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Esko Martikainen

Environmental Factors Influencing Effects of Chemicals on Soil Animals

Studies at Population and Community Levels

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ABSTRACT

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Effects of abiotic environmental factors like soil organic matter content, soil moisture and temperature on the toxicity of chemicals to soil animals were studied in laboratory experiments. An insecticide, dimethoate, and two fungicides, benomyl and propiconazole, were used as reference chemicals in the experiments. Two types of experiments were conducted: single species tests and microcosm experiments. Single species experiments revealed that soil organic matter content affects substantially the toxicity of dimethoate to collembolans. Increasing organic matter content decreased dimethoate concentration in the soil pore water, and hence its toxicity. Lowering the temperature increased the toxic effects, but only slightly. Population level effects lasted longer at low temperature due to slower reproduction of collembolans. Decreasing the soil moisture either decreased (dimethoate) or increased (benomyl) the toxic effects on an enchytraeid worm. In the microcosm experiments pesticide application and drought decreased different soil animal groups resulting in lower total soil animal numbers than exposured to either of these stressors alone. Both fungicides had only minor effects on soil animal communities and soil processes, possibly due to relative low significance of fungal based energy channel in agricultural soil. It was revealed that both single species tests and microcosm experiments are needed when assessing ecotoxicological effects of chemicals in the environment.

Key words: Microcosms; organic matter; pesticides; single species tests; soil moisture; soil organisms; temperature.

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List of original publications

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I	Martikainen, E. 1996: Toxicity of dimethoate to some soil animal species in different soil types. - Ecotoxicology and Environmental Safety 33: 128-136.
II	Puurtinen, M. & Martikainen, E. 1997: Effect of soil moisture on pesticide toxicity to an enchytraeid worm *Enchytraeus* sp. - Archives of Environmental Contamination and Toxicology, 33: 34-41.
III	Martikainen, E. & Krogh, P.H. 1998: Effects of soil organic matter content and temperature on toxicity of dimethoate to *Folsomia fimetaria* (Collembola: Isotomiidae). - Environmental Toxicology and Chemistry (in press).
IV	Martikainen, E. & Rantalainen, M.-L. 1998: Temperature-time relationship in collembolan response to chemical exposure. - Ecotoxicology and Environmental Safety (in press).
V	Martikainen, E., Haimi, J. & Ahtiainen, J. 1998: Effects of dimethoate and benomyl on soil organisms and soil processes - a microcosm study. - Applied Soil Ecology 9: 381-387.
VI	Martikainen, E., Krogh, P.H., Ahtiainen, J., Haimi, J. & Mäntykoski, K. 1998: Pesticide application and drought as stress factors to soil decomposer community and its function. - Manuscript.

Responsibilities of Esko Martikainen in the articles of this thesis

Paper I. I was responsible for all phases of the experiment and wrote the article.

Paper II. The idea for the study was mine, and the experiments were planned, designed and set up together with Mikael Puurtinen, who also analysed the data and wrote the draft of the article. The article was then completed together.

Paper III. The idea for the study was mine, and the experiments were planned, designed and set up together with Paul Henning Krogh. The data was analysed together, and I wrote the draft of the article, which was then completed together.

Paper IV. The idea for the study was mine, and the experiment was set up with Minna-Liisa Rantalainen. I analysed the data and modified the manuscript from the Finnish version (MSc-thesis) written by Minna-Liisa Rantalainen.

Paper V. The experiment was planned together with Jari Haimi. I was responsible for the setting up the experiment, handling of the data and writing the draft of the manuscript, which was then completed with Jari Haimi and Jukka Ahtiainen.

Paper VI. The idea for the study was given by Heikki Setälä. I and Jari Haimi planned the experiment and I was responsible for setting up the experiment. Part of the experiment was conducted by Paul Henning Krogh. I analysed the data and wrote the draft of the manuscript, which was then completed with Jari Haimi, Paul Henning Krogh and Jukka Ahtiainen.

Jyväskylä, September 18, 1998

Esko Martikainen

1 INTRODUCTION

1.1 Chemicals in the environment

Chemicals play important part in our life. In this century chemical industry has become one of the most important branches of industry in the world. Over 10 million different synthesized chemicals are known and ca. 120 000 of them are in general use (Paasivirta 1991). Out of these, ca. 100 000 can be classified as environmental chemicals. They can be defined as substances that either enter the environment as a result of human activity, occur in the environment as a consequence of human activity or occur there in much higher concentrations than they naturally would (Römbke & Moltmann 1996). Emissions into air and water are well known and their harmful effects in these compartments have been extensively studied. Less attention has been paid to the third compartment, soil.

1.2 Importance of soil processes and soil organisms

Soil is a milieu for processes that are vital for life on earth. Dead organic material is partly decomposed and mineralised in soil to mineral nutrients, carbon dioxide and water, and the rest is transformed to more persistent humic compounds. Decomposition together with rock weathering provide essential mineral nutrients for primary producers that transform solar energy and carbon dioxide to biomass and oxygen. These two processes, production of organic material (i.e. biomass) by primary producers and their consumers, and decomposition of the biomass by decomposing organisms, are processes that roughly balance each other. Without one, the other would not be possible in the long run.

Diversity of decomposing organisms in soil is enormous, ranging from the smallest bacteria to the largest earthworms. Species numbers are largely unknown, but it has been estimated that there are e.g. 30 000 bacterial species,

1 500 000 fungal species, 10 000 protozoan species and 500 000 nematode species (Hawksworth & Mound 1991). Bacteria and most microfauna inhabit soil pore water that surrounds soil particles, while larger animals and fungal hyphae live in cavities between the soil particles. Microflora, mainly bacteria and fungi, contribute ca. 85% of the soil biomass and ca. 90% of soil biological activity expressed as CO_2-production (Reichle 1977). They are also responsible for nutrient mineralisation and it has been estimated that e.g. in grasslands and agricultural fields ca. 70% of nitrogen mobilisation in soil is due to microbial activity and 30 % is directly due to soil fauna (Verhoef & Brussaard 1990).

Soil microfauna consists of protozoans (flagellates, amoebae, ciliates), nematodes and tardigrades. They are often the main consumers of soil microbes and therefore they form an important link between the primary decomposers (i.e. microflora) and the larger fauna in the detritus food-web. Their numbers in the soil are high; nematode density can reach 10^9 inds./m^2. Microarthropods, mainly collembolans and mites, and enchytraeid worms are the main groups of mesofauna, that together with microfauna are the primary agents for the release of the nutrients immobilised in the soil microflora (Gupta & Yeates 1997). They can also regulate the activity and composition of the microbial community with the microfauna (Hendrix et al. 1986). Numbers of microarthropods in the soil can reach 10^7 inds./m^2 (Lal 1991) and numbers of enchytraeids up to 145 000 inds./m^2 (Didden 1993). Soil macrofauna consists of earthworms, millipedes, spiders, beetle larvae etc. They are usually either top predators that consume microfauna, or detritivores that modify soil structure by their burrowing and comminuting activity. In some habitats earthworms may comprise over 95 % of soil invertebrate biomass (Hendrix et al. 1986, Didden et al. 1994).

The abundance and species composition of soil organisms varies both geographically and seasonally. In general, the largest biomasses are found in tropical and temperate soils. Land use has often great impact on soil fauna, and cultivated fields have lower numbers of soil animals than grasslands and forests in the same area. Altogether, soil microflora and fauna form a complicated detrital food-web whose structure and fuction have been studied extensively during the last few decades (e.g. Persson & Lohm 1977, Hendrix et al. 1986, Verhoef & Brussaard 1990, Didden et al. 1994, Heal et al. 1996). These studies emphasize the importance of soil fauna in soil organic matter dynamics and nutrient cycling.

1.3 Soil contamination

Soil is at least a long term, sometimes even permanent storage for many environmental chemicals. Airborne contaminants from combustion, chemical production, metal processing, traffic etc., are spread worldwide by air currents. A large part of these contaminants ends up on the soil surface from where they may leach into deeper soil layers. They cause a small but continuous strain, the damages of which can usually be detected only after a longer period. There are some good examples of soil heavy metal contamination in industrial areas, e.g. around smelters (Strojan 1978, Kilham & Wainwright 1981, Bengtsson &

Rundgren 1982, Fritze et al. 1989, Vanhala & Ahtiainen 1994, Haimi & Siira-Pietikäinen 1996), where abundance and diversity of soil microbes and fauna and hence soil decomposition activity have been greatly reduced. On the other hand, excesses of essential substances, e.g. nutrients, can pose problems. Atmospheric nitrogen deposition has become a great problem in central Europe and it constitutes a significant threat to oligotrophic ecosystems (Koshiek et al. 1993).

Another source of pollutants entering soil is accidents in which a large amount of chemical, e.g. oil, may enter the soil causing damage to soil microflora, fauna and plants. The effects are usually detected in a restricted area, but usually they are serious. Also long term leakage of pollutants into soils due to improper waste management has contaminated numerous sites all over the world. It has been estimated that even in Finland there would be 25 000 sites that are suspected to be contaminated, out of which ca. 1200 sites need to be cleaned up (Puolanne et al. 1994). Typical contaminated sites are soils around saw-mills, wood impregnating plants and gas stations. In the vicinity of these sites reductions in faunal populations and decomposition activity have been found (e.g. Yeates et al. 1995)

A third, somewhat different type of stress factor for soils are pesticides used in agriculture and forestry. They are applied either onto foliage or soil in order to prevent damages for crop or sapling stand caused by fungal diseases, weeds and pests. Their use increased after the Second World War and peaked in the mid-1970s (Nimmo & McEwen 1993). In Finland their usage peaked in 1980 when the annual sale (active incredients, a.i.) was ca. 2500 tn (Hynninen & Blomqvist 1997). After that the usage has decreased (less than 1000 tn in 1996 in Finland), mainly because of increased toxicity and effectiveness of new pesticides (Pimentel et al. 1991). Also people's concern about the threats of persistent pesticides to the environment and to humans themselves has increased. In 1994 0.7 kg pesticides (a.i.) were used per cultivated hectare in Finland. Corresponding figures in Germany, Spain and the Netherlands were 2.4, 4.7 and 12.6 kg/ha, respectively (Laitinen 1997).

Today there are ca. 160 different pesticides (a.i.) on the market in Finland (Laitinen 1997). The number of trade formulations is even higher. Worldwide there are hundreds of pesticides in everyday use. Pesticides can be grouped according to their target organisms. Herbicides are intended to eliminate weeds, insecticides are for pest insects, nematicides are for plant parasitic nematodes, fungicides are for fungal diseases etc. They can also be grouped by their chemical structure (organophosphates, acetanilides, carbamates, pyrethroids etc.). Some of the pesticides are strictly targeted to specific pests and some of them are more or less equally toxic to several faunal or microbial groups.

Several pesticides with different modes of action are applied to agricultural fields during the growing season. Seeds treated with fungicides are sown in spring and soon after that herbicides are applied for weed control. During the summer fields are sprayed several times with insecticides to prevent damage caused by insect pests, and also growth regulators are usually applied. In addition to pesticides, agricultural soils are subjected to several other measures like ploughing, fertilizing etc., all of which disturb the normal activities of soil organisms.

Some proportion of any pesticide ends up on the soil surface either directly or through the vegetation, and subsequently into the soil via rainwater. Pesticides disappear from the soil through degradation by microorganisms, evaporation from the soil surface and leaching into deeper soil layers. Before the disappearance of the pesticides, soil organisms are exposed to them. Indeed, pesticides have been shown to cause adverse effects on the abundance and activities of soil organisms (e.g. Edwards & Thompson 1973, Edwards & Bohlen 1992, 1995).

Many chemical stressors mentioned above affect the soil environment, its faunal and microbial commuties and their function. Because of these threats it has been essential to study adverse effects caused by those contaminants in soils.

1.4 Ecotoxocological research and testing in soil environment

Ecotoxicology studies the effects of chemicals and other foreign substances in the environment (e.g. Moriarty 1988, Levin et al. 1989). It is closely related to environmental chemistry, ecology and toxicology. Ecotoxicological effects can be detected at many levels of biological organisation from biochemical changes in individuals up to changes in ecosystem functioning. Effects at individual or lower levels (organs, nerve system etc.) are usually targets of interest in traditional toxicology. Ecotoxicological research is more interested in population and higher level (community, ecosystem) consequences of environmental contamination. Therefore ecotoxicology is close to ecological research, which studies phenomena that determine the abundance and distribution of organisms (Krebs 1985). According to Eijsackers (1994), ecotoxicological research includes the distribution and behaviour of contaminants in the environment, and the impacts of contaminants on the environment, organisms, and the interrelations between the organisms and their environment.

