixed litter is easily covered by microarthropods and enchytraeids, and so the fauna can more effectively exert their influence at this scale." (Sulkava & Huhta 1998.) It was also thought that the smaller positive animal effect in the patchy litter could be related to the effects of grazing on fungi: Fungi "have the ability to translocate nutrients from a high-nutrient source [---] to a high-energy material [---], and so contribute to the decomposition of both kinds of substrate", and grazing animals might therefore have lowered the fungal activity by disrupting the fungal connections between patches with different litter types (Sulkava & Huhta 1998).

On the other hand, substrate homogenisation might also lower the ability of soil fungi to compensate for grazing. Bengtsson et al. (1993) conducted an experiment in which two species of fungi were added to sterilised soil in a maze of vials and tubes, creating "patches" of fungi separated by sterile areas. Fungivorous springtails (*Onychiurus armatus* (Tullb.)) were introduced to the maze, and the effects of fungal patch size and distance between patches on the total fungal respiration activity were measured. Bengtsson et al. (1993) found that patchiness led to an increase in the total respiration in relation to a control in which the fungus was homogeneously distributed, and concluded that a fragmented distribution may increase the effect of the mechanisms by which fungi compensate for grazing, by allowing for regrowth during periods of low disturbance when the grazers feed in other patches.

The results of Bengtsson et al. (1993) and those of Sulkava & Huhta (1998) (discussed above) do not seem to agree. As a suggestion, the difference could perhaps be partly related to the fact that the fungal patches in Bengtsson et al. (1993) all grew on the same type of substrate. Nutrient translocation in the hyphae between different patches may therefore have been less important than in the study by Sulkava & Huhta (1998).

Mixing different layers in the soil horizon could also create problems. Anderson et al. (1985) observed that the effect of soil animals on nitrogen mineralisation was larger in the L layer than in the F and H layers of the soil horizon. "The partitioning of these materials according to time-depth relationships is therefore critical for exper-

imental work since the combination of resource types with different net nitrogen mineralisation potentials will influence the interpretation of animal and microbial effects. [---] We now feel that small differences in the combination of these resources, whether a feature of sampling procedure or through seasonal variations in animal activities, can influence the outcome and interpretation of these experiments." (Anderson et al. 1985.) A similar comment was made by Verhoef (1996), who noted that "The effect of the addition of soil animals into these [i.e. microcosm] systems differs with the degradation phase of the litter: in the early stages these soil animals decrease decomposition and cation leaching and increase N immobilization. In later stages they increase decomposition, cation leaching, and N mineralization. These effects are density dependent: higher densities of soil animals tend to decrease these effects, underlining the importance of *combining* these studies with studies of naturally fluctuating population densities."

ABIOTIC CONDITIONS IN THE MICROCOSMS

Since the microcosms are generally kept indoors in the laboratory, the climatic factors of the soil environment, i.e. temperature, moisture, and light, must be simulated.

The moisture level of the microcosm soil is clearly of importance. For example, some soil invertebrates are air-living, whereas others live in the soil water, and changes in the moisture content will result in changes in the relative suitability of the environment for the animals. This is illustrated in an experiment by Sulkava et al. (1996), where microcosms containing microflora, microfauna, and the enchytraeid *Cognettia sphagnetorum* (Vejd.), were incubated with or without the additional presence of a diverse microarthropod fauna, at different temperature and moisture levels. In wet conditions, the presence of the microarthropods had no effect on the numbers of enchytraeids or nematodes in the microcosms, but in dry conditions the numbers of both enchytraeids and nematodes were lower when microarthropods were present than when they were absent. At the end of the experiment, both nematodes, enchytraeids, and most microarthropods were more numerous at the highest temperature (15°C) than at the lowest (2°C) (Sulkava et al. 1996). Similarly, Huhta et al. (1998b), studying several different combinations of *C. sphagnetorum* and microarthropods in microcosms at varying moistures, found that the presence of a mixed microarthropod community resulted in lower enchytraeid populations, especially at low moisture levels, whereas the presence of *C. sphagnetorum* did not affect the microarthropod fauna at any moisture level.

