Biol Fertil Soils (1995) 19:257–262


ORIGINAL PAPER

Alexander Bruckner · John Wright
Christian Kampichler · Roswitha Bauer · Ellen Kandeler

# A method of preparing mesocosms for assessing complex biotic processes in soils

Received: 21 January 1994

**Abstract** Equipment and handling methods for the preparation of soil mesocosms were developed. The mesocosms were used to investigate the interrelationships between mesofauna and microflora in a coniferous forest soil. Soil monoliths were taken from the ground, defaunated by deep-freezing, wrapped in nets to control reimmigration of different faunal size-classes, and replanted in the field for 8 months. In a practical test the technique described here proved to be an inexpensive field method for producing a replicated series of mesocosm in a short time. Deep-freezing is appropriate for defaunating soil monoliths. The fine nets effectively exluded meso- and macrofauna. No significant differences were found in the abundance of Enchytraeids and Collembola between recolonized mesocosms and the undisturbed control at the end of the study period. In contrast, oribatid mite abundace was still greatly reduced in the recolonized mesocosms. Dominance structure and species composition of the more dominant oribatid species in the different treatments were apparently similar. To compensate for the low colonization ability of oribatids, a reintroduction of selected animal size-classes to defaunated monoliths is recommended.

A. Bruckner (✉) · R. Bauer
Institute of Zoology,
University of Agriculture,
Gregor-Mendel Strasse 33,
A-1180 Vienna, Austria

J. Wright
Wright Eco-Tools,
A-2620 Wartmannstetten 85, Austria

C. Kampichler
Institute of Zoology,
University of Vienna,
Althanstrasse 14,
A-1090 Vienna, Austria

E. Kandeler
Federal Institute for Soil Management,
Denisgasse 31,
A-1200 Vienna, Austria

**Key words** Mesofauna-microflora interaction · Defaunation · Monolith · Deep-freezing · Coniferous forest soil · Simulation

## Introduction

Microcosm and mesocosm techniques are different experimental approaches that can be applied to understand ecological processes and to generate models about them. Microcosms are additively assembled from specific elements (e.g. litter, fungi, nematodes) removed from their natural environment. Typically, they contain a limited number or abnormal amount of the original elements. Where and when the living elements in microcosms are active may not be representative of the larger whole. Mesocosms (according to our definition; Odum 1984) are enclosed outdoor systems that are partially permeable to their surroundings. They are sections cut out of ecological systems as whole "blocks", treated, and planted back in the natural environment. The mesocosm method allows one to assess the effects of a particular variable on the system by eliminating that variable and observing the subsequent system responses (Ingham et al. 1986). As far as possible, all other elements of the system are left undisturbed. Mesocosm approaches are therefore well suited for overall simulations of multicomponent processes with a high level of interdependence between components.

Despite the apparent complexity of soil phenomena, there have been only singular applications of mesocosms in soil ecology and definitions of the term vary (Edwards and Lofty 1978; Elliot et al. 1986; Teuben and Verhoef 1992; Sheppard and Evenden 1994). This is probably due to technical difficulties in the preparation and treatment of more or less undisturbed soil mesocosms. The preparation of monoliths is easier in more compact agricultural soils than in loose forest soils. Furthermore, many ecological parameters exhibit low spatial variability in agricultural soils. It is thus possible to design studies with very small numbers of replicates ( = numbers of mesocosms). The soil monoliths used in these investigations are rather

large (Belford 1979; Figge 1992), difficult to handle, and therefore expensive.

We have developed techniques and devices specifically for the production and manipulation of mesocosms in forest soils. These monoliths are small and can be handled easily by two or three persons. The equipment is inexpensive to construct and use and hence great numbers of replicates can be produced in a short period of time. It is thus possible to conduct ecological research even on parameters with high spatial variability.