Soil ecotoxicology has lagged behind aquatic ecotoxicology and only recently has there been a growing interest in ecotoxicological research in soils. Largely because of serious impacts of organochlorine pesticides in the environment during 1960s and 1970s, effects of pesticides also on soil organisms have been studied from the 1960s (see reviews by e.g. Edwards & Thompson 1973, Eijsackers & Van de Bund 1980). Later on the impacts of other stressors like heavy metals, soil acidification etc. have gained growing interest in soil ecotoxicology.

Toxicity testing using soil biota has been developed during the last couple of decades. In a test an organism is exposed to the chemical in a controlled environment, and the response of the organism to the chemical is measured. These tests produce relevant, reproducible and standardized information about the toxicity of a chemical. It is then possible to evaluate potential harmful effects of chemicals before they are either intentionally (e.g. pesticides) or accidentally released to the environment. Most of the test methods described are laboratory methods, although some field tests are also available. There has been a large number of different toxicity tests developed for aquatic organisms (see Calow 1993). This is mainly due to the fact that adverse effects of chemicals were first

observed in aquatic environments. Several aquatic ecotoxicological test methods have been modified for use in terrestrial systems by changing the test medium and test species.

1.4.1 Single species tests

Most of the test methods are single species tests since they are usually cost effective, relatively easy to perform and standardize. They are therefore practical methods for assessing relative toxicities of chemicals to the species tested. The first standardized test procedure for soil fauna was an earthworm acute toxicity test adopted by the Organisation for Economic Co-operation and Development (OECD 1984). The substrates used in the test are filter paper, that enables direct contact of the test organism with the chemical, and artificial soil, that mimics contact in natural soil. It contains quartz sand (ca. 69 %), kaolin clay (20 %), *Sphagnum* peat (10 %) and calcium carbonate (ca. 1 %). Later on a modified version of this test has also been adopted by the International Standardisation Organisation (ISO 1992). Other media used in soil animal testing include nutrient agar (Westheide et al. 1991), amorphous silica gel (Artisol) (Ferriere et al. 1981), field soils, (e.g. Van Gestel & Ma 1988) and saline water (Ronday & Houx 1996).

Acute toxicity tests are, however, relatively insensitive for predicting possible population consequences of chemicals, because adverse effects on the test organisms usually appear in substantially lower concentrations than mortal effects. In general, the trend in test development is from acute toxicity tests towards more relevant sublethal tests, where the endpoints are growth and reproduction. The earthworm test mentioned above has been further developed to a reproduction test (ISO 1996). In addition, a collembolan reproduction test is in its final stage (ISO 1997) and an enchytraeid worm reproduction test (Römbke 1998) is under international evaluation at the moment. Also many other soil animal species have been used in soil ecotoxicological research in national testing programmes (see reviews by e.g. Van Straalen & Van Gestel 1993, Römbke & Moltmann 1996).

1.4.2 Multispecies tests and microcosm tests

Single species tests do not, however, allow the study of interactions (competition, predation) between the species. In addition to the single species tests, two species systems (Hamers & Krogh 1997) and microcosms or microecosystems containing either several introduced species (Salminen et al. 1997) or indigenous soil fauna (e.g. Edwards et. al 1994, Parmelee et al. 1993, 1997) have been developed for studying effects of chemicals on soil organisms. The soil in the microcosms can be intact soil cores that have been taken from the field with minimum disturbance, or the soil can be homogenised to minimise variation between the replicates (see review by Morgan & Knacker 1994).

The advantage of these test systems is that they more closely resemble the actual situation in the field than the single species tests do, and yet they are relative easy to replicate adequately. They also have a more or less diverse soil fauna and therefore it is possible to study, not only the reactions of individual

species, but also the reactions of different animal groups (Parmelee et al. 1997), relations between the species (Hamers & Krogh 1997) and community responses (Salminen et al. 1996, 1997, Salminen & Haimi 1997) to the chemical application. It is also possible to measure functional parameters such as nutrient mineralisation and carbon dioxide production, that give better insight into possible changes in community functioning and hence explanations for e.g. nutrient leakage to ground water or weak growth of plants. Results obtained from microcosm experiments have, in general, been found parallel the results of field experiments (Teuben & Verhoef 1992, Römbke et al. 1993).

Some limitations in microcosm studies, however, exist. They are usually far more difficult to standardize and more laborious than single species tests. In addition, the interpetation of the results is more difficult due to manyfold interactions between the species involved. In addition, one can argue whether the microcosms really mimic real conditions in the field. In spite of this, they have proven to be useful tools for the study of the effects of chemicals on higher than individual level responses in soil ecosystems (Salminen & Haimi 1997, Salminen et al. 1997).

1.4.3 Field tests

The third way of studying effects of pesticides on soil organisms and fuctioning is field tests. There is at least one standard method in its final stage for studying effects of chemicals on earthworms in the field (ISO 1997). For other soil organisms these standards do not exist. In spite of the lack of standards numerous field experiments have been done in order to assess effects of certain chemicals, e.g. new pesticide products, on soil biota. The applicability of these studies to other environments is, however, questionable because of different environmental conditions in other parts of the world. The costs of large field tests are also so high that these experiments are conducted only for the chemicals that have been shown to be potentially hazardous in the laboratory tests.

1.5 Influence of environmental conditions on toxic effects

One of the major objectives in the development of testing systems (either single species or multi species tests), is to standardise test conditions (soil texture, temperature, moisture, illumination etc.). In this way it is possible to compare both relative toxicities of chemicals and the toxicity results of different laboratories. Contact tests are performed in an aquatic medium and there are also some test procedures where the tests are performed in silica gel or agar (see above). These tests maximise contact of the test organism with the test substance, but their ecological relevance is at least questionable. As an improvement, a standard soil mixture has been developed for soil animals (OECD 1984, see above). Soil moisture (usually 50% of water holding capacity) and incubation temperature (+20°C) are kept constant during the tests. In general, the incubation conditions are usually kept optimal for the test organisms.

However, soil itself seldom has homogeneous structure, chemical composition, moisture or temperature. In the field, quality and content of soil organic matter, clay content, pH and particle size may change even within a distance of some millimeters. There can also be quite extensive changes in soil moisture and temperature within a short time period. Due to climatic conditions these factors vary tremendously also in geographical scale. Characteristics for high latitudes (Scandinavia, Canada etc.) are long, cold winters and short, cool summers, which results in relatively slow degradation of organic substances (e.g. pesticides) in the soil. Also organic matter content of the soils is relatively high in the north. For instance, organic matter content of agricultural soils in central Europe is typically 2-3% (Briggs & Courtney 1985) while in Finland it is 5-7% (Rajala 1995). In the northern coniferous forests the organic matter content of the humus layer is substantially higher, over 50%. Variation in organic matter content influences the sorption of chemicals and hence their toxic effects in the soil. Also the geological history of the area has had impact on overall soil formation in the past (glaciation, sea level changes etc.).

Variations in soil environmental conditions inevitably influence both the fate of a chemical in the soil and the behaviour of the exposed animals. Adsorption and desorption of a chemical as well as evaporation, leaching and degradation are all dependent on soil properties and climatic factors. Also animals have their optimal soil conditions, and deviations from these cause changes in their survival, growth and reproduction. Changes in conditions may also change their sensitivity to chemicals. All these factors affect toxic effects of the chemical on the exposed animals.

Soil organic matter content has been shown to be an important factor determining chemical toxicity to soil animals (Ma 1984, Van Gestel & Van Dis 1988, Van Gestel & Ma 1988, 1990, Crommentuijn 1994). Also soil pH (Crommentuijn 1994, Van Gestel et al. 1995), moisture (Harris 1964, Monke & Mayo 1990, Van Gestel & Van Diepen 1997), and temperature (Harris & Turnbull 1978, Demon & Eijsackers 1985, Everts et al. 1991, Heimbach & Balogh 1994, Smit & Van Gestel 1997) have influence on toxic effects. In addition, the presence of other substances may affect the toxicity of one substance (Van Gestel & Hensbergen 1997). For example, pesticide formulations often contain mixtures of two or several active ingredients and other additives, e.g. solvents. Also most contaminated sites are polluted by several contaminants simultaneously, e.g. by heavy metals and aromatic hydrocarbons.

Because of seasonal and spatial variation in soil quality, it is practically impossible to assess the toxicity of a chemical to the whole spectrum of soil organisms in different environmental conditions on the basis of single species tests conducted in standardised test conditions. Threfore, in addition to studies with several soil animal groups, it is of importance to study the effects of abiotic factors on chemical toxicity. Only a limited number of studies on the influence of environmental conditions on the toxicity of chemicals to terrestrial organisms has been published thus far (Van Gestel 1997). However, information obtained from this kind of study might improve the risk assessment of chemicals.

1.6 Objectives of the thesis

A main theme of this thesis is the study of the effects of some abiotic factors on the toxicity of chemicals to soil organisms at both population and community levels. The main questions were:

1. How do abiotic factors (soil organic matter, temperature, soil moisture) affect chemical behaviour in the soil and the organisms exposed, and what are the implications for toxic effects?
2. Does a closer examination of population development and population structure (size distribution) give any new information about toxic effects?
3. Are there any differences between the species in their responses to chemical exposure?
4. What are the effects of chemical application on the soil animal community under stress caused by either another chemical or drought?

The thesis comprises six papers (I-VI) in which the effects of soil quality (I, III), temperature (III, IV), soil moisture (II,VI) and presence of another chemical (V) have been studied. Emphasis has also been put on improvement of the test methods, e.g. by measuring size distributions of juveniles (II-IV) and by determining chemical disappearance from the test systems (I-VI). Applicability of the current tests is evaluated on the basis of these experiments, and the results of single species experiments (I-IV) and the microcosm experiments (V-VI) are compared.

2 MATERIALS AND METHODS

2.1 Experimental systems

All experiments were conducted in the laboratory. They were either single species experiments with one soil animal species (I, II, III, IV) or microcosm experiments with diverse soil fauna, microflora and a plant species (V, VI). Procedures of the experiments were modified partly from the international standard test guidelines (OECD 1984, ISO 1993, 1997a,c) and partly from individual papers (e.g. Krogh 1995, Salminen et al. 1996, Edwards et al. 1996).

2.1.1 Single species experiments

In the first experiment (I) the effects of an insecticide on three different soil animal species in three different soils were studied in order to determine species and soil specific variation in toxic effects. In the second experiment (II) the effects of soil moisture on pesticide toxicity were studied with an enchytraeid worm. In the last two single species experiments the effects of soil organic matter content (III) and temperature (III and IV) on insecticide toxicity to a collembolan were studied. These experiments were conducted for more thorough investigation of the questions arising from the previous experiments.

Closed glass (I, II, IV) or plastic (III) vessels were used as test containers. The soils used in the experiments were standard artificial soil (OECD 1984) (I, III), standard LUFA 2.2 field soil from Germany (III), or field soils collected from organically farmed fields near Jyväskylä, central Finland (I-IV). Field soils were defaunated before the experiments, except the soils of the earthworm experiment (I), where microbial activity was followed as one effect parameter. Defaunation would have also affected microbial community.

The species used in the experiments were an earthworm *Aporrectodea caliginosa* ssp. *tuberculata* (Eisen) (I), an enchytraeid worm *Enchytraeus* sp. (I, II), and two collembolan species *Folsomia candida* (Willem) (I, IV) and *F. fimetaria* (Linné) (III). The earthworms were collected from a garden soil in Jyväskylä during the autumn ploughing and they were stored at +5°C until the experiment.

Also the enchytraeid worms originated from the same soil. Later on a monoculture of the enchytraeid species was established and cultured in defaunated field soil (II). The culture was kept in a climate chamber at + 16°C in constant darkness and fed with rolled oats.

Both collembolan species were cultured in Petri dishes containing plaster of Paris/charcoal mixture (ISO 1993). *F. candida* originated from a culture from the Free University of Amsterdam, The Netherlands, and it was cultured in Jyväskylä in the same conditions as *Enchytraeus* sp. *F. fimetaria* was originally collected from an agricultural field near Silkeborg, Denmark, and it was cultured in the National Environmental Research Center (NERI), Silkeborg, Denmark, in a climate room at +20°C. The collembolan cultures were fed with dry granulated baker's yeast.

Pesticide solutions/emulsions were mixed homogenously into the soil before adding animals. Different concentrations were used in the experiments depending on the aim of the experiment and on the known toxicity range obtained from the literature, range-finding tests or previous experiments. Control (without pesticide addition) + two (IV), four (I: collembolan and enchytraeid worm) or five (I: earthworm, II, III) concentrations were used in the single species experiments.

Animals were introduced into the vessels next day after the pesticide mixing. Earthworms (I) and enchytraeids (I, II) were introduced from the storage vessels or from the permanent cultures due to difficulties in rearing batches of animals of exactly the same age. For the collembolan experiments (I, III, IV) synchronised cultures were used. Ten specimens were introduced into each vessel except in the earthworm experiment, where five specimens were introduced. Duration of the experiments varied from 14 days (I, earthworm) up to 56 days (III, IV). Also temperature varied from +10°C to +20°C depending on the experiment.