In a microcosm experiment by Persson (1989), in which the influence of soil arthropods on carbon and nitrogen mineralisation of organic soil from a spruce stand was measured at various levels of temperature and moisture, it was found that the absolute contribution of the arthropods to nitrogen mineralisation was more or less the same at all moisture levels in the study. Since the total N mineralisation was much higher at high moisture (60% of WHC) than at low moisture (15% of WHC), this meant that the relative influence of the arthropods on the nitrogen mineralisation was much larger in the dry than in the wet soil.

In the field, the soil moisture and temperature will fluctuate according to weather and season, and it is difficult to reproduce this accurately on an artificial basis. This may influence the decomposition process: Teuben & Verhoef (1992) conducted a study in which soil microcosms and field studies were compared and found that "in the microcosms respiration and cellulase activity is double than that in the field, probably partly due to the higher experimental temperature, absence of a diurnal rhythm or moisture fluctuations in the microcosms." Also, as commented by Taylor & Parkinson (1988), shallow microcosm soil cannot draw moisture from water in deeper soil layers and may therefore dry out if not watered enough. Taylor & Parkinson (1988) suggest some ways of dealing with this problem.

Even if one succeeds in creating the appropriate moisture conditions, there is the problem that the animals can migrate vertically or horizontally to some extent in a natural soil when the conditions in one site are unsuitable, while this clearly is not always possible in a microcosm where, in addition, the homogenisation of the substrate that is often done will reduce the amount of microhabitats with different moisture levels.

The temperature regime could be a problem for the same reason: The researcher must necessarily choose a particular temperature range for the incubation of the microcosms, and in a system involving a diverse microflora and fauna the chosen temperature may not be equally acceptable to all species. This could possibly mean that the species whose temperature optimum is close to the incubation temperature may gain an unnatural advantage over those whose temperature preference is further from the incubation temperature.

If the drainage in the microcosms is not sufficiently efficient, the natural leaching away of such substances as nitrate may be reduced, and the substances could accumulate in the microcosm soil. In the same way, an absence of plant roots could also lead to high nutrient concentrations in microcosms (cf. Faber & Verhoef 1991, Mikola & Setälä 1998). It seems plausible that this, in some cases, might affect not only the amount of available nutrients for the microflora (and plants, if these are present), but also possibly the pH of the soil water and the osmotic balance for the soil water fauna and microflora.

COMMUNITY STRUCTURE OF MICROFLORA, ANIMALS, AND PLANTS

Diversity of organisms

The diversity of both animals, plants, and microflora in a laboratory system is necessarily lower than the diversity in a natural soil ecosystem. According to Drake et al. (1996), it may not be possible

to create a natural species assembly by introducing organisms into sterilised or defaunated soil: "Assembly processes have proven so important to community development that laboratory attempts to reconstruct an extant community state from the component species alone generally fail [---]. As a consequence, serious community reconstruction and restoration attempts necessarily involve the production of a number of preliminary states that include species and community configurations not present in the final community." (Drake et al. 1996.) Even if one should succeed in introducing a relatively realistic species diversity at the outset of the experiment, extinction within the microcosms coupled with an absence of immigration will gradually lead to a situation with relatively few species (cf. Hågvar 1995). The latter problem could perhaps to some extent be reduced by "multiple seeding" (Beyers & Odum 1993, pp. 7, 47), i.e. introduction of "many more species than are needed or sustainable", from different areas of a similar type, to the microcosms, or by "cross seeding" (Beyers & Odum 1993, p. 9), i.e. regular transfer of contents between replicate microcosms to counter differences in development between replicates.

In microcosms with a low number of trophic groups, the effects of an organism on, for instance, decomposition or plant growth may not be the same as in situations with a larger number of trophic groups (cf. review by Huhta et al. 1998a). For an animal, for example, it may be that the organisms preferred as food are absent in the microcosms, so that the diet of the animal is not representative of a natural soil. Alternatively, the preferred food source could be enormously abundant in the microcosms owing to reduced competition, leading in its turn to an unrealistic number of the animal in question. The absence of competitors or predators in the microcosms could have a similar effect (Petersen & Luxton 1982 p. 355, Setälä 1990, Hågvar 1995, Huhta et al. 1998a).