We tested the soil mesocosm technique in a field study that was designed to investigate the interactions between mesofauna and microflora in a forest soil. We tried to establish one treatment of mesocosms with and another set without mesofauna (besides two control treatments). Nutrient contents and enzymatic activities in the two treatments were compared at the end of the experiment. To fulfil the constitutive definition of mesocosms given above, it was crucial that the fauna in the treatment with mesofauna was nearly identical to the fauna in the undisturbed soil.

In this paper, we describe the equipment and procedures used to prepare soil mesocosms and report on our practical experience with it in the field study. We discuss (1) whether the soil mesofauna was successfully excluded from the treatment without mesofauna and (2) whether it was re-established in the treatment with mesofauna by recolonization of defaunated monoliths.

Microbiological results from this study have been presented by Kandeler et al. (1994). Bruckner et al. (1993) and Kampichler et al. (1994) reported preliminary zoological results.

## Materials and methods

### Principle of the mesocosm technique

For the preparation of mesocosms, soil monoliths are removed from the ground. In the field, they are partially sterilized (defaunated) by deep-freezing and replanted in the ground. The re-immigration of faunal size-classes is controlled by wrapping the monoliths in nets of various mesh sizes. Each of four mesocosm treatments is therefore provided with a different set of faunal size-classes (no meso- or macrofauna, only mesofauna, mesofauna + macrofauna, or full set of soil fauna). Differences in chemical and microbial properties among treatments thus can be explained by the presence or absence of a particular faunal size-class.

### Equipment

*Cutting frames.* The soil monoliths are cut out of the ground using two chromium steel frames (250×250×220 mm, an inner and an outer one). The frames fit into one another tightly with only a few millimeters between them for the netting (see below). Both frames are provided with a cutting edge (75° chamfer) on the bottom. Two concentric holes are drilled into the opposite sides of each frame to accommodate bolts.

*Bar-handle cover.* To ease manipulation of the soil monolith, a footboard with bar-handles can be fastened into the top of the inner frame (Fig. 1). This device consists of a steel band (700×50 mm) that fits into the inner side of the inner frame. A footboard is welded on the upper edge of the steel band. Two retractable bolts jut out from the steel band. They are connected to the handles and can be levered under the surface of the steel band. The bolts can be clicked into the boreholes of the inner frame and the elements are fixed together (Fig. 2).

*Cutting sheet.* The soil monolith inside the inner frame is cut free from the underlying soil with a metallic sheet (255×255 mm).

*Platform cover.* To drive the outer cutting frame in the ground a footboard-shaped tool is fitted over the outside. It consists of a steel band that can be slipped over the outer frames. A footboard is welded on the top of the steel band. The footboard is open in the middle to permit removal of soil material (Fig. 3).

*Hydraulic lifting-jack.* Considerable force is necessary to detach the inner frame from the frozen soil monolith (see below). A hydraulic jack (hand-pumped, 4500 kg carrying capacity; Fig. 4) is used for this. Two cantilevers are welded onto the sides of the jack. On each cantilever, a drawing lever is hinged. The levers can be bolted to the inner cutting frames. The piston of the jack is capped with a ground plate that pushes against the monolith while the inner frame is drawn out (Fig. 5).

*Mechanical jack.* The outer cutting frame is removed from the ground by a mechanical-jack lift. Cantilvers with drawing levers hinged on are welded on the jack. Like the hydraulic jack, the draw levers can be bolted to the outer frame. The jack is provided with a footing so that it can be supported on the ground (Fig. 6).

*Exclusion nets.* To control the re-immigration of fauna, each frozen monolith is net-wrapped (see below). The nets must be prepared before field work begins. Net sheets are heat-glued together using a glue gun to form the sides of the monoliths.

### Sequence of operations

The outer cutting frame is used to cut out a cavity in the ground which will later hold the manipulated mesocosm. The frame is placed on the ground and the platform cover is put over it and attached (Fig. 3). One person stands on the platform cover and slowly presses the frame 15 cm deep into the ground. Simultaneously, roots are cut off with pruning shears and soil material is dug out inside the frame. The cutting of roots is especially important in forest floors with dense root layers because it greatly reduces any physical disturbance of the surrounding soil. The soil and root material is completely removed and scraped out down to the bottom of the frame.