2.1.2 Microcosm experiments

The microcosm experiments were established for investigating effects of two simultaneous or subsequent stressors on soil animal community and its function. The effects of two pesticides applied separately or together were studied in the first microcosm experiment (V). In the second microcosm experiment (VI) the effects of pesticide application and subsequent drought on the soil animal community were studied.

The microcosms were prepared from acrylic cylinders (V) or from plastic bottles (VI). Soil for the microcosms was collected from the same site as the soil used in the single species experiments (II) and (IV). The soil was either sieved carefully in order to cause as little damage as possible for the indigenous fauna (V), or sieved and defaunated in the bottles in order to eliminate all undesired animals (VI).

In experiment V indigenous fauna was amended with a random selection of arthropods collected from the same site as the soil and with *Enchytraeus* sp. In the experiment (VI) an artificial faunal community was introduced into the microcosms. The community consisted of four collembolan species (*Folsomia fimetaria* Linné, *Tullbergia macrochaeta* Rusek, *Hypogastrura assimilis* Krausbauer, *Isotoma anclicana* s.lat), one predatory mite (*Hypoaspis aculeifer* Canestrini), one

enchytraeid worm (*Enchytraeus* sp.) and five nematode species (*Prionchulus punctatus* Cobb, *Acrobeloides tricornis* Thorne, *Aphelenchoides saprophilus* Franklin, *Aphelenchus avenae* Bastian, *Caenorhabditis elegans* Dougherty). *I. anglicana* was collected from agricultural fields near Silkeborg, while the other collembolan species and the predatory mite were from the permanent cultures at the NERI, Denmark. The predatory nematode *P. punctatus* (Cobb) was collected from soil near Jyväskylä and the other nematodes were reared from inoculations of the permanent cultures at the Department of Biology and Environmental Science, University of Jyväskylä. The enchytraeid worms were from the same culture as those used in the single species experiments.

Pesticides were sprayed on the soil surface some weeks after the introduction of animals in order to let the community to stabilize. Doses used were 10 times higher than normal application doses, and they were added in one (V) or two (VI) applications. Number of replicates was higher that in the single species experiments, 5-6 (V) or 6-7 (VI) per treatment.

The microcosms were incubated in climate chambers with diurnal illumination and temperature cycles. Evaporated water was replenished when needed by spraying deionised water on the soil surface. Duration of the experiments was 13 (V) or 34 (VI) weeks.

2.2 Pesticides used in the experiments

Dimethoate [0,0-dimethyl-S-(N-methykarbomoyl-methyl)-phosphorodithionate] was used as a representative of pesticides in all experiments. It is the most used insecticide in Finland (Hynninen & Blomqvist 1997). It is an organophosphate that inhibits cholinesterase activity(WHO 1989) and its toxicity to terrestrial arthropods is well known (e.g. Powell et al. 1985, Unal & Jepson 1991, Krogh 1994). Either a commercial formulation (III) or technical dimethoate (I-II, IV-VI) was used in the experiments. A desired amount of dimethoate was mixed with deionised water before the applications.

Also commercial formulations of benomyl (II, V) and propiconazole (VI), Benlate and TILT 625, respectively, were used in the experiments. Benomyl [methyl-1-(butylcarbamoyl)benzimidazol -2- ylcarbamate] is a systemic fungicide that is highly toxic especially to terrestrial annelids (e.g. Heimbach 1984, Römbke 1989, Van Gestel et al. 1992). Propiconazole [(±)-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1H-=1,2,4-triazole] is a rather new systemic triatzole fungicide that is used against fungal diseases in agriculture, forestry and golf courses. Information about its toxic effects in the soil environment is scanty, but it has been reported to be possibly harmful to earthworms (Edwards & Bohlen 1992), and it has been shown to be toxic to algae and fish (Tomlin 1994). Benomyl formed an emulsion with water while propiconazole formed a solution.

2.3 Analyses and measurements

Earthworms (I) were sorted manually, counted and weighed (fresh mass). Enchytraeids were extracted with a wet funnel method from whole soil samples that were in the incubation jars (I, II), or from subsamples taken from the soil of the microcosms (V, VI). Nematodes were also extracted with the wet funnels from subsamples (V, VI). Microarthropods were extracted with modified high gradient extractors from whole soil samples (I, III, IV) or from subsamples (V, VI).

A digital image processing (DIP) method was used for the size determinations of enchytraeids. The worms were preserved, dyed and scanned as images on a computer hard disk. The images were converted to data files from which approximate lengths of individuals were calculated (II). Another DIP-method (Krogh et al. 1998) was applied to the collembolans in experiment III. In experiment IV the sizes of collembolans were measured manually with a stereomicroscope.

In the microcosm experiments collembolans were identified to species level. Other animal groups were counted only (V) or counted and identified to species level (VI). Microbial biomass was measured by analysing ATP-content of the soil (Vanhala & Ahtiainen 1994). Acid and basic phosphatase enzyme activities (VI) were measured with the methods modified from Tabatai & Bremner (1969). Aboveground biomasses of barley *Hordeum vulgare* (V) and grass weed *Poa annua* (VI) were determined.

Soil respiration (CO_2-production) was measured by taking air samples from the test vessels (I) or from the microcosms (V, VI) and analysing CO_2-contents of air samples with an infrared carbon analyser. Nutrient concentrations of the microcosm soils were measured photometrically from 2M KCl extracts (V, VI). Soil moisture content (+105°, 16h), loss on ignition (+550°C, 5h) and pH (water) were measured in most cases from the subsamples. In order to get information about pesticide degradation, pesticide concentrations in the soil were analysed from separate samples (I-IV) or from soil subsamples (V-VI) taken 3-4 times during the experiments. Samples from one (I, III, V, VI) or two concentrations (II, IV) were analysed and the method was modified from Andersson & Pålsheden (1991).

2.4 Statistics

Effect concentrations (LC_x- and EC_x-values) were calculated with several methods: probit method (I), a linear interpolation method for sublethal toxicity (Norberg-King 1993) (I) and by fitting the logistic equation to the data (III). Dunnett's test was used for the calculations of the highest no observed effect concentrations (NOEC-values) (I, II). Size distributions of the juveniles (IV) were tested with non-parametric Kruskall-Wallis H-test and Mann-Whitney U-test.

Either factorial analysis of variance (ANOVA) (II, IV, VI) or non-parametric Kruskall-Wallis ANOVA (V, VI) were used to determine differences between the treatments. Mann-Whitney U-test (V) or one way ANOVA (VI) were applied to pairwise comparisons. Collembolan communities (V) and whole soil animal communities (VI) of the microcosms were analysed with canonical discriminant analysis. Soil respiration results were analysed with MANOVA for repeated measurements. Dimethoate degradation rates were calculated with regression statistics (I, IV).

U.S. EPA software package, SPSS for Windows Release 6.1, SAS procedure NLIN and Microsoft Excel software were used for calculations and statistical analyses.

3 RESULTS

3.1 General toxicity of the chemicals studied

Dimethoate proved to be highly toxic especially to soil arthropods. Calculated or tentative LC_{50}- (survival) and EC_{50}-values (reproduction) for collembolans were 1-6 mg/kg in all soils (I, III, IV). Also nominal concentrations of 3.7-6.5 mg/kg in the whole soil column of the microcosms supressed collembolan populations substantially (V, VI). There were clear differences between the collembolan species in their sensitivity to dimethoate (V, VI): some species, e.g. *Isotoma notabilis* (V) and *I. anglicana* (VI) seemed to suffer from dimethoate applications while *Tullbergia*-species were somewhat more resistant. Mites also showed high sensitivity to this pesticide.

On the other hand, other soil animal groups were not as sensitive to dimethoate as arthropods. To the earthworm *A. caliginosa* dimethoate proved to be moderately toxic with EC_{50}-values for biomass change being 14-43 mg/kg (I). To an enchytraeid worm *Enchytraeus* sp. it was not toxic except at extremely high concentrations. The lowest observed effect concentrations (LOEC-values) for growth and reproduction for this species were 400 mg/kg (II).

Dimethoate application also affected soil respiration (VI) in the microcosms. It reduced CO_2-production in the dimethoate treated microcosms compared to the control microcosms. The reduction lasted some weeks before the CO_2-production recovered to the same level as in the controls.

Benomyl showed abrupt toxic effects on *Enchytraeus* sp. at a concentration of 32 mg/kg (II). Ten times normal application doses (ca. 5 mg/kg) did not show any clear effects on enchytraeids, although their numbers tended to be lower in benomyl treated than in dimethoate treated or control microcosms (V). Benomyl did not affect total numbers of collembolans, but did change species composition and their abundances. Because those changes targeted less abundant species, the effects were not possible to detect statistically at species level. The CDA- analysis revealed those changes (V, Fig. 2), which were apparently due to changes in the microbial food resources of collembolans.

Propiconatzole proved to be harmless to the soil animals in the concentrations studied. No effects were found on microarthropods or on enchytraeid worms in the single species tests when the highest concentrations tested were 30-32 mg/kg. In the microcosm experiment no effects were detected on these species, either, when the nominal concentrations in the soil column were 1-2 mg/kg (VI). This concentration, however, reduced CO_2-production compared to the controls (VI).

3.2 Abiotic factors affecting toxicity

3.2.1 Soil type

In experiment I three different soil types were studied. It was shown that in clay soil toxicity of dimethoate to the earthworm was somewhat higher than in artificial or humus rich sandy soil. The reason for the difference was apparently differences in organic matter contents. Clay soil contained ca. 50% less organic matter than the two other soils. Effect of organic matter on toxicity was studied in more detail in the experiment (III), where collembolans (*F. fimetaria*) were exposed to dimethoate in three artificial soils containing different amounts of organic matter. It was found that toxicity increased with increasing organic matter content. Approximate dimethoate concentrations in the soil pore water of these soils were also calculated and revealed that the dimethoate concentration was lower in the soil pore water in soil with high organic matter content. The artificial soils were wery similar in terms of soil pore water concentrations.

This held true, however, for the artificial soils only, which were otherwise similar to each other except for their organic matter content. When field soils were studied the situation was more complicated. Humus rich sandy soil and LUFA 2.2 standard field soil showed somewhat differing toxicities in terms of recalculated soil pore water concentrations (III). The toxic effects were lower in the LUFA 2.2 soil than expected based on the organic matter content.

3.2.2 Temperature

Effects of temperature on toxicity of dimethoate to collembolans were studied in experiments III and IV. It was found that temperature affected collembolan reproduction, and a decrease of only a few degrees slowed the reproduction drastically. Therefore it was not possible to use the same incubation periods for different temperatures if approximately the same reproductive output was necessary in the controls at different temperatures. The problem was partially avoided by using longer incubation periods for the lower temperatures (III). This allowed the same physiological time (degree-days) for collembolan growth and reproduction. In experiment IV the problem was solved by taking samples in two weeks intervals.

In general, variations in the incubation temperatures did not have a substantial influence on toxic effects. It seemed, however, that dimethoate toxicity

decreased when temperature increased. Toxic effects on adult survival (III) and on adult growth (IV) were lower at higher temperatures. For reproduction this effect was, however, dependent on the timing of sampling. Apparently the collembolans laid eggs at certain periods of time producing one or two clutches of juveniles in the samples by the end of the experiments. If only the first clutch had hatched at the end of the incubation period, as at the lowest temperature (+10°C) in the experiment (III), the calculated EC_{50}-value was higher than if two clutches had hatched. Also in the experiment (IV) were no differences in numbers of juveniles between 1 and 3 mg/kg dimethoate concentrations right after the first clutch had hatched, but soon after that almost all juveniles died at 3 mg/kg while the rate of population increase at 1 mg/kg was close to that in the controls. The reason for this is that adult collembolans lay eggs also at higher concentrations immediately after the exposure has started, but laying will cease after some time and the second clutch remains smaller than at lower concentrations or in controls (III), or is absent (IV). Therefore EC_{50}-values were higher at +10°C than at +15°C, although the temperature was lower (III, Table 4), or there were no differences between the concentrations immediately after the first juveniles had hatched (IV).

In experiment IV it was also found that toxic effects tended to last longer at low temperatures. At +13°C the adults were relatively smaller at 1 mg/kg than in the control during the whole experiment while at +16°C and +19°C the differences were observed on the first sampling occasion only, if at all.

Temperature also affected growth rate of adult collembolans (IV). At low temperature they grew faster, apparently because at higher temperature they allocated resources to reproduction instead of growth. Also dimethoate degradation rate was slower at low temperature (III). This might also have had some implications for collembolan exposure to the chemical, which, in turn, could have increased toxic effects at low temperatures.

3.2.3 Soil moisture

Soil moisture *per se*, as well as temperature, affected the well-being of the soil animals. In both experiments where the effects of soil moisture were studied (II, VI), growth and reproduction of *Enchytraeus* sp. were clearly suppressed by low soil moisture content. Temporary drought also affected the whole soil animal community by decreasing the total numbers of animals in the dried microcosms (VI).