Low biodiversity in a microcosm may also lower the efficiency of nutrient cycling when life conditions change, by restricting the possibilities of successional replacement of dominant species and takeover of their functional roles by other species that were originally relatively rare: "By facilitating an efficient substitution of species, a gene pool helps maintain functions during times of change. After initial colonisation has maximised what can be done with explosive exponential and exclusionary stages of growth, succession tends to develop specialisation and diversity [---] A pool of extra species allows continual fine-tuning of ecosystems in response to small changes. In ecological theory, as in economics, a greater diversity of processes and functions facilitated by a diversity of specialists, contributes to improved total system function." (Beyers & Odum 1993, p. 91.)

Setälä (1995) studied the interactions between soil fauna, ectomycorrhizal fungi and the growth of birch and pine seedlings in microcosms, and found that the plants grew better in the presence of soil fauna even though the biomass of the mycorrhizal fungi decreased with animals present. He commented that this result did not agree with those found by several other researchers (who found that the growth of mycorrhiza-infected plants was reduced at high faunal densities), and suggested that the disagreement might be caused by the fact that the other studies he cited used single-species populations to represent each functional group, while Setälä (1995) used more diverse communities as regards both mycorrhizal fungi and animals. According to Setälä (1995), "there is evidence that increased complexity of detrital food webs is associated with improved decomposition and nutrient turnover rate [---] and primary production [---]. Therefore, it is not surprising that results originating from experiments with fundamental differences in community and food web configuration give rise to contradictory interpretations."

In microcosms without plants, such factors as carbon-rich root exudates, a separate rhizosphere microflora and fauna, herbivorous animals, and mycorrhizal and phytopathogenic fungi, will be scarce or completely absent, resulting in a soil environment that is rather different from that in a naturally occurring soil. Mikola & Setälä (1998) commented that "Studying decomposer food webs in the absence of plants is somewhat problematic, since plants offer microbes energy in the form of litter and root exudates, and also compete with them for nutrients. As a result, heterotrophic experimental decomposer systems tend to suffer from energy shortage and commonly become heavily loaded with nutrients." To compensate for this problem, Mikola & Setälä (1998) added glucose to their microcosms, commenting that "When sugar is offered, microbes can immobilize available nutrients, which eventually decreases the amount of free nutrients found in soil."

According to Huhta et al. (1998a), it is even possible that stimulation of N mineralisation by the soil fauna, which has often been the subject of microcosm studies without plants (e.g. Huhta et al. 1988, Persson 1989, Abrahamsen 1990, Setälä et al. 1990, Sulkava et al. 1996), might be relatively unimportant for nutrient cycling in boreal forests. The major part of the nitrogen supply of trees in these forests may be by uptake of organic N by ectomycorrhizal fungi, and correspondingly faunal effects on the growth of mycorrhizal fungi through grazing may be more important than faunal effects on N mineralisation (Huhta et al. 1998a).

Even if faunal effects on N mineralisation are presumed to be important, the presence or absence of roots might be crucial for the results: Faber & Verhoef (1991) studied litter decomposition with different animal assemblages in field enclosures with and without pine tree roots. They observed that "In rooted plots, particularly in the L- and F-layers, N tends to increase in fauna treatments relative to the defaunated control, as opposed to a frequent tendency towards a fauna effected decrease in N relative to the control in non-rooted plots." In other words, the faunal effect was reversed depending on whether roots were present or absent.

If plants are added to microcosm systems, however, yet another unknown factor is introduced to the experimental situation. For example, Setälä & Huhta (1991) conducted a microcosm experiment to study the effect of the soil fauna on the growth of birch

(*Betula pendula* Roth) seedlings, and commented that if the soil animals in the treatment with fauna stimulated microbial respiration throughout the incubation period (this could not be determined), "the plants in the refaunated microcosms may have benefited from the increased CO_2 concentrations inside the chambers".

In microcosm studies where plant growth is studied in treatments with and without soil animals, an increased CO_2 concentration in the replicates with fauna could perhaps also lead to a higher transfer of carbohydrates from the plant roots to the soil, which again would stimulate parts of the microflora (Körner & Arnone 1992, Diaz et al. 1993; cited by Lawton 1995). This factor could perhaps lead to somewhat misleading results, since such effects would not occur in the open air in the field.