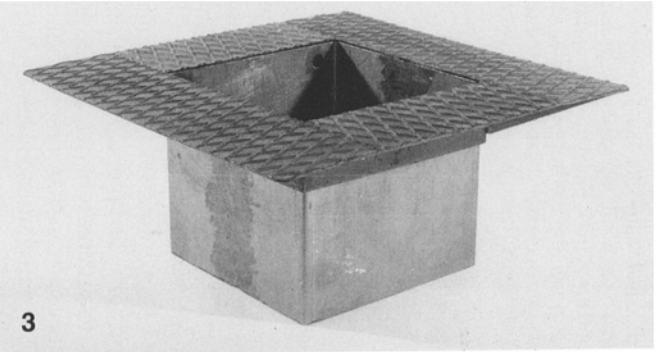
The inner cutting frame is used to cut the soil monolith, to remove it from the ground, and to handle it during subsequent operations. The inner frame is placed on the ground and the bar-handle cover is attached (Fig. 2). One person stands on the cover. While the frame slowly glides into the ground, roots are cut off simultaneously. When the frame is 15 cm into the ground, it is dug free on one side. The monolith is freed from the underlying soil by hammering the cutting sheet under the frame.

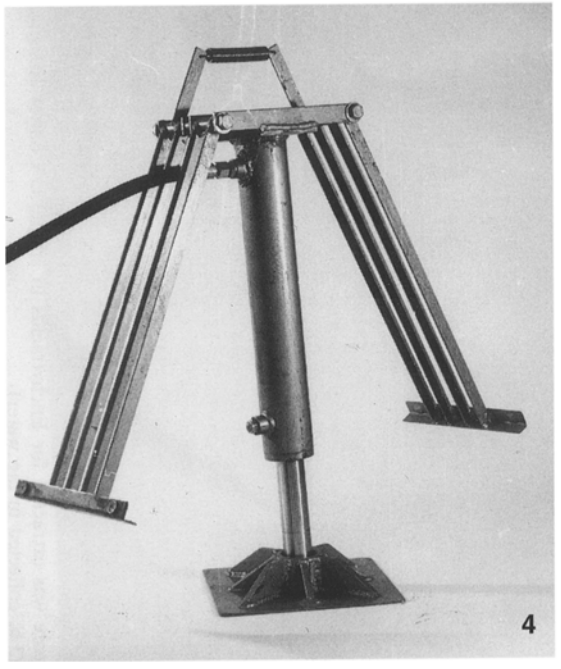
To eliminate soil fauna in the monoliths, we use a deep-freeze technique in the field. The monolith, still inside the inner cutting

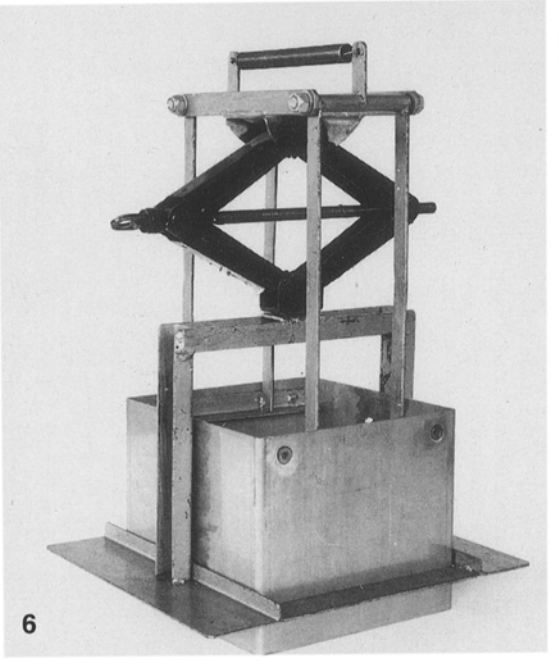
**Figs. 1–6** Equipment for preparing soil mesocosms: **1** Bar-handle cover to inner cutting frame; **2** bar-handle cover attached to inner cutting frame; **3** outer cutting frame with platform covering attached to it; **4** hydraulic jack and ground plate; **5** hydraulic jack raises inner cutting frame, showing a white exclusion net between the outer and the inner frame; **6** mechanical jack and support footing pulls outer cutting frame out of ground