Toxicity of pesticides was also affected by drought, but in an unpredictable way. Dimethoate toxicity to *Enchytraeus* sp. decreased when soil moisture decreased. On the other hand, toxicity of benomyl increased with decreasing soil moisture (II). In the microcosm experiment both the dimethoate treatment and drought reduced total animal numbers through their impacts on arthropods (dimethoate) and enchytraeids (drought) (VI).

Low soil moisture clearly reduced soil respiration (VI). There was also some evidence that low soil moisture content extended the duration of reduced respiration caused by the chemical application. Reduction in CO2-production lasted longer in the dried microcosms than in the moist microcosms when dimethoate treated and control microcosms were compared.

3.2.4 Simultaneous application of two pesticides

Effects of simultaneous applications of two pesticides with different modes of action were studied in the microcosm experiment (V). Both pesticides, dimethoate and benomyl, affected soil collembolan communities. Dimethoate reduced the total numbers while benomyl altered the collembolan community structure. When both pesticides were applied simultaneously, effects of dimethoate on collembolan community were direct and so severe that the possible effects of benomyl remained unnoticed.

4 DISCUSSION

4.1 General features

It has been shown throughout the thesis that responses of animals to chemical exposure vary between the species. This emphasizes the importance of an adequate set of tests with different types of species in the risk assessment of chemicals (Eijsackers & Løkke 1992). It has been argued that soil ecological function is sufficiently protected when all species are protected (Van Straalen & Van Gestel 1993). Therefore it is necessary to develop test procedures with different species and try to standardize them as far as possible. On the basis of these tests it is possible to statistically estimate the concentrations in the soil at which most (e.g. 95 %) of the species are not affected and hence the ecological functioning of the soil is not endangered (Van Straalen & Denneman 1989, Wagner & Løkke 1991, Aldenberg & Slob 1993).

The species studied proved to be as sensitive in the single species tests as in the microcosm experiments. Thus, it cannot be concluded that single species tests would be more insensitive than multispecies tests. Salminen et al. (1996) found, that single species tests were even more sensitive than microcosm experiments when studying the effects of terbuthylazine on soil fauna in forest soil. Multispecies tests or community tests are, however, closer to the natural situation where species interact with each other in a heterogeneous environment. The indirect effects found in the microcosm experiments, the change in collembolan community structure caused by benomyl (V) and increased numbers of enchytraeids and one nematode species under dimethoate contamination (VI), would not have been noticed in single species tests.

Although dimethoate caused significant changes in soil arthropod populations in both microcosm experiments (V-VI), no clear effects were detected in fuctional parameters like nutrient contents, pH etc. Soil communities are assumed to be functionally redundant, i.e. activity of some lost species can be replaced by other species (Mikola & Setälä 1998, Setälä et al. 1998). Hence the net function of the community may remain unchanged despite large changes in

numbers of animals at species level. It is also possible that the sampling methods, subsamples from carefully mixed soil, were too robust for detecting slight alterations in soil nutrient balance etc. There was, however, some indication that severe alterations in soil animal community, reductions in collembolan populations and increase in enchytraeid population, may have implications for plant growth (VI). The exact causal mechanisms are, however, difficult to detect.

4.2 Abiotic factors

4.2.1 Soil quality

In general, environmental conditions played somewhat less significant role in toxicity than expected. It has been noticed earlier, that soil quality has an important role in chemical toxicity to soil animals (Van Gestel 1992, Van Gestel & Ma 1988, 1990). Soil organic matter content determines adsorption of most chemicals and hence exposure of animals to them. As experiments I and III showed, increased organic matter content decreased dimethoate toxicity to the earthworm *Aporrectodea caliginosa* (I) and the collembolan *Folsomia fimetaria* (III). At high organic matter content more dimethoate was bound onto the soil and therefore collembolans were less exposed to bioavailable dimethoate.

Van Gestel & Ma (1990) applied the pore-water hypothesis to soil and showed it to be valid. I used another method to calculate soil pore water concentrations and got results similar to Van Gestel & Ma (1990). In experiment III it was shown that recalculation of soil porewater concentration explained the differences in dimethoate toxicity to *F. fimetaria* between the artificial soils containing different amounts of peat (organic matter). There has been little information about the validity of the pore-water hypothesis for other soil animals than earthworms. It has been argued that soil pore water concentration determines the toxicity mainly for soft bodied animals, like earthworms, enchytraeids, nematodes etc. They live within pore water or in close contact to it and therefore take up pollutants through their cuticle (Van Gestel & Van Straalen 1994). The results of experiment III showed that the soil pore water hypothesis can also be applied to collembolans.

Differences in dimethoate toxicity between the soils were 3-4 fold when soil organic matter content varied between 1.8% and 8.6% (III). In the field, soil organic matter content can vary from low organic matter agricultural soils to high humus forest soils or to agricultural fields drained from peat bogs. In those high humus soils the toxicity is evidently substantially lower than in the soils with low organic matter content. As mentioned earlier, soils in the northern latitudes are usually more humus rich than the soils in the mid-latitudes. Therefore, in general, acute toxic effects may be lower in the northern soils.

Differences between the field soils (III) imply that soil organic matter content is not the only factor that determines the toxicity. Clay content, organic matter quality and degradation rate of the chemical caused by differing microbial activity may cause some variation in the toxicity results. For instance, in experiment III

dimethoate degraded faster in the LUFA-soil than in the artificial soils, which caused lower toxicity than expected based on the organic matter content. Also soil clay content and pH can be important factors, especially for toxicity of heavy metals (Crommentuijn 1994, Van Gestel & Ma 1988). Effects of pH were not, however, studied in this thesis, but pH was kept close to 6.0, which evidently is optimal for most soil animals in agricultural soils.

4.2.2 Temperature

Temperature has influence on many biological and chemical processes in soil, for example physiological processes and population dynamics of soil animals. Relationship between temperature and population development differs between species (Van Straalen 1995) and also the threshold temperature for inactivity is species dependent (Venette & Ferris 1997). In general, activity of soil animals starts to increase when temperatures increases a few degrees above 0°C, but increase in activity is not necessarily linear (Johnsson & Wellington 1980, Van Straalen & Joosse 1985). Gregoire-Wibo & Snider (1983) showed that collembolans optimize survival at low temperatures by slowing down their growth, which delays reproduction and maximizes longevity. At high temperatures they optimize population growth by rapid development and high fecundity.

Also behaviour of chemicals in soil is temperature dependent. Usually increasing temperature increases chemical losses from soil by increasing desorption and subsequent leaching, degradation and evaporation (Edwards 1973). Because temperature also affects detoxification rates in organisms exposed (Janssen & Bergema 1991, Howe et al. 1994), it has a two-fold effect on toxicity of chemicals. At low temperatures, when activity of animals is low, the possibility of coming into contact with chemicals is lower than at high temperatures with higher activity. On the other hand, detoxification and degradation rates are slower at low temperature. When temperature increases, activity of animals increases, but also detoxifying and excretion mechanisms and degradation are accelerated (Heimbach & Balogh 1994, Smit & Van Gestel 1997). Increased temperature may also have indirect effects on chemical excretion efficiency through increased growth of animals (Eberhardt 1978).

Relatively little information is available on the effect of temperature on the toxicity of chemicals to terrestrial invertebrates. Heimbach & Edwards (1983) did not find any significant influence of temperature (10-26°C) on 2-chloroacetamide or benomyl toxicity to an earthworm *Eisenia fetida* in acute toxicity tests, but the duration of the test is relatively short (2 weeks), and substantial decreases in concentrations of the chemicals concerned may not occurred. Also sublethal effects on reproduction cannot be detected in the acute test. Sandifer & Hopkin (1997) did not find any clear differences in toxicity of heavy metals to *F. candida* at temperatures of 15 and 20°C, either. They concluded that although 20°C is a somewhat higher temperature than in the field in England and northern Europe, it gives the same results as experiments conducted at 15°C, but in shorter time.

On the other hand, Heimbach & Balogh (1994) tested effects of three different pesticides on a carabid beetle *Poecilus cupreus*, and they found a clear negative correlation between temperature and toxicity for all chemicals. Also Smit

& Van Gestel (1997) found a negative correlation between temperature and zinc sublethal toxicity to *F. candida*. Their study revealed, however, that the effect of temperature on toxicity is dependent on the parameter measured. Toxic effects of cadmium increased at low temperature when adult growth and reproduction were considered. Effect on adult survival was, however, decreased when temperature was decreased. In both temperature experiments (III and IV) in this thesis toxic effects of dimethoate showed a weak negative correlation with temperature. In experiment III both the LC_{50}-value for adult survival and the EC_{50}-value for reproduction were decreased at 15°C compared to 20°C, but at 10°C the LC_{50}-value again increased. This indicates that adult collembolans changed their strategy from high reproduction to maximizing of survival when the temperature was low enough (see Gregoire-Wibo & Snider 1983). In experiment IV the toxic effect of dimethoate on growth of *F. candica* lasted longer at low temperature, which is in accordance with the results of Smit & Van Gestel (1997).

It seems that duration of the experiment plays a significant role when the effects of temperature on toxicity are studied. In general, it is necessary to have more or less the same number of juveniles (population increase) at the end of the experiment in the controls of all temperatures. This means that incubation periods should be extended substantially at lower temperatures. This was clearly demonstrated in experiments III and IV. Smit and Van Gestel (1997) used the same degree-day technique (Axelsson 1997) for compensating for slower growth and reproduction as it was done in experiment III, sampling lower temperatures later than higher ones. If the experiments had been sampled at the same time at all temperatures, the EC_{50}-values for reproduction (III) or numbers of juveniles (IV) would have been misleading because of different strategies at different temperatures. For instance, the higher EC_{50}-value for reproduction at 10°C than at 15°C (III) can be explained by the presence of only one clutch of juveniles at 10°C compared to two clutches produced at 15°C. Under chemical exposure the second clutch remains smaller than the first clutch due to gradually reducing reproduction capacity of the adults. This increases the toxic effect on the whole population and hence decreases the EC_{50}-value (III).

In general, the trade-off between growth and reproduction of animals, e.g. collembolans, at different temperatures (Gregoire-Wibo & Snider 1983) is somewhat problematic from the ecotoxicological point of view. Optimizing survival at low temperature and population growth at high temperature evidently affects their strategy to cope with toxic stress. Investing for survival at low temperatures through inactivity increases the survival under chemical stress. This affects, however, population increase since reproduction is slower at low temperature. Hence population recovery from chemical stress may be delayed at low temperatures although adult survival is somewhat better. It seems that temperature may substantially alter the toxic effects of chemicals. Therefore it should be taken into account when assessing possible environmental risks of chemicals by means of ecotoxicological testing. This could be done by conducting the tests at two different temperatures.

4.2.3 Soil moisture

In the field, the effect of temperature on toxicity is closely related to soil moisture, since high temperature usually increases evaporation and hence affects toxicity indirectly through drought stress (Everts et al. 1991). Effects of soil moisture content *per se* on chemical toxicity are also complex. Soil moisture content affects the distribution of a chemical between soil air, soil water and soil particles (Harris 1964). In dry soil chemical adsorption onto soil particles is stronger because of lack of water molecules that would compete with chemical molecules for adsoption sites (Edwards 1973). Soil moisture also affects the rate of biological and chemical transformation/degradation of a chemical (Monke & Mayo 1990) as well as the physiology and behaviour of animals (Everts et al. 1991). Soil animals, e.g. collembolans and enchytraeids, are greatly dependent on soil moisture (Verhoef & Van Selm 1983, Lagerlöf & Strandh 1997). As soft bodied animals they are susceptible to desiccation and therefore drought may cause severe stress to them.

Toxicity of chemicals has been found to increase with increasing soil moisture (Harris 1964, Mowat & Coaker 1967), which was also the case with dimethoate in experiment II. Also opposite findings have been reported (Demon & Eijsackers, 1985 Monke & Mayo 1990) and this was found with benomyl in experiment II. In addition, in some studies, no clear effects have been reported (Heimbach & Edwards 1983, Van Gestel & Van Diepen 1997). In some cases toxicity has been lowest at moderate soil moistures and higher in dry and very wet soils (Everts et al 1991).

It seems that several independent mechanisms affect both chemical bioavailability and well-being of exposed animals. In dry soil adsorption of a chemical is stronger and hence bioavailability is reduced. Some species can also avoid drought stress through dormancy (e.g. nematodes) and hence reduce their exposure to the chemical at the same time. Species without this ability may suffer from drought stress (desiccation) and are therefore more susceptible to toxic effects (Everts et al. 1991). The converse is also true; chemical stress decreases the drought tolerance of soil animals. Holmstrup (1997) demonstrated that sublethal concentrations of three different chemicals increased mortality with decreasing soil moisture.