Microflora composition

Many soil microcosm studies involve separate treatments with and without animals. In this case, the method used to remove unwanted organisms may influence the treatment effects by changing the later composition of the microflora. To study effects of the presence and absence of soil meso- or macrofauna, without specifying the nature of the microflora, it may be possible to use a partial sterilisation, for instance by heating, freezing/thawing, or drying/rewetting (e.g. Bengtsson et al. 1988, Setälä et al. 1988, Persson 1989, Abrahamsen 1990, Huhta & Setälä 1990, Teuben & Roelofsma 1990, Teuben 1991, Setälä et al. 1991, Huhta et al. 1998b), which eliminates most animals but leaves some of the microflora intact. However, since this procedure could lead to unintended changes in the substrate, such as rupture of plant cell walls if the substrate is frozen when moist (cf. Swift et al. 1979 p. 256), and since it may fail to eliminate parts of the microfauna (Setälä 1990), the substrate could also be sterilised completely with gamma rays (e.g. Hågvar 1988). If the animals to be studied are earthworms, slugs, or other relatively large animals that can be removed by hand-picking or sieving, it may be unnecessary to sterilise the substrate at all (e.g. Haimi & Huhta 1990).

Defaunated microcosms can be reinoculated with microflora by (a) introducing just a few selected species of bacteria and fungi to a completely sterile substrate (e.g. Elliott et al. 1980, Anderson et al. 1981, Mikola & Setälä 1998), or (b) adding an unspecified and reasonably diverse microflora (e.g. Hågvar 1988, Persson 1989, Brussaard et al. 1990, Setälä 1990, Sulkava & Huhta 1998). In alternative (a), one has the advantage of knowing exactly which species are present, which makes it easier to know what is happening in the microcosms, but the system will be so simplified that it is difficult to compare it with a natural soil. In alternative (b) one may get a more realistically composed microflora, but the fact that one cannot know exactly what is in the filtrate might cause some problems:

Firstly, the filtrate will probably contain microfauna such as protozoa and small nematodes. In experiments comparing microcosms with or without soil mesofauna, this could lead to large abundances of the accidentally introduced microfauna in the "non-faunal" treatments, due to the absence of predators or competitors (Setälä 1990). Effects of these accidentally introduced animals in "non-faunal" treatments could cause misinterpretations of the effects of a purposely introduced fauna in other treatments (Setälä 1990). A mesh size that is small enough to shut out these animals will probably also be too small to allow a reasonable diversity of bacteria and fungi in the filtrate: According to Bamforth (1988), flagellates and small amoebae can occupy soil pore spaces down to a diameter of 8 μm.

Secondly, the different species of bacteria and fungi that are introduced to a sterilised habitat without competition might not all have the same opportunity of colonising the habitat because of differences in reproduction rate. Fast-reproducing, "opportunistic" bacteria and fungi could achieve an unrealistic dominance in the microcosms at the cost of more slowly reproducing species, which as a rule are better adapted to extracting energy from the more resistant substrates (Garrett 1951, 1963, cited by Swift et al. 1979 p. 82). However, it may be possible that species belonging to later successional stages that are introduced in small numbers at the start, may find better opportunities when the microcosm system has stabilised (cf. Beyers & Odum 1993, p. 91).

Many soil animals may carry viable bacteria and fungal fragments in their guts or on the outside of their bodies (e.g. Visser 1985, Moore 1988). Adding animals to partially or totally sterilised soil, to which a microflora filtrate has been introduced, could therefore result in accidental introduction of extra bacterial and fungal species along with the animals. It seems possible that a relatively large proportion of these extra species could be present on or in the animals because the animals prefer them as food, and the densities and growth rates of such microflora species may therefore be more heavily influenced by faunal grazing than the density or growth rate of most other species. If the study also involves control treatments without animals, some of these microflora species could be absent in the controls, and the study results might exaggerate faunal effects. Some mechanisms of faunal addition, such as extracting a diverse arthropod fauna from fresh soil cores directly into defaunated microcosms (e.g. Hågvar 1988, Setälä et al. 1988, Persson 1989, Huhta & Setälä 1990), might be more vulnerable to such problems than other methods.