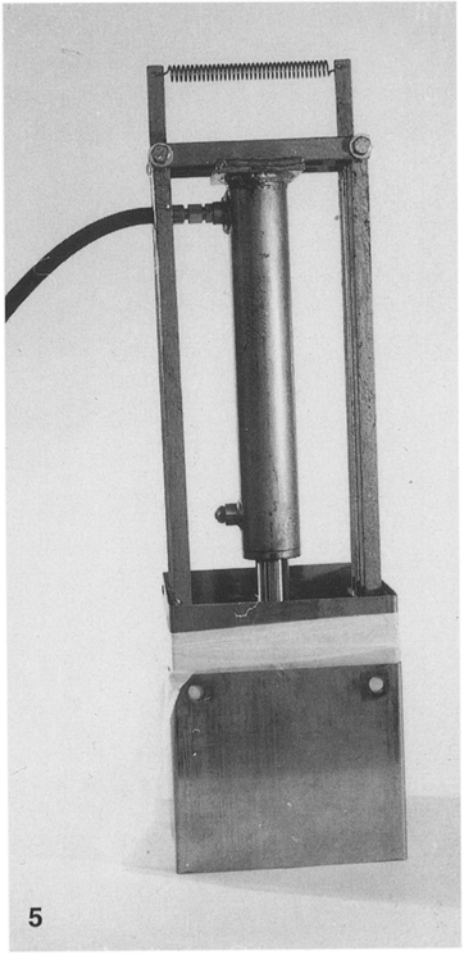

1


2


3


4


6


5

frame, is placed in an insulated container that can hold two monoliths at the same time. Crumbled pieces of dry ice (solid $CO_2$, gaseous at −78.5 °C) are shoveled into the container around the frames. The monolith is cooled down to −15 °C, measured at the center of the block. Previous tests indicate that the whole meso- and macrofauna of organic forest soils is killed off at this temperature. To ensure easy handling of the monoliths and to reduce damage to the soil structure, the following operations can be performed while the monolith is still frozen.

After freezing, the bottom of the monolith should be roughened by detaching clods of earth. This may be necessary since work with the cutting sheet might fill soil pores and thus alter the water-draining properties of the monolith. In order to free the inner frame from the frozen monolith, the frame is defrosted with a blowtorch once or twice. A net of a particular mesh-size (see below) is drawn over the inner frame (containing the monolith) like a stocking.

The bar-handle cover is attached to the netted inner frame (with monolith inside). The frame is lowered down into the cavity held open by the outer cutting frame. This procedure requires extreme care, since there is very little room between the inner and the outer frame and the net must not tear or become gappy. The bar-handle cover is removed from the inner frame when it is fully inside the outer frame.

The hydraulic lifting-jack is used to free the soil monolith from the inner frame. A ground plate is put on the surface of the monolith. The draw levers of the jack are bolted into the boreholes of the inner frame. The piston of the jack is centered in a short tube, welded on in the middle of the ground plate. The jack is pumped up and the draw levers raise the frame (Fig. 5). The pate holds back the monolith in its position on the floor. The mechanical lifting-jack is used to remove the outer cutting frame from the ground. The support footing of the jack is positioned outside the frame and the draw levers are bolted to the boreholes of the frame (Fig. 6). The top and side sheets of netting are glued together. The mesocosm is thus entirely wrapped in a net.