When soil moisture increases substantially, also chemical uptake increases causing higher internal concentrations and hence increased toxicity. In moist soil bioavailability of a chemical may be greater, but also degradation by microbial metabolism is accelerated (VI). Also activity and hence chemical uptake of an animal is usually higher in moist and hence favourable conditions. Therefore acute toxicity can be higher but due to rapid degradation, duration of exposure is shorter and overall toxicity may be at the same level as in drier soil.

Van Gestel & Van Diepen (1997) concluded that (within the moisture range chosen) moisture content had no great influence on the bioavailability and toxicity of cadmium to the collembolan *F. candida*. In their study collembolan reproduction was highest at the lowest soil moisture (25% of water holding capacity, WHC). In experiment II the lowest soil moisture content was 40% of WHC and this moisture substantially decreased both growth and reproduction of

the enchytraeid worm compared to higher moistures. In general, enchytraeids are more sensitive to drought than collembolans. Therefore the question about the effect of soil moisture on toxicity is highly species spesific.

In experiment VI effect of drought on the animal community was clear although not distinct. While dimethoate reduced arthropod numbers, drought decreased enchytraeid numbers. These two simultaneous stresses resulted in the lowest total animal numbers. This indicates the possibility that although a chemical itself does not decrease all faunal groups evenly, some other stress factor (drought in this case) may exaggerate the decrease in total animal numbers, and thus affect the total population densities.

4.3 Evaluation of the methods used

4.3.1 Single species experiments

All the experiments except experiment III were conducted in small glass beakers that were rather convenient to use. The only disadvantage was the absence of a proper lid. Parafilm sheets were not very durable and they had to be replaced with new ones almost every time when food was added and evaporated water was replenished. The vessels used in experiment III were especially constructed for this kind of experiment. Both the lids and the bottoms were removable. They also had mesh at the bottoms which enabled direct transfer of the vessels to the high gradient extractor after the bottoms had removed. The advantage of this construction was that it was not necessary to remove the soil from the incubation vessel and hence possible damage to the animals in the soil can be avoided. The vessel was constructed at the NERI, Silkeborg, Denmark, where experiment III was conducted.

High gradient extraction of collembolans was used instead of the flotation method (ISO 1997) in all the experiments for counting the animals. This allowed easy counting of animals after they had been collected into cups with plaster of paris/charcoal bottom, and deepfrozen. The collembolans were counted and their length was measured under a stereomicroscope. The extraction method also enabled digital counting and size measuring (III). Because a dark background was needed for reliable distinguishing of whitish collembolans from the background, the flotation method was not feasible. The floating method was tried in preliminary trials of the experiment (IV), but without success. Foam formed on the water surface and the collembolans, especially the smallest juveniles, were difficult to distinguish on the surface even by eye. From the photographs taken it was nearly impossible. Some juveniles were under the foam and could not be counted at all.

One disadvantage of the extraction method is that its efficiency is not always known. When almost all adults can be found in the controls one can be relative sure that the extraction efficiency was high (III and IV). On the other hand, if the numbers of adults are low in the controls (I), it is not possible to know whether it is due to low survival of adults or poor extraction efficiency. Sometimes survival

of adult collembolans can be less than the 80% required in the standard test (ISO 1997c) even in the controls (Sandifer & Hopkin 1997, Smit 1997, Van Gestel & Van Diepen 1997) and therefore low numbers in the extracted samples are not necessarily due to low extraction efficiency. It seems, thus, that both methods are satisfactory and can produce reliable results in ecotoxicological studies.

Digital counting and image processing are not often used in ecotoxicological studies. The more sophisticated method used in experiment III was developed at the NERI, Denmark (see Krogh et al. 1998). It enabled differentiation of the two clutches of *F. fimetaria* juveniles (III). Without the method it would not have been possible to explain the somewhat inconsistent results of the temperature experiment (III). The more robust method used in experiment II was not as successful. The same conclusions could have been drawn by merely counting the adults and the juveniles. It was also quite a laborious method since the samples had to be cleaned and the enchytraeids had to be dyed. In conclusion, the digital counting and subsequent data processing is a promising method to study ecotoxicological effects. It needs, however, some improvements of the equipments before it will be a widely used standard tool in ecotoxicological research.

4.3.2 Microcosm experiments

The microcosms used in both experiments (V-VI) proved to be feasible for ecotoxicological studies. The plastic bottles used in experiment VI were especially suitable. The construction was inexpensive, it was relatively easy to introduce soil, animals and plants into the bottles, and it was possible to close the bottles for CO_2-measurements. Both methods of introducing animals, indigenous fauna with random additions of extra animals (V) and a completely artificial animal community (VI), were successful and both methods produced soil animal communities which could be maintained for several months. It seems that it would be relatively easy to standardise a set of 10-15 species of soil animals which could be cultured in a laboratory and repeatedly used in microcosm studies. Neither of these experiments were dose-response experiments, but both methods could be also used for that kind of experiments. The use of intact soil cores has been emphasized by many researhers (Fredrickson et al 1991, Morgan & Knacker1994), but also homogenised soils with indigenous fauna (Parmelee et al. 1993, 1997, Edwards et al. 1996) or constructed soil communities (e.g. Mothes-Wagner et. al 1991, Salminen et al 1996) have successfully been used. Especially when both the chemical and community structure effects on soil processes have been the objectives of study, constructed soil communities have been useful (Salminen et al. 1995, Salminen & Haimi 1997, Salminen et al. 1997).

5 CONCLUSIONS

In general, it can be concluded that slight variations in environmental conditions do not usually cause large changes in the toxic effects of chemicals on soil animals. Species specific sensitivity of different species to chemicals seems to be more important when evaluating effects of chemicals in the soil environment. It is clear, however, that critical conditions, like severe drought or freezing temperatures, may force soil animals to use extra resources for survival, and their ability to resist chemical stress may temporarily be weakened. In those situations effects can be detrimental for the population.

Although there has been a lot of criticism of single species tests in ecotoxicological research, they seem to be valuable for studying other aspects of toxicity than only chemical toxicity *per se* to the test species. We need single species experimental systems for our studies when we study those countless aspects that affect toxic effects of chemicals entering soil. In addition, microcosms proved to be good tools for higher than population level ecotoxicological studies. At present they are, however, individual cases of experiments without any standardization and field validation, but they can be elaborated further towards more standardized methods. Only then will the results of studies in different laboratories be comparable.

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YHTEENVETO

Ympäristötekijöiden merkitys kemikaalien maaperäeläimiin kohdistuvissa haitoissa – tutkimuksia populaatio- ja yhteisötasoilla

Tutkimukseni tavoitteena oli selvittää erilaisten abioottisten ympäristötekijöiden, kuten maan humuspitoisuuden, lämpötilan, kosteuden ja toisen kemikaalin läsnäolon vaikutuksia torjunta-aineiden ekotoksisiin vaikutuksiin maaperäeläimiin, sekä tätä kautta myös maaperän prosesseihin. Tutkimuksia tehtiin sekä populaatio- että yhteisötasolla. Populaatiotason tutkimukset olivat ns. yhden lajin toksisuustestejä, joissa koe-eläimiä altistettiin torjunta-aineille joko luonnonmaassa tai keinotekoisessa maassa. Useimmat yhden lajin testeistä olivat ns. annos-vaste -testejä. Maan kosteutta, lämpötilaa tai humuspitoisuutta vaihdeltiin riippuen tutkittavasta asiasta. Tutkimuksissa käytettiin merkkiaineena pääasiassa dimetoaattia, joka on yleisesti käytetty hyönteistuholaisten torjunta-aine eli insektisidi. Myös kahta sienitautien torjunta-ainetta eli fungisidia (benomyyli ja propikonatsoli) tutkittiin. Koe-eläiminä olivat peltoliero (*Aporrectodea caliginosa*), änkyrimato (*Enchytraeus* sp.) ja hyppyhäntäiset (*Folsomia candida* ja *F. fimetaria*).

Yhden lajin kokeissa havaittiin, että maan orgaanisen aineen pitoisuudella on huomattava merkitys torjunta-aineen haitallisuudelle: mitä enemmän maassa on orgaanista materiaalia, sitä haitattomampaa aine on. Maan humus sitoo haitallisia aineita tehokkaasti vähentäen kemikaalin pitoisuutta maan huokosvedessä, joka on kemikaalin pääasiallinen kulkeutumiskanava maasta eläimeen. Alhaisen lämpötilan havaittiin jonkin verran lisäävän kemikaalin haittavaikutuksia, lähinnä pitkittämällä eläinten palautumista kemikaalistressistä. Kuivuudella havaittiin olevan, torjunta-aineesta riippuen, joko toksisuutta lisäävä (dimetoaatti) tai vähentävä (benomyyli) vaikutus änkyrimatoihin.

Yhteisötason tutkimukset tehtiin ns. mikrokosmoksissa, joissa torjunta-aineelle altistettiin joko luonnollinen tai keinotekoisesti muodostettu eliöyhteisö. Ensimmäisessä mikrokosmoskokeessa benomyylin todettiin muuttavan maan hyppyhäntäisyhteisön rakennetta, vaikka kokonaisyksilömääriin sillä ei ollut vaikutusta. Dimetoaatti puolestaan pienensi selvästi mikroniveljalkaisten (punkit, hyppyhäntäiset) määriä. Toisessa mikrokosmoskokeessa kuivuuden ja dimetoaatin yhteisvaikutus eläimiin oli voimakkaampi kuin kummankin stressitekijän vaikutus erikseen, sillä kuivuus vähensi änkyrimatojen ja dimetoaatti mikroniveljalkaisten määriä. Vaikka altistuksen havaittiin aiheuttavan selkeitä muutoksia eläinyhteisöissä, pystyivät ne palautumaan muutamassa kuukaudessa. Myöskään selkeitä suoria tai epäsuoria vaikutuksia maaperän prosesseihin, kuten ravinnekiertoon, ei torjunta-aineilla yleensä havaittu olevan. Kokeiden perusteella näyttää siltä, että perinteiset yhden lajin testit ovat tarpeeksi herkkiä ilmentämään kemikaalin mahdollisia haittavaikutuksia maaperässä, mikäli kemikaali myös häviää maasta kohtuullisen ajan kuluessa. Molempia, sekä yhden lajin testejä että mikrokosmostutkimuksia, tarvitaan kuitenkin arvioitaessa kemikaalien ekotoksikologisia vaikutuksia maaperässä.

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Original papers

I

Toxicity of dimethoate to some soil animal
species in different soil types

by

Esko Martikainen

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II

Effect of soil moisture on pesticide toxicity to an enchytraeid worm *Enchytraeus* sp.

by

Mikael Puurtinen and Esko Martikainen

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III

Effects of soil organic matter content and temperature on toxicity of dimethoate to *Folsomia fimetaria* (Collembola: Isotomiidae)

by

Esko Martikainen and Paul Henning Krogh

Environmental Toxicology and Chemistry (in press), 1998

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IV

Temperature - time relationship in collembolan response to chemical exposure

by

Esko Martikainen and Minna-Liisa Rantalainen

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V

Effects of dimethoate and benomyl on soil organisms and soil processes - a microcosm study

by

Esko Martikainen, Jari Haimi and Jukka Ahtiainen

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VI

Pesticide application and drought as stress factors to the soil decomposer community and its function

by

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Jari Haimi and Keijo Mäntykoski

Manuscript

PESTICIDE APPLICATION AND DROUGHT AS STRESS FACTORS TO THE SOIL DECOMPOSER COMMUNITY AND ITS FUNCTION

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Abstract

Effects of two stressors, pesticides and temporary drought, on the soil decomposer community and its function were studied in a microcosm experiment. A constructed soil animal community was allowed to adapt for 1-2 months in the soil before pesticide applications (5 x normal dose, applied twice) and subsequent drying periods. Dimethoate, an insecticide, altered animal community structure by decreasing arthropod numbers substantially. It enabled enchytraeid worms and one nematode species to increase in numbers due to reduced predation by the mites and/or reduced competition with the collembolans. Sensitivity of arthropods to dimethoate was species specific, and it correlated with the sensitivity found in single species dose-response tests performed. Decreased arthropod populations recovered, however, within three months. Dimethoate application also reduced soil respiration. Plant growth tended to be enhanced in the dimethoate treated soils but not in the other soils, probably due to induced nutrient mineralisation by the enchytraeids and/or reduced fungal and root grazing of collembolans.

The influence of propiconazole (a fungicide) on animals and soil processes was less consistent, and only soil respiration was somewhat decreased. Drought decreased arthropod and enchytraeid numbers and soil respiration. It also slowed pesticide disappearance evidently by decreasing microbial activity. Because dimethoate and drought reduced different animal groups, their combined action reduced the total animal numbers more than either of these stressors alone. In spite of rather clear effects of the stressors on soil animals, no substantial effects on microbial parameters other than CO_2-production (i.e. ATP, enzyme activities) or soil nutrient concentrations were found.