An example related to the above is accidental introductions of protozoa in earthworm guts: Bamforth (1988) referred to various studies that indicated that protozoa may be a part of earthworm diets, and that protozoan cysts (as opposed to active protozoa) can pass through the earthworm intestine unharmed; and suggested that "the following scenario can be developed for protozoan-earthworm relationships. Ingested material contains active and encysted protozoa. The former are digested by the worm and the latter pass out in the feces. Under moist conditions, some of the protozoa excyst and multiply as they exploit the bacteria in the feces. Worms re-ingest feces, again digesting the active protozoa and passing out the protozoan cysts in their feces. Thus, earthworms can obtain maximum

nutrition from organic matter they ingested initially." In other words, what appears to be the effect of earthworms alone in the microcosms is actually a combined effect of earthworms and protozoa introduced along with the worms.

Balance between species populations in the microcosms

Evidently, in a simplified microcosm ecosystem, one cannot expect that the artificial additions of microflora and fauna will be balanced exactly like the population densities in the field. For example, species with short generation times and a large number of offspring may reach unrealistic abundances at the cost of species with slower reproduction rates when they are added to previously sterilised soil. According to Drake et al. (1996), the species compositions and dominance hierarchies of organism communities in the field are partly a result of the history of a specific site, and "Our studies of assembly, for example, show that radically different community configurations are possible simply by assembling communities using different sequences of species introduction" (Drake et al. 1996).

The outcome of the interactions between different organisms may to some extent depend on the relative biomasses of the species present (Huhta et al. 1998a). Furthermore, since functional diversity in a microcosm is not necessarily the same as taxonomic diversity (Huhta et al. 1998a), the total biomasses of several species in the same trophic group might perhaps overexploit a food source in a similar way to a high biomass of one species.

An example of such a density effect is grazing by soil animals on bacteria and fungi. If the grazing pressure is sufficiently low, the net result may be an increase in microbial activity ("apparent predation", Moore 1988), possibly due to changes in the fungal growth form (Hedlund et al. 1991), or to release through grazing of scarce nutrients that are immobilised in bacterial and fungal tissue and therefore unavailable to the actively growing microflora (cf. Moore 1988). That this effect depends on the grazing pressure is seen, for instance, by the results of Hanlon (1981), who allowed different numbers of the springtail *Folsomia candida* (Willem) to graze on colonies of the fungus *Botrytis cinerea* (Pers ex Fr.) kept at different nutrient levels. Grazing resulted in decreased fungal respiration at low substrate nutrient levels, with the largest number of animals resulting in the largest decrease, whereas grazing at high nutrient levels increased fungal respiration with the largest number of animals resulting in the largest increase. At intermediate substrate nutrient levels a low grazing pressure resulted in a higher fungal respiration rate than both the high grazing pressure treatment and the treatment without animals.

Studies where animals are added to some, but not all, treatments will also involve a higher absolute amount of animal tissue, and therefore of nutrients, in the faunal than in the non-faunal treatments. Death and decay of these extra animals could give a fertiliser effect in the animal-containing microcosms (cf. Abrahamsen 1990, Haimi 1993).

CONCLUDING COMMENTS

The many potential inaccuracies associated with the use of soil microcosms lead to the question of whether this research method is a sufficiently good simulation of a natural soil to be useful in research on complex ecosystem functions involving many parameters. However, although it might be better in theory to study the ecology of soil animals in their natural environment, this approach is difficult since their feeding habits and other aspects of their behaviour cannot be observed directly, and because of the high number of potential biotic and abiotic influences on the various species. Although microcosms have several drawbacks, the practice of simplifying the soil ecosystem and studying one or a few factors at a time under controlled conditions may therefore give information that is difficult to obtain by other methods.

On the basis of the points discussed previously, a few suggestions might be put forward regarding some of the choices of method when designing microcosm experiments:

Substrate

Homogenisation of the microcosm soil may lead to reduced habitat heterogeneity and destruction of the soil structure. Also, it may not always lower the variance between replicate microcosms (cf. Ausmus & O'Neill 1978), which is a main argument for choosing a homogenised substrate in favour of intact soil cores. Intact soil cores of an appropriate volume, cut off at the bottom in such a way that they all have the same height, in addition to containing the same soil layers in similar proportions, could perhaps be a better solution.