After removing all instruments, the millimeter-wide gap remaining between the mesocosm and the surrounding soil is filled with mixed and sieved (5 mm) humus material from the L/F layers.

### Current study

Our investigations were carried out in a 100×40 m site in a 40-year-old *Picea abies* forest (Poschawald) in Gumpenstein, Styria, Austria (47°29′N, 14°7′E, National Grid Reference BMN 5702-0860-4 b, 730 m above sea level). The mean annual temperature in the region of Gumpenstein is 6.8 °C, and the mean annual precipitation is 1010 mm. The soil was a dystric cambisol (Silikatische Braunerde). The humus form was raw (mor) with distinct L (≈2 cm depth), F (≈2 cm), and H (≈6 cm) layers. The F layer was densely rooted. The ground vegetation was sparsely developed and consisted mainly of mosses.

In October 1991, 30 soil monoliths were taken from an approximately 50×50 m area from the periphery of the study site. Four different treatments were applied: (1) Ten monoliths were frozen and wrapped in fine nets (mesh size 35 µm). (2) Ten monoliths were frozen and wrapped in coarse nets (mesh size 1 mm). (3) Ten monoliths were frozen and left without a net wrapping. (4) The positions of 10 plots were marked out on the ground, but these plots were not manipulated and thus served as a control for the effects of freezing and manipulation.

The mesocosms were replanted randomly in the study site and the control plots were designated randomly. After an exposure time of 8 months, the mesocosms were destructively sampled in June 1992. Two cores (⌀ 7 cm, 10 cm depth) were taken from each mesocosm and control plot for zoological analysis. The cores were brought to the laboratory within 5 h. One core from each mesocosm and control plot was extracted for microarthropods in simple Tullgren funnels (10 days; collecting fluid 80% ethanol). The other core was extracted for Enchytraeids in simple O'Connor funnels (3 h; collected in tap water).

Statistical analysis was performed using the Statgraphics 2.6 package. When applying statistical tests, differences between data sets were judged to be significant at $P \leq 0.05$. Since the animal data sets for the treatments were not normally distributed we used the non-parametric Kruskal–Wallis test for overall comparison and the non-parametric Nemenyi test for pairwise comparisons between treatments.

## Results

### Practicability of the methods

After 8 months, the refilled gaps had no subsided and we could not find any macroscopic differences between the monoliths and their surroundings. No new tree roots had grown into the monoliths of any treatment.

All operations described above were easily performed by a team of two or three persons. The preparation of one mesocosm in a coniferous forest soil took about 1 h, excluding freezing time. Thus one team can prepare about 8–10 mesocosms a day. The most time-consuming step was defaunating the monoliths. The duration of freezing depends on the amount of dry ice in the freezing containers and the structure and water content of the soil. It took 4–6 h to cool down the monoliths taken from the *Picea abies* plot. In agricultural soil, 8–10 h was required. With such long freezing periods, each team should be equipped with at least four insulated containers (including one container used to store dry ice) and six or eight pairs of steel frames to defaunate several monoliths simultaneously.

Approximately 50 kg dry ice per day per team was needed. If the ice cannot be deposited in a cold-storage depot for longer periods, it must be delivered daily. Given an adequate supply of dry ice, deep-freezing is a suitable method for field work.

The size of our experimental units, compared with those of Hågvar (1988), Huhta et al. (1991), and Teuben and Verhoef (1992), proved adequate because (1) they can be handled easily in the field, (2) many microbial and zootic parameters can be measured simultaneously in the soil/humus material of each monolith, (3) the volume of the disturbed humus material in the periphery of each monolith is small compared to its total volume, and (4) as in undisturbed soil, mosaics of microsites with different biological activity may be present and a mean value can be obtained from each monolith. Smaller units are probably more susceptible to over- or underestimation of the effects of interactions since they may be "hot spots" of biotic processes due to overcrowding phenomena (Hågvar 1988; Huhta and Setälä 1990).