Our results suggest that drought may have even counteracted the effects of the pesticide by forcing animals to move downward in the soil column, where dimethoate concentrations were lower than near the soil surface.

Introduction

Pesticides are applied on agricultural fields either before or during a growing season. Since the weather on the following days after application cannot be predicted accurately, the fate of pesticides is partly unpredictable. Weather conditions at the time of application are especially important because they control the rate at which the pesticide is washed into, through and out of the soil, and also influence processes of retention and degradation (Briggs & Courtney 1985). In certain weather conditions a considerable proportion of a pesticide enters the soil where it may affect soil biota and soil decomposition processes. When soil microbes and fauna are stressed by adverse abiotic conditions, the effects of chemical contamination may differ from those in optimal conditions (e.g. Everts et al. 1991ab, Holmstrup 1997). When it is warm and moist in the soil, a pesticide usually degrades quickly and the effects may be less severe (Monke & Mayo 1990). On the other hand, under such conditions organisms are active and may become exposed to pesticides, although for a shorter period. In cold, dry conditions pesticide degradation is slower, and due to slower reproduction the effects on soil organisms may be more drastic.

Assessment of the chemical effects in the environment is one of the most important tasks of the contemporary ecotoxicological research. Simple single species testing of chemicals is necessary for getting the first insight into possible harmful effects of a chemical on biota. Several testing methods have been or are being developed for terrestrial organisms (reviewed by e.g. Van Gestel & Van Straalen 1994). There has been much discussion about the need for testing systems of higher organisation levels, and some have already been described (Mothes-Wagner et al. 1992, Parmelee et al. 1993, 1997, Edwards et al. 1996). These microcosms have been used in specific studies concerning the effects of xenobiotics on populations and communities of soil organisms, on their mutual interactions and hence soil system function (Parmelee et. al 1993, 1997, Salminen & Haimi 1997, Salminen et al. 1997, Martikainen et al. 1998). Besides enabling studies of the structure and function of the soil ecosystem (Teuben & Verhoef 1992, Morgan & Knacker 1994) they are also considered to predict effects of the chemicals in the field more accurately than the single species tests (Römbke et al. 1994).

In most ecotoxicological experiments environmental conditions have been kept stable and optimal for the organisms. Soil organisms are, however, exposed to several natural stresses (drought, freeze etc.), and are particularly susceptible to soil desiccation. It has been shown that altering soil moisture may also affect the toxicity of chemicals to soil organisms (Harris 1964, Ma 1986, Monke & Mayo 1990, Everts et al. 1991b, Puurtinen & Martikainen 1997, Van Gestel & Van Diepen 1997). Likewise, exposure to a chemical may reduce their drought tolerance (Holmstrup 1997).

We designed a microcosm experiment in which the effects of pesticide application and subsequent drought on the soil decomposer community and soil processes were studied. From a wide variety of pesticides two representatives

with different modes of actions were selected: an insecticide with direct toxic effects on soil arthropods, and a fungicide that may have indirect effects on animal community and hence on soil processes. Our aims were to study: (1) short term effects of two pesticides and drought on soil microbial and faunal community structure, (2) recovery of the community after the stress, and (3) indirect effects of those stress factors on nutrient mineralisation and hence on plant growth. We hypothesized that the pesticide and drought will affect different soil faunal groups and therefore a combined sress by the pesticide and drought on the soil community may differ from the effects of either of these stressors alone. Potentially enhanced effect of the pesticide to soil animals under drought stress was also of interest.

Materials and methods

Soil and microcosms

The soil used in the experiment (loamy sand, pH 6, loss on ignition 5%) was taken from an organically cultivated field in central Finland. It was first sieved to homogenise the soil structure, and then defaunated by drying at +65°C for three days. After drying the soil was sieved again to break crumbs and, and then stored at +4°C. Oat straw was used as resource for microbes and as food for soil animals. The straw was chopped into small pieces, dried at +65°C and sieved through a 5 mm mesh.

Seven replicates of microcosms were prepared for each treatment (see treatment procedures below) and sampling occassion and one set of six microcosms for the sampling before the treatments, totalled 132 microcosms. Soil (300.0 g dry mass), chopped straw (2.0 g d.m.) and deionised water (70.0 ml) were mixed in a glass bowl and put into each microcosm. One liter transparent plastic bottles with cotton plugs at the top were used as microcosms. The microcosms were closed with screwable stoppers and heated at +65°C overnight in order to eliminate indigenous soil animals. After that the microcosms were stored at +4°C until the start of the experiment (day 0). Then the stoppers were removed and the microcosms were transferred into a climate room in which the illumination cycle was 12 h 2500-3000 lux, 1 h 1000-1500 lux, 10 h less than 200 lux and 1 h 1000-1500 lux. Temperature varied between +17°C (low light) and +20°C (high light).

Introduction of microbes and animals

A microbial inoculate was prepared from the same fresh soil as used in the microcosms. The soil was first deepfrozen at -80°C to eliminate animals. After thawing 500 g (fresh mass) soil was mixed with 800 ml of deionized water in a household mixer for 30 seconds. The mixture was poured into a glass jar and allowed to settle for one hour. The upper light coloured suspension column (2/3 of the total height) was filtered through a 50 µg nylon mesh and then three times

through a 10 µm nylon mesh to prevent contamination of the microcosms by nematode eggs. Deionised water was added to the suspension and 5 ml of the mixture was sprayed onto the soil surface of each microcosm on day 5.

Bacterial feeding nematodes, *Acrobeloides tricornus* (Thorne) and *Caenorhabditis elegans* (Dougherty), and fungal feeding nematodes, *Aphelenchoides saprophilus* (Franklin) and *Aphelenchus avenae* (Bastian), were cultured at +16°C on agar plates containing appropriate food for each species (see Mikola and Setälä 1998). Nematodes were extracted from the agar plates with a wet funnel extractor (Sohlenius 1979) before addition to the microcosms. They were mixed with ca. 150 ml autoclaved water and one ml of nematode inoculate of each species was added into every microcosm on days 6-7 (Table 1). Predatory nematodes *Prionchulus punctatus* (Cobb) were extracted from soil collected from a spruce forest in central Finland. They were transferred twice into clean water in order to avoid contamination by other nematodes, and inoculated in the same way as the other nematodes on day 29 (Table 1).

Three collembolan species, *Folsomia fimetaria* (Linné), *Tullbergia macrochaeta* (Rusek) and *Hypogastrura assimilis* (Krausbauer), originated from permanent cultures of the National Environmental Research Institute (NERI), Silkeborg, Denmark. The fourth species, *Isotoma anglicana* (s.lat), was collected from agricultural areas near Silkeborg. The collembolans were added into the microcosms on days 11-13 (Table 1). Also predatory mites *Hypoaspis aculeifer* (Canestrini) originating from a culture in the NERI were inoculated at the same time as predatory nematodes (Table 1). Specimens of a small enchytraeid species, *Enchytraeus* sp., were introduced into the microcosms at the same time as the collembolans (Table 1). The species has not been identified to a species level, but most likely it is an undescribed species closely related to *E. crypticus*. Originally the species was collected from a field in Jyväskylä in 1993, and it has been cultured in the Institute for Environmental Research since then.

Treatment procedures

A two-way factorial design was used in the experiment, with the factors being pesticide application and soil moisture. The treatment combinations were: 1. no pesticide, constant moisture, 2. dimethoate, constant moisture, 3. propiconazole, constant moisture, 4. no pesticide, temporary drought, 5. dimethoate, temporary drought, and 6. propiconazole, temporary drought.

Two types of pesticides were studied in the experiment. Dimethoate is a widely used organophoshate insecticide shown to be very toxic to soil arthropods, but relatively non-toxic to the enchytraeid species used in the present experiment (Unal and Jepson 1991, Martikainen 1996, Puurtinen and Martikainen 1997, Martikainen and Krogh 1998). Propiconazole is a systemic fungicide and information about its toxicity to soil organisms is scanty. It has been reported to be possibly harmful to earthworms (Edwards & Bohlen 1992), but Reicher et al. (1997) did not find any effects on earthworm cast production. Propiconazole is, however, toxic to algae and cladocerans (Tomlin 1994).

Single species tests for both pesticides were also conducted with each arthropod species and for propiconazole also with *Enchytraeus* sp.. The same soil was

used in the single species tests as in the microcosm experiment. Arthropod tests were performed with the following procedure: 10 adult microarthropods (five replicates) were exposed in 30 g of moist soil to six doses of pesticide for one week. Then they were extracted over two days in a high gradient extractor (Petersen 1978). For the enchytraeid testing method, see Martikainen and Puurtinen (1997).

Animals were allowed to adapt to the conditions in the microcosms for 1-2 months. On day 64, the pesticides (1.1084 mg dimethoate or 0.2960 mg propiconazole) were sprayed in 5 ml deionised water onto the soil surface. Technical dimethoate (Cheminova Agro AS, Denmark) and TILT625 (625 g/l a.i., Kemira, Finland) were used. The doses corresponded to five times the recommended dosage (dimethoate 400 g/ha and TILT 200 g/ha), which corresponded calculatory concentrations of 3.7 mg dimethoate/kg dry soil and 1.0 mg propiconazole/kg dry soil in the soil column. The controls were sprayed with 5 ml of deionised water.

One week after the pesticide applications half of the microcosms were dried slowly. An air pump (Edwards ECB1 vacuum pump/compressor) was connected to a PVC pipe (length 125 cm, Ø 12 cm) having 62 outlets for 100 cm plastic tubes. The tubes were placed in the microcosms, 15 cm above the soil surface. Light air flow (0.2-0.3 L/min.) was maintained in the microcosms for 13 days, after which ca. 60 % of the moisture had evaporated. The microcosms were rewetted to the original moisture content with deionised water the day after the drying had finished. The rewetting took three days, days 85-87 (10 ml + 25 ml + the rest needed). Also the non-dried microcosms were moistened to the original weight on day 87 (ca. 3-5 ml/microcosm).

Because of the rather weak effects of drought on the animals (see the results) the treatment procedure was repeated. On day 95 the same amounts of pesticides as in the first application were added and the drying procedure was started eight days later. The drying period lasted 25 days, and after that ca. 85 % of the soil moisture had evaporated. The rewetting procedure on days 128-130 was similar to the first rewetting (20 ml + 20 ml + the rest needed). After that the soil moisture was checked three times during the rest of the experiment and the evaporated water was replenished.

Three weeks after the second rewetting procedure 20 grassweed (*Poa annua*) seeds were strewn on the soil and moistened with 5 ml of deionized water. After the seeds had germinated, 13 seedlings per microcosm were allowed to grow.

Samplings and analyses

The first destructive sampling was done on day 60, just before the pesticide applications. After the first treatment procedure the microcosms were sampled on day 88 and after the second treatment procedure on day 131. After a recovery period the third sampling was done on day 242. A microcosm was cut and the soil was poured into a glass bowl. The soil was gently mixed and subsamples were taken for different animal extractions and analyses: enchytraeid worms (80.0 g f.m.), microarthropods (80.0 g), nematodes (10.0 g), moisture (ca. 10 g), pH (20.0

g), nutrients (20.0 g), pesticides (ca. 20 g) and microbial analyses (rest of the soil).

Enchytraeid worms were extracted in wet funnels for 4 h (O'Connor 1962). The worms were preserved in 70% ethanol and dyed with bengal red for easier counting. A modified wet funnel method was used to extract the nematodes (Sohlenius 1979), after which they were counted and a subsample of 100 specimens was identified. Microarthropods were extracted with a modified high gradient extractor (McFadyen 1961), counted and identified to species level.

On the last sampling date plant aboveground biomass was measured by cutting the grass from just above the soil surface and the dry mass of the plants was determined by drying them at +65°C for three days.

For nutrient analyses a subsample was mixed with 100 ml of 2 mol KCl-solution, then stored at +5°C overnight and filtered through Whatman® glassfibre filters. NO_3-, NH_4- and PO_4- concentrations were analysed according to Finnish standards (SFS 5752, SFS 3032 and SFS 1189, respectively).

Soil respiration was measured twice a week during the experiment starting one week before the first treatment procedure. When plant seedlings had germinated, the measurents were stopped due to CO_2 assimilation of the growing plants. When measuring the respiration a microcosm was closed with a stopper and one ml air sample was taken through the stopper with a syringe. Another sample was taken after 240 minutes. Before the treatment procedure the respiration of 10 randomly selected microcosms was measured, whereafter CO_2-production of all microcosms was measured.