A third alternative was suggested by Huhta & Setälä (1990). They used several homogenised substrates, put together afterwards in a mosaic in which the exact amount of each component was known. It is possible that this solution could avoid some of the problems both of a homogenised substrate (low habitat heterogeneity) and of intact soil cores (large differences in substrate content between replicates).

Moisture conditions and drainage

If a water potential range corresponding to suitable natural conditions in the soil type in question is determined before the incubation start, the corresponding moisture content can be maintained by weighing the microcosm before and after watering. This is not ideal (natural temporal and spatial moisture variations are lost). However, it might be the best solution available unless an exact replication of natural moisture is feasible. It should be noted that if the substrate

has a high organic content, the same amount of water will give a higher moisture late in the incubation than at the start, due to substrate mass loss with decomposition.

The drainage should be efficient in order to avoid a high concentration of excess salts in the water (at least if the natural soil environment simulated in the microcosms is well drained). In addition to a hole or plastic tube leading from the bottom of the microcosm box, the substrate could be placed on a porous fundament. Setälä et al. (1990) conducted experiments with substrates simulating the organic part of a natural podsol soil, resting on a layer of either alcathene granules or defaunated mineral soil. (The concentrations of salts in the leaching water were, however, dependent on whether plastic granules or mineral soil was used.) To remove the substrate, or parts of it, from this "fundament" for measurements during incubation (e.g. to determine the water content), the substrate could be enclosed in a "litterbag" (cf. Huhta and Setälä 1990). If such removals have to be made without danger of contamination, the mesh size of this "litterbag" should ideally be small enough to shut out unwanted organisms, as well as large enough to allow drainage.

Introduction of microflora and animals

In microcosms containing partially or completely sterilised soil reinoculated with microflora, a comparison of treatments with and without animals could be biased due to the presence of microflora species in the faunal treatments, accidentally introduced along with the animals, that are absent in the treatments not containing fauna. The problem could perhaps be minimised (though probably not completely eliminated) by introducing the animals in such a way that they are as "clean" as possible (i.e. empty guts and few particles clinging to the outside of their bodies), and by introducing as many as possible of the fauna-associated microflora species to all treatments to minimise the number of absent species in the non-faunal treatments. One possible method is to extract animals onto moist filter paper (e.g. Hågvar 1988, Setälä et al. 1988, Setälä & Huhta 1991), or put them in clean water (e.g. Setälä et al. 1991), and let them empty their guts there for a period before adding them to the microcosm soil. After examination (or in the case of water, filtering) to ensure that live animals and eggs are removed, the paper or water could then be introduced to the non-faunal replicates (cf. Abrahamsen 1990, Mikola & Setälä 1998). In addition, excrements from the animal species in question, which are likely to contain some of the "animal-associated" microflora, might be added to the replicates without animals. If the animals added to the microcosms belong only to a few known species, these can be kept in cultures with a limited bacterial or fungal food source for a period before introduction to the microcosms (e.g. Setälä et al. 1991), and the microbial food source can be introduced to all treatments (e.g. Mikola & Setälä 1998).

To put slowly-growing microflora at the least possible disadvantage in relation to more rapidly growing species, and to avoid possible exaggerated animal effects due to more efficient transport of microflora to sterile substrates in faunal than in non-faunal microcosm treatments, one might let the addition of microflora take place over a long stretch of time. This could, for example, be done by allowing a continuous ingrowth of fungal hyphae into the sterilised soil through a filter (Hågvar 1988), so that those species that need time to establish themselves are not prevented from doing so. The technique of "cross seeding" described in Beyers & Odum (1993, p. 9) could possibly reduce the rate of species extinctions to some extent, as well as reducing differences in species composition between replicates within the same experimental treatment. (A high degree of species similarity between the replicates in each treatment is not necessarily an advantage, however. Huhta et al. (1998a) discussed the problem of distinguishing the effects of a general increase in biodiversity in microcosms from the specific effects of the extra species added. To avoid confusing these two factors, they concluded that "every replication of a diversity experiment should contain different (randomly assembled) species combinations.")