### Zoological results

Only negligible numbers of animals were found in the fine-mesh treatment 1 (Table 1). Thus deep-freezing is an appropriate method for eliminating mesofauna from soil monoliths. The fine nets effectively excluded meso- and macrofauna throughout the study period.

**Table 1** Medians and 95% confidence intervals (according to Sachs 1984, table 69) of abundance data of mesofaunal groups in mesocosms at time of sampling. Treatment 1: frozen monoliths, wrapped in fine nets; treatment 2: frozen, coarse nets; treatment 3: frozen, without nets; control: no manipulation. *Values* partly recalculated after Bruckner et al. (1993). *Opp. & Suct.* oribatid mite families Oppiidae and Suctobelbidae

| | Treatment 1 (fine net) | Treatment 2 (coarse net) | Treatment 3 (no net) | Control (no cut) |
|---|---|---|---|---|
| Enchytraeidae | 2.5 (1–13) a | 106.0 (79–174) b | 148.0 (97–169) b | 132.0 (59–187) b |
| Collembola | 1.5 (0–4) a | 91.5 (46–185) b | 126.5 (58–162) b | 75.0 (36–144) b |
| Acarina juvenile | 12.0 (5–22) a | 37.0 (30–69) b | 159.0 (83–266) c | 805.0 (486–1205) d |
| Oribatida adult | 2.0 (0–3) a | 58.0 (37–173) b | 111.0 (44–144) b | 261.0 (199–358) c |
| Opp. & Suct. | 0 a | 2 (1–3) b | 6.5 (3–11) bc | 74.5 (42–118) c |

Values followed by different letters are significantly different ($P \leq 0.05$)

Mites dominated the soil mesofauna in all treatments. Oribatids made up 90–100% of the adult mite fauna. For Enchytraeidae and Collembola, no significant differences were found between treatments 2 and 3 or between either set and the control. All treatments differed highly significantly from treatment 1 (Table 1). With regard to juvenile mites, all treatments differed highly significantly from each other. The average abundances of adult oribatid mites were not significantly different in treatments 2 and 3, but in all other pairs of treatments a highly significant difference was found. The differences between treatments were not equally pronounced for all adult oribatid taxa. A faunistic comparison between treatments and control revealed small numbers of the deep-dwelling families Oppiidae and Suctobelbidae (sensu Schatz 1983) in treatments 2 and 3 (Table 1).

Despite these differences in abundance, the dominance structure of the adult oribatid mite populations in treatments 2, 3, and the control were quite similar (Fig. 7). Moreover, the dominance ranks of the more abundant species (more than 10 individuals treatment$^{-1}$) corresponded significantly in the three treatments (Spearman rank correlation between species ranks: $r = 0.86^{***}$ for comparison of treatments 2 and 3; $r = 0.49^{**}$ for 2 vs control; $r = 0.69^{***}$ for 3 vs control). The correlations between dominance ranks were closer when the Oppiidae and Suctobelbidae were removed from the data set (Spearman rank correlation between species ranks: $r = 0.94^{***}$ for 2 vs 3; $r = 0.75^{***}$ for 2 vs control; $r = 0.86^{***}$ for 3 vs control).