Microbial biomass was estimated by measuring ATP-content of the soil. For soil ATP analysis, 10 g (f.m.) of sieved soil was placed in a 100 ml vial containing 10 ml 20% trichloroacetic acid and 10 ml 8 mM EDTA and shaken vigorously for 30 min (Vanhala and Ahtiainen 1994). Suspended solids were removed by filtration (Schleicher &Schuell 604 paper filter). A subsample (0,5 ml) of this filtrate and 0,5 ml of 0.1M Tris + 2mM EDTA buffer were mixed in Eppendorf tubes. These tubes were kept on ice. For the measurement the sample was further diluted 50 times in the same buffer. The ATP concentration was measured with BioOrbit luminometric assay kit with BioOrbit 1251 luminometer (BioOrbit, Turku, Finland). ATP content was then calculated on the basis of fresh weight of the soil sample.

Alkaline and acid phosphatase activity were measured with the modified soil phosphatase activity method based on the release of phosphate from p-nitrophenyl phosphate (PNP-P) (Tabatai and Bremner 1969). A sample of 2 g fresh, homogenized soil was incubated for two hours with p-nitrophenyl phosphate at pH 7.75 (0.1M Tris + 2mM EDTA buffer) for alkaline phosphatase and at pH 5,0 (0,1M acetic acic + 0,1M sodium acetate buffer) for acid phosphatase activity at room temperature. Concentration of p-nitrophenyl (PNP) was measured by spectrophotometer and phosphatase activity was calculated by PNP concentrations compared to blank samples.

Soil moisture contents during the drying procedures were monitored by weighing the microcosms regularly.

Statistical analyses

Treatment induced effects on animal community structure were analysed with canonical discriminant analysis (CDA). Two-way analysis of variance (2-ANOVA) was applied for comparisons of numbers of different animal species, plant germination and biomass, microbial parameters, pH and nutrient concentrations. One-way ANOVA was used for pairwise comparisons when interaction of the two main factors was found. Data were log transformed if requirements for normality and/or homogeneity of variances were not met. Respiration results were analysed with MANOVA for repeated measurements. SPSS for Windows software package (Release 6) was used for all statistics.

Results

Community level

Both soil moisture and chemical application had clear effects on animal communities in the microcosms. In CDA-analysis the effects of both factors were evident. After the first treatment procedure (day 88) moist and dried dimethoate treatments were separated from the other treatments by the first discriminant function, while soil moisture had no noticeable effects (Fig. 1a). After the second treatment procedure (day 131) four distinct treatment groups formed: the first function separated the dimethoate treated soils from the others, while the second function separated moist and dried soils from each other (Fig. 1b). Propiconazole had no effects on the communities. On the last sampling date (day 242), several months after the treatment procedures, still the same groups could be found, but variation between the replicates had increased which increased overlap between the groups (Fig. 1c).

Soil respiration showed clear responses to the treatments (Table 2. Fig. 2). During the first treatment procedure both chemicals produced a slight inhibiting effect on respiration, while drought decreased respiration rate substantially. In the dried microcosms respiration rate decreased more rapidly than in the moist ones. After rewetting respiration increased in the dried microcosms over the moist ones. During the second treatment procedure both chemicals, especially dimethoate, decreased respiration. The dimethoate effect was stronger in the moist soils. Soil moisture seemed to have no effect, but when time was taken into account, an interaction between soil moisture and time was clear. The dried and then rewetted microcosms initially respired more than the moist ones, but then respiration of the rewetted microcosms decreased again during the second drying procedure. During the last measurement period dimethoate still decreased respiration rate, while propiconazole had no effect. Rewetting again increased the respiration rate in the dried soils, but respiration soon decreased to the same level as in the moist soils. Altogether, dimethoate seemed to supress soil respiration somewhat more than propiconazole.

Analyses did not reveal any large effects of either soil moisture or pesticide application on microbial parameters. Soil ATP content, that indicates microbial biomass, varied between 0.1 and 0.2 nmol/g (d.m.) soil during the experiment (Table 3) without any treatment effects (Tables 4 and 5). Acid and basic phosphatase entzyme activities tended to increase during the experiment (Table 3). After the second treatment (day 131) acid phosphatase enzyme activity was higher in the dimethoate treated moist soil than in the moist control soil (1-ANOVA, $p < 0.05$), but the reverse was true with basic phosphatase enzyme activity (1-ANOVA, $p < 0.05$). In the dried soils there were no differences between the dimethoate treated soils and the control soils. On the last sampling date (242) acid phosphatase activity was lower in the propiconazole treated soils than in the control soils (Table 5).

Species level

Microarthropods

Only dimethoate had clear effects on microarthropod populations in the microcosms. *Folsomia fimetaria* was the most abundant collembolan species and the numbers increased until day 131 in all but dimethoate treated soils (Fig. 3). In the dimethoate treated microcosms their numbers were significantly lower than in the controls after both treatment procedures (Table 4), but by the last sampling date their numbers increased to the same level as in the controls. Numbers of *T-ullbergia macrochaeta* increased towards the end of the experiment (Fig. 3). Dimethoate, and drought to some extent, decreased their numbers (Tables 4 and 5). On the last sampling date there were more *T. macrochaeta* in the dimethoate treated microcosms that in the others (Fig. 3), but no statistical differences were found between the treatments due to large variations between the replicates.

Isotoma anglicana did not tolerate dimethoate and no specimens were found in the dimethoate treated soils except in one sample on the last sampling date (Fig. 3) while in the propiconazole treated and control soils they were regularly found. However, due to extremely low numbers of the species, it was not possible to detect statistical differences between the treatments. *Hypogastrura assimilis* could not maintain its populations in the microcosms; only a few individuals were found on the first two sampling dates with no differences between the treatments.

Numbers of the predatory mite *Hypoaspis aculeifer* increased steadily during the experiment (Fig. 3). The first treatment procedure (day 88) did not clearly affect their numbers, but after the second treatment procedure (day 131) dimethoate decreased their numbers (Table 4). By the last sampling date their numbers recovered and no clear treatment induced differences were found, although there tended to be fewer mites in the dimethoate treated soil than in the control (Table 4).

The single species tests revealed that *I. anglicana* was the most susceptible species to dimethoate (Table 6). Also the other collembolan species were affected at the concentration level used in the microcosm experiment. *H. aculeifer* appeared to be the most tolerant arthropod species to dimethoate (Table 6). No effects of propiconazole on the arthropods were found when the highest concentration tested was 30 mg/kg.

Enchytraeids

Numbers of *Enchytraeus* sp. increased substantially during the early weeks of the experiment reaching the highest numbers by the first sampling date (day 60) before the treatments (Fig. 4). The first treatment procedure (day 88) did not affect the enchytraeid numbers (Tables 4 and 5), which was the main reason for repeating the chemical application and subsequent drying procedure. After the second treatment procedure (day 131) there were fewer enchytraeids in the dried microcosms than in the moist ones (Tables 5 and 6). Dimethoate treatment increased their numbers compared to the control (Table 4). At the end of the experiment there tended to be more enchytraeids in the dimethoate treated soil than in the control (Table 5) and their numbers were higher in the dried soils than in the moist soils (Tables 5 and 6).

In the single species test no effects of propiconazole on *Enchytraeus* sp. survival or juvenile numbers were found at the highest concentration tested, 32 mg/kg.

Nematodes

Acrobeloides tricornus was the most numerous nematode species consisting ca. 90% of the total numbers of nematodes. Its numbers peaked on the second sampling date (day 88) when there were less *A. tricornus* in the moist soils than in the dried ones (Fig. 5, Tables 4 and 5). The interaction between the treatments on day 88 was due to differences in the dried soils: the numbers were higher in the control than in the dimethoate treated soil (1-ANOVA $p < 0.05$). In the moist soils there were no differences. On days 131 and 242 no effects were found.

Also *Aphelenchoides saprophilus* was relatively abundant. On day 88 there were somewhat more *A. saprophilus* in dried soils than in the moist ones (Fig. 5, Table 5). On days 131 and 242 their numbers were increased in the dimethoate treated soils compared to the control soils (Fig. 5, Table 4). On the last sampling date (day 242) the effect was different at different moistures (2-ANOVA, interaction $p < 0.01$), which was due to higher numbers in the dimethoate treated dried soils than in the dried control soils ($p < 0.001$). In the moist soils there were no statistical differences. In the propiconazole treated soils *A. saprophilus* numbers were lower than in the control soils on day 88 (Fig. 5, Table 5). On the last sampling date (day 242) both propiconazole and drought decreased their numbers (Fig. 5, Table 5). The propiconazole effect was due to lower *A. saprophilus* numbers in the moist propiconazole treated soils than in the moist control ($p < 0.05$) while in the dried soils no differences were detected.

Only low numbers of both *Aphelenchus avenae* and *Caenorhabditis elegans* were found in the samples (Fig. 5) and no treatment effects on *A. avenae* were found (Tables 4 and 5). Dimethoate application seemed to decrease *C. elegans* after the first treatment procedure (Fig. 2, Table 4), but due to low numbers statistical differences found using two way ANOVA are not reliable. There were several replicates without the species, which violates the assumption that data are normally distributed. After the second treatment procedure no *C. elegans* were found in the dried soils (Fig. 5).

Plant germination and biomass

Out of 20 *Poa annua* grassweed seeds 13-20 seedlings emerged. Pesticide application did not affect grassweed germination, but somewhat higher number of seedlings tended to emerge in the dried soils (2-ANOVA drought, $p = 0.067$). Grassweed biomass was not significantly affected by soil moisture, but dimethoate application tended to increase biomass compared to the control (2-ANOVA dimethoate, $p = 0.071$), while propiconazole had no effect (Tables 4 and 5, Fig. 6).

Nutrients

Soil PO_4-P was below the detection limit on days 60 and 88, increased somewhat on day 131, and decreased again by the last sampling date (Table 7), with no treatment effects (Tables 5 and 6). NH_4-N and NO_3-N concentrations in the soils decreased during the experiment (Table 7), also with no clear treatment effects (Tables 4 and 5).

Fate of the chemicals

Behaviour of the two chemicals in the soil differed from each other (Fig. 7). Dimethoate concentration was ca. one third of the initial nominal concentration after the first treatment prodecure (day 88), remained at the same level after the second treatment procedure (day 131), and disappeared completely by the last sampling date. Degradation of dimethoate was strongly dependent on soil moisture. Dimethoate concentration was clearly higher in the dried soil than in the moist soil on day 131 (1-ANOVA, $p < 0.001$), and on day 88 the tendency was similar ($p = 0.078$). Propiconazole concentrations increased after both treatment procedures and ca. half of the nominal initial concentration was present even at the end of the experiment. Also propiconazole concentrations were higher in the dried soil than in the moist soil on the last sampling date (1-ANOVA, $p < 0.01$).

Discussion

Effects of dimethoate

Dimethoate as an insecticide clearly affected the soil animal community structure. Dimethoate was toxic especially to arthropods, which has earlier been noted in several studies (Krogh 1996, Martikainen 1996, Martikainen et al. 1998). The toxic effect was highest immediately after the applications. Because of the rapid degradation of dimethoate faunal populations recovered by the end of the experiment.

Response of soil arthropods to dimethoate in the microcosms was similar to the responses in the single species tests. *I. anglicana*, the most sensitive species in the single species tests, suffered most from the dimethoate application. The initial

nominal concentration of 3.7 mg/kg was well over the nominal LC_{50}-value of 1.6 mg/kg found in the single species test. In addition to high sensitivity to dimethoate, as an epiedaphic species it was apparently readily exposed to sprayed dimethoate and subsequent high concentrations in the upper soil layer. Numbers of the other arthropod species having higher LC_{50}-values did not decrease substantially until after the second treatment procedure.

On the other hand, the enchytraeid worm *Enchytraeus* sp. and the nematode *A. saprophilus* clear benefitted from the dimethoate application. This can be explained either by reduced predation pressure by the predatory mite or by reduced competition for food by the collembolans, or both. The predation hypothesis is supported by the fact that the numbers of predatory mites, that are known to prey upon enchytraeids (Walter et al. 1988) were lower in the dimethoate treated microcosms and hence their predation pressure on the prey was reduced. Competition may also explain the increase in *Enchytraeus* sp. and *A. saprophilus* numbers since soil respiration (=microbial activity) decreased to a very low level within some weeks from the start of the experiment. Therefore food resources for the microbial feeders may have been low in the microcosms. Because of the decreased numbers of fungal grazing collembolans there was more food available for *Enchytraeus* sp. and the fungal feeding nematode *A. saprophilus* in the dimethoate treated soils. The present experiment also confirmed the finding of Puurtinen & Martikainen (1997) that dimethoate is not particularly toxic to this enchytraeid species.

Suppression of respiration by dimethoate was probably indirect. Stimulation of microbial activity by grazing (see Hanlon & Anderson 1979) may decrease due to a decrease in collembolan numbers, thus resulting in lowered soil respiration in the dimethoate treated soils. In another microcosm experiment Martikainen et al. (1998) found no effects of dimethoate on respiration at concentrations comparable to those in the present experiment. In that experiment, however, plants (barley) were growing from the beginning of the experiment and CO_2-assimilation by them evidently diminished differences between the treatments.