Although a completely "natural" assemblage of organisms is probably impossible to create artificially, the importance of the artificiality might perhaps be determined: In a field enclosure experiment by Faber & Verhoef (1991), measurements of litter mass loss and N dynamics in previously defaunated enclosures, with artificial "complete" species assemblages, were compared to corresponding measurements on unconfined forest floor. Teuben & Verhoef (1992) made similar comparisons between microcosms in the laboratory, microcosms that were kept in the field and separated from the natural soil by a 200 μm mesh ("mesocosms"), and natural field conditions. Such comparisons between artificial species assemblages in laboratory microcosms, and unsterilised microcosms containing samples of the natural soil incubated under the same conditions, could perhaps partly quantify the difference between the naturally and artificially assembled communities. (The problem of species extinctions, however, would also appear in the unsterilised microcosms.)

General comments

Several of the problems discussed above are difficult, or perhaps even impossible, to avoid. For this reason, since it seems probable that different microcosm designs could lead to partly different results, the lack of a standardised procedure in microcosm studies could possibly be an advantage. Microcosm studies vary with respect to the litter types, substrate treatments, sterilisation methods, abiotic conditions, and selections of organisms that are used. In all probability, the effects of some potential sources of error, most notably those that depend on the relative quantities of the organisms present, the temperature and moisture regime and partly also the size of the model ecosystem, will not be exactly the same in different studies. Thus, the average results of several independent experiments, trying to find an answer to the same question, may be less influenced by these biases than the results of each single experiment.

On the other hand, the problem of a poor species composition in the microcosms is probably not averaged out over several experiments, since it seems plausible that most such studies are afflicted with it in a somewhat similar manner. Several important functional groups of organisms could be missing in the microcosms. In sterilised or defaunated microcosms reinoculated with microflora and animals, it is not unlikely that it will often be the same categories, i.e. slowly-growing organisms characteristic of late successional stages, that are missing in different studies. Furthermore, it is difficult to add a diverse microflora to a microcosm substrate without at the same time adding protozoa and nematodes.

Generally, the best solution would probably be for each individual researcher to conduct several experiments in the same type of habitat and with the same theoretical aim, using alternative methods that differ enough from each other to make it probable that most errors do not point in the same direction (e.g. Teuben & Verhoef 1992, Verhoef 1996). However, this procedure requires a lot of time and resources to accomplish. When conducting microcosm studies involving a large number of species interactions, without having sufficient capacity to conduct several parallel experiments, the best *practical* solution could therefore perhaps be to avoid making the experiment in exactly the same way as others have done when studying the same problem, so that comparisons with other studies are as unbiased as possible. Differences in the results could then be discussed on the basis of the influence of the differences in the research methods.

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SAMMENDRAG

En kritisk gjennomgang av mikrokosmos-metoden ved studier av jordbunns-dyrenes økologi

Mikrokosmos-eksperimenter brukes mye i undersøkelser av jordbunnsdyrenes økologi, siden de muliggjør kontrollert manipulering av fauna, klima og substrategenskaper. Imidlertid kan det bli vanskelig å vurdere resultatene av slike forsøk, ettersom mikrokosmoset lett kan få et biotisk og abiotisk miljø som avviker fra det naturlige økosystemet det er ment å skulle representere. På grunnlag av en litteraturgjennomgang ble mulige påvirkninger av de følgende faktorene diskutert: (1) Mikrokosmosets størrelse og varigheten av inkubasjonsperioden. (2) Preparasjon av substratet i mikrokosmoset, med vekt på valget mellom et homogenisert substrat eller intakte jordpropper. (3) Fuktighet, temperatur og drenering i løpet av inkubasjonsperioden. (4) Biotisk diversitet i mikrokosmoset, med vekt på (a) det generelle problemet med lav biodiversitet i mikrokosmos-forsøk, (b) den unaturlige situasjonen man har hvis det ikke er planter i mikrokosmoset, (c) forskjeller i mikroflora-sammensetning mellom behandlinger "med dyr" og "uten dyr", og (d) relative tettheter av forskjellige arter og grupper av organismer. Til sist ble det diskutert noen mulige måter å kontrollere disse problemene på.

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