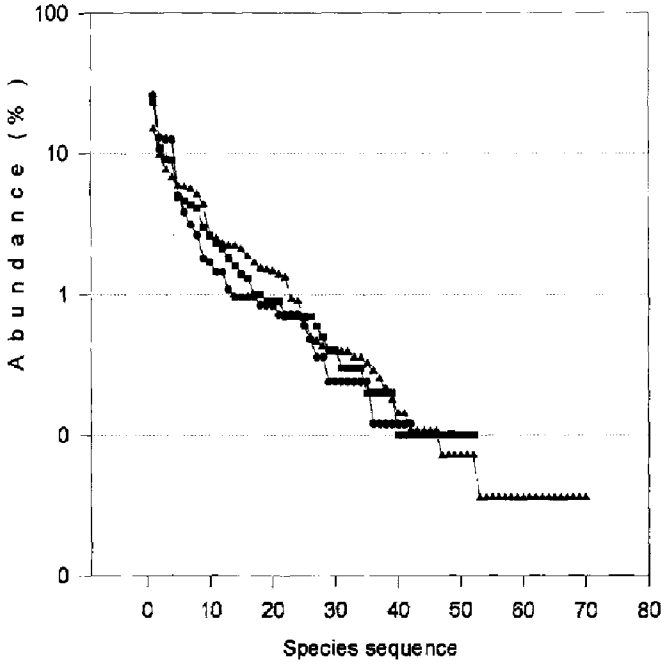



**Fig. 7** Rank/abundance graph of the dominance structure of adult oribatid mites in treatments 2 (●), 3 (□), and the control (△). The abundance of each species (logarithmic scale) is plotted against the species rank, ordered from the most to the least abundant species. Treatment 2: monoliths frozen, wrapped in coarse nets; treatment 3: frozen, without nets; control: no manipulation

## Discussion

For decades, soil ecologists have carried out microcosm studies to analyze the complex interrelationships between mesofauna and microflora. None of the mechanisms involved in mesofauna–microbial interactions have been well quantified yet (Lussenhop 1992) and it is still not possible to say without doubt whether mesofaunal activities are "noise or necessity for soil processes" (Anderson 1987).

Naturally, due to non-target manipulation effects, mesocosm systems do not behave exactly like undisturbed systems. This is exemplified in the present study. The number of mites in the defaunated monoliths lagged behind mite abundance in the control mesocosms. Since oribatids were the most abundant fungivorous microarthropod taxon on the study site, we cannot eliminate the possibility that the effects of grazing were also delayed. Further, the small numbers of deep-dwelling Oppiidae and Suctobelbidae in treatments 2 and 3 indicated that the three humus layers (L, F, H) were recolonized with different degrees of success.

On the basis of these results, we suggest two modifications to the developed mesocosm technique. First, to compensate for the apparently low colonization ability of

soil fauna, selected microarthropod size-classes should be re-introduced into the monoliths immediately following defaunation. Second, a prolonged application of the mesocosms (up to several years) may result in an adjustment of biotic parameters. For the faunal component, the corresponding community structure of oribatid mites in treatments 2 and 3 and the control in this study supports this assumption.

However, the present soil mesocosm techniques are a promising way of partly overcoming the experimental simplicity and deficiencies of conventional microcosm systems. They are spatially complex (Leonard and Anderson 1991), are not set up with sieved or mixed soil substrate (Wright et al. 1989), and can include the full number and combination of taxa and functional groups (Brussaard et al. 1991; Setälä et al. 1991). With further experience it should be possible to minimize disturbances due to manipulation and to construct mesocosms that resemble the field situation closely enough to make adequate generalizations about the natural soil situation.

**Acknowledgements** We thank G. Eder, W. Hein, B. Krautzer, and the staff of the Federal Research Institute for Agriculture in Alpine Regions in Gumpenstein, Styria, for cooperation and help during field work. M. Eisenhut described the soil profile. M. Roithmair and E. Christian made helpful suggestions that significantly improved the text. J. Plant corrected the English. The study was supported by the Austrian Federal Ministry of Science and Research.

## References

Anderson JM (1987) Interactions between invertebrates and microorganisms: noise or necessity for soil processes? In: Fletcher M, Gray TRG, Jones JG (eds) Ecology of microbial communities. Cambridge University Press, Cambridge, pp 125–145

Belford RK (1979) Collection and evaluation of large soil monoliths for soil and crop studies. J Soil Sci 30:363–373

Bruckner A, Kampichler C, Wright J, Bauer R, Kandeler E (1993) Using mesocosms to investigate mesofaunal–microbial interactions in soil: Re-immigration of fauna to defaunated monoliths. Mitt Dtsch Bodenkd Ges 69:151–154

Brussaard L, Kools JP, Bouwman LA, de Ruiter PC (1991) Population dynamics and nitrogen mineralization rates in soil as influenced by bacterial grazing nematodes and mites. In: Veeresh GK, Rajagopal D, Viraktamath CA (eds) Advances in management and conservation of soil fauna. Oxford & IBH Publishing Company, New Delhi, pp 517–523

Edwards CA, Lofty JR (1978) The influence of arthropods and earthworms upon root growth of direct drilled cereals. J Appl Ecol 15:789–795

Elliott ET, Hunt HW, Walter DE (1986) Microcosms, mesocosms and ecosystems: Linking the laboratory to the field. In: Megusar S, Gantar M (eds) Perspectives in microbial ecology. Proc Int Symp Microb Ecol IV, Slovene Society of Microbiology, Ljubljana 1986, pp 472–480

Figge K (1992) Facilities for the examination of the degradation and distribution of chemical compounds in sections of terrestrial ecosystems. In: Führ F, Hance RJ (eds) Lysimeter studies of the fate of pesticides in the soil. BCPC Monograph 53. British Crop Protection Council, Farnham, pp 83–99

Hågvar S (1988) Decomposition studies in an easily-constructed microcosm: Effects of microarthropods and varying soil pH. Pedobiologia 31:293–303

Huhta V, Setälä H (1990) Laboratory design to simulate complexity of forest floor for studying the role of fauna in the soil processes. Biol Fertil Soils 10:155–162

Huhta V, Haimi J, Setälä H (1991) Role of the fauna in soil processes: Techniques using simulated forest floor. Agric Ecosyst Environ 34:223–229

Ingham ER, Trofymow JA, Ames RN, Hunt HW, Morley CR, Moore JC, Coleman DC (1986) Trophic interactions and nitrogen cycling in a semi-arid grassland soil. II. System responses to removal of different groups of soil microbes or fauna. J Appl Ecol 23:615–630

Kampichler C, Bruckner A, Kandeler E, Bauer R, Wright J (1994) A mesocosm study design using undisturbed soil monoliths. Acta Zool Fenn (in press)

Kandeler E, Winter B, Kampichler C, Bruckner A (1994) Effects of mesofaunal exclusion on soil microbial biomass and enzymatic activites in field mesocosms. In: Ritz K, Dighton J, Giller KE (eds) Beyond the biomass: Compositional and functional analysis of soil microbial communities. Wiley and sons, Chichester, pp 181–189

Leonard MA, Anderson JM (1991) Growth dynamics of Collembola (*Folsomia candida*) and a fungus (*Mucor plumbeus*) in relation to nitrogen availability in spatial simple and complex laboratory systems. Pedobiologia 35:163–173

Lussenhop J (1992) Mechanisms of microarthropod–microbial interactions in soil. Adv Ecol Res 23:1–33

Odum EP (1984) The mesocosm. Bio Science 34:558–562

Sachs L (1984) Angewandte Statistik, 6th edn. Springer, Berlin

Schatz H (1983) Catalogus Faunae Austriae. Teil IXi: U-Ordn: Oribatei, Hornmilben. Verlag Österr Akad Wiss, Wien

Sheppard SC, Evenden WG (1994) Simple whole-soil bioassay based on microarthropods. Bull Environ Contam Toxicol 52:95–101

Setälä H, Tyynismaa M, Martikainen E, Huhta V (1991) Mineralization of C, N and P in relation to decomposer community structure in coniferous forest soil. Pedobiologia 35:285–296

Teuben A, Verhoef HA (1992) Relevance of micro- and mesocosm experiments for studying soil ecosystem processes. Soil Biol Biochem 24:1179–1183

Wright DH, Huhta V, Coleman DC (1989) Characteristics of defaunated soil II. Effects of reinoculation and the role of the mineral component. Pedobiologia 33:427–435