The somewhat better growth of the grass weed in the dimethoate treated soils may be associated either to the better survival of enchytraeids or decreased survival of collembolans. Enchytraeids have been found to increase soil mineral nitrogen concentration (Abrahamsen 1990, Setälä et al. 1991), improve soil structure by their burrowing activity (Didden 1990) and hence improve soil fertility and increase plant growth (Didden 1993). Salminen and Haimi (1996) hypothesized that reduction in the numbers of enchytraeids in high pentachlorophenol concentration could partly explain, through reduced nutrient mineralisation, the lower plant biomass. In addition, high numbers of collembolans may damage plant roots or overgraze the root associated mycorhizal fungi, and hence reduce plant growth (Getzin 1985, Harris & Boerner 1990). Food shortage for collembolans in the control soils may have forced them to also graze roots, and in the dimethoate treated microcosms this grazing was evidently reduced.

Effects of propiconazole

Only some weak effects of fungicide propiconazole on nematodes was found. There is little information about propiconazole toxicity to soil animals. Normal application doses have been found to be either possibly harmful (Edwards & Bolhen 1992) or harmless (Reicher et al. 1997) to earthworms. Martikainen (unpubl.) tested effects of propiconazole on some soil animal species in artificial soil (OECD 1984). For the earthworm *Eisenia andrei* he found an LC_{50}-value of 472 mg/kg for survival and an EC_{50}-value of 384 mg/kg for biomass change. For the enchytraeid worm *Enchytraeus* sp. the LC_{50}-value for adult survival was >320 mg/kg and the EC_{50}-value for juvenile reproduction was 140 mg/kg. For the collembolan *Folsomia candida* the corresponding LC_{50}- and EC_{50}-values were >320 mg/kg and 220 mg/kg, respectively. Thus, propiconazole is toxic to soil animals in very high concentrations only.

As pointed out by Martikainen et al. (1998), indirect effects on the soil faunal community through changes in the microbial community are possible, although the pesticide is not directly toxic to soil animals. Soil respiration was somewhat suppressed in the propiconazole soils compared to the control soils, which indicates direct inhibitive effects on soil microbes. Also acid phosphatase enzyme activity was lower on the last sampling date (day 242) in the propiconazole treated soils than in the control soils. Elmholt (1991) found that propiconazole, applied at both normal and 10 x normal doses, decreased length of fungal hyphae in a field test. Her study also revealed that the effect was delayedonly appearing one month after the applications. However, agricultural soils usually have bacterial based energy channels while fungal based channels are typical for forest soils (Moore & Hunt 1988). Therefore possible effects on fungi had no extensive effects on the decomposer communities in these bacterial dominated systems.

Effects of drought

Effects of drought were less severe than expected. Only microbial activity decreased substantially during both the drying periods. This also led to a reduced pesticide degradation rate in the dried microcosms. In general, adsorption of pesticides onto soil particles is stronger and volatilisation as well as degradation are slower in dry soils that in moist soils (Edwards 1972).

For the soil animals the effects were only observed after the second, more severe drying when the soil moisture reached less than 10 % of WHC at the end of the drying procedure. Four species, *T. macrochaeta*, *H. aculeifer*, *C. elegans* and *Enchytraeus* sp. decreased due to drought.

Enchytraeids are relatively sensitive to low soil moisture content because of their soft body and permeable cuticle (Didden 1991). Lagerlöf et al. (1989) revealed that in an arable field enchytraeids decreased drastically during summer droughts, but due to high number of drought resistant cocoons in the soil, the populations increased rapidly when the conditions became favourable. Also in the present experiment *Enchytraeus* sp. recovered in the dried soils after rewetting, evidently due to drought resistant cocoons in the soil, and its numbers even exceeded those in the moist soils by the last sampling date.

One collembolan species (*T. macrochaeta*) appeared to suffer from drought to some extent. Collembolans have relatively low resistance to desiccation due to their soft bodies and high rates of cuticular water loss (Holmstrup 1997). Many species have, however, different mechanisms to avoid desiccation during dry periods (Hopkin 1997). It is possible that in spite of extreme water loss during the drying period, the effects were not fatal due to some moist patches in the soil, thus providing refugia to soil fauna during the drought.

Nematodes are capable of tolerating drought due to their ability to become inactive and form resting stages. Therefore the effects of drought on nematodes were not substantial.

Combined effects of dimethoate and drought

In general, few additional effects of drought on pesticide toxicity were noticed at species level. However, numbers of *T. macrochaeta* were clearly lower in the dried dimethoate treated than in the moist dimethoate treated soil after the second treatment procedure. This indicates reduced tolerance of *T. macrochaeta* to drought under the dimethoate stress. Holmstrup (1997) has found that drought tolerance of a collembolan *Folsomia candida* was lower in animals exposed to sublethal chemical stress. Also Everts et al. (1991b) have shown that sensitivity of an ergonid spider *Oedothorax apicatus* to drought was increased by a pyrethroid insecticide deltamethrin. *T. macrochaeta* is small, slender bodied and unpigmented species adapted to live in deeper soil layers, where humidity is higher than on soil surface, and therefore it is sensitivie to drought.

For the other arthropods drought did not cause stress in addition to dimethoate stress. It is possible that dimethoate application and drought counteracted the adverse effects of each other. Because drying began at the soil surface on which the pesticides were sprayed, arthropods may have reduced their exposure to the pesticide by moving downwards in the soil column. Therefore they became exposed only to low dimethoate concentrations in the lower part of the soil column. In the moist soil arthropods were exposed to high dimethoate concentration when they were active near the soil surface. Krogh (1995) has found that the collembolan *Folsomia candida* cannot detect dimethoate in the soil and thus cannot avoid contact with it. The net effect of higher exposure in the moist soil may therefore equal the lower exposure but increased drought stress in the dried soil. This implies that in the field combined stresses may actually not cause increased but decreased stress.

At the community level, dimethoate application together with drought changed the soil animal community structure which was due to the effects of these factors to different target species. As dimethoate decreased mainly arthropods and drought decreased enchytraeids, decrease in total numbers of animals was somewhat higher than the decrease caused by either of these factors alone. Because soil invertebrate species and populations have different life history characteristics (e.g. generation times and seasonality of reproduction [Luxton 1981]), it may not be possible to predict the effects of more than one stress factor simultaneously affecting the decomposer community. The biological properties will evidently lead to differences between species in their responses to stress.

Although drought suppressed respiration, it did not mask the chemical effect completely. While the differences in the respiration rate between the treated soils and the control soils disappeared soon after the second treatment period in the moist soils, they were detected longer in the dried soils (Fig 2). This can be explained by the higher dimethoate concentration in the dried soil. It seems, therefore, that drought suppressed dimethoate degradation and hence the negative effect on microbes was longer in the dried soil than in the moist one.

Microbial parameters

It was somewhat unexpected that no substantial effects were detected in the microbial parameters like ATP-content or acid and basic phosphatase activity, while clear changes in the soil respiration were observed. It is evident that the microbes reacted to chemicals and/or drying immediately. Because the microcosms were sampled some weeks after the pesticide applications and some days after rewetting, microbial populations may have recovered from disturbances by the sampling dates. Sum parameters, like soil ATP content and phosphatase activities, can indicate severe acute effects on total microbial biomass and activity but fail to indicate possible harmful changes in microbial community structure.

Conclusions

1. Soil animal communities were altered by dimethoate and drought together, resulting in somewhat lower total numbers of animals than either of these factors alone. Thus, the combined stress of factors with different modes of action (dimethoate and drought in this case) may cause at least short term changes in soil animal communities, and those changes cannot be predicted if only one of these factors is studied at a time. We also found some indications of delayed pesticide degradation due to the severe drought, which caused prolonged pesticide effects.

2. Dimethoate alone altered the community structure by decreasing arthropod numbers, but increasing some other groups, such as enchytraeids and one nematode species. Again, this is an effect that cannot be detected in single species experiments. The arthropods recovered within a few months after the chemical stress had finished.

3. Severe temporary drought can decrease numbers of some species, and it may even exacerbate decreases (*T. macrochaeta*) under pesticide stress. On the other hand, drought stress seemed to counterbalance the effects of the chemical by forcing animals to move downwards to moister soil and thus avoid the chemical applied on the soil surface.

4. Functional changes were relatively weak, but differences in soil respiration and to some extent in plant growth revealed that soil community structure and its fuctioning are interlinked.

5. Propiconazole had only minor effects on the soil decomposer community, which reveals that the bacterial based channel is more important than the fungal based one in agricultural soil, and therefore not seriously affected by fungicides.

6. Most introduced species thrived in the microcosms, but animal communities changed throughout the experiment. As a whole this kind of microcosm can support an adequate soil animal community for several months. If the species used in the experiment have relative short generation times, it is possible to study population and community level effects of chemicals in this kind of microcosm experiment.

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Table 1. Time of introductions and numbers of animals introduced into each microcosm. Numbers in parentheses indicate the lowest and highest numbers in the three samples checked.

Species	Days from the start	inds. per microcosm
Microbial inoculate	5	
Nematodes		
Aphelenchus avenae	6	2000 (1838-2087)
Caenorhabditis elegans	6	400 (375-430)
Aphelenchoides saprophilus	6	1600 (1596-1632)
Acrobeloides tricornus	7	800 (745-867)
Prionchulus punctatus	29	21 (21-22)
Collembolans		
Folsomia fimetaria	11	30
Isotoma anglicana	11	20
Tullbergia macrochaeta	12-13	15
Hypogastrura assimilis	12-13	20
Predatory mite		
Hypoaspis aculeifer	29	7m/8f *
Enchytraeid worm		
Enchytraeus sp.	12-13	30

* m=males, f=females

Table 2. Repeated MANOVA of the effects of chemical treatment, moisture and time on soil respiration in the microcosms. Statistical significances: (–), (+), (*) = p<0.10; – , +, * = p<0.05; – – , ++, ** = p<0.01; – – – , +++, *** = p<0.001. + = positive effect, – = negative effect, * = interaction effect.

	Source of variation	Dim	Prop
Soil respiration between days 63 and 86	Between subjects effects:		
	chemical	(–)	–
	drought	– – –	– – –
	chemical by drought	n.s.	n.s.
	Within subject effects:		
	time	– – –	– – –
	chemical by time	n.s.	n.s.
	drought by time	***	***
	chemical by drought by time	(*)	(*)
Soil respiration between days 90 and 130	Between subjects effects:		
	chemical	– – –	–
	drought	n.s.	n.s.
	chemical by drought	*	n.s.
	Within subject effects:		
	time	– – –	– – –
	chemical by time	*	*
	drought by time	***	***
	chemical by drought by time	n.s.	*
Soil respiration between days 134 and 167	Between subjects effects:		
	chemical	– –	n.s.
	drought	+++	+++
	chemical by drought	n.s.	n.s.
	Within subject effects:		
	time	– – –	– – –
	chemical by time	n.s.	n.s.
	drought by time	***	***
	chemical by drought by time	n.s.	n.s.

Table 3. ATP-content (nmol/g d.m.) and acid and basic phosphatase activity (μmol PNP/g(d.m.)/h) of the soils in the microsoms. Statistical differences between the treatments, see Tables 4 and 5.

		day 60	day 88	day 131	day 242
ATP	Moist	0.137 (0.012)	0.204 (0.013)	0.121 (0.010)	0.169 (0.024)
	Dim.		0.203 (0.012)	0.128 (0.007)	0.164 (0.017)
	Prop.		0.192 (0.015)	0.117 (0.006)	0.192 (0.015)
	Dry		0.192 (0.009)	0.117 (0.004)	0.164 (0.021)
	Drydim.		0.226 (0.015)	0.136 (0.011)	0.204 (0.016)
	Dryprop.		0.205 (0.017)	0.102 (0.005)	0.185 (0.008)
Acid phosphatase activity	Moist	0.124 (0.035)	0.216 (0.034)	0.116 (0.011)	0.626 (0.132)
	Dim.		0.211 (0.075)	0.261 (0.034)	0.596 (0.099)
	Prop.		0.190 (0.045)	0.216 (0.034)	0.481 (0.060)
	Dry		0.306 (0.127)	0.210 (0.070)	0.781 (0.091)
	Drydim.		0.159 (0.018)	0.144 (0.028)	0.594 (0.096)
	Dryprop.		0.170 (0.069)	0.309 (0.126)	0.295 (0.078)
Basic phosphatase activity	Moist	0.264 (0.040)	0.182 (0.059)	0.282 (0.026)	0.272 (0.121)
	Dim.		0.119 (0.043)	0.077 (0.051)	0.184 (0.219)
	Prop.		0.231 (0.033)	0.182 (0.034)	0.320 (0.119)
	Dry		0.139 (0.055)	0.222 (0.031)	0.418 (0.126)
	Drydim.		0.254 (0.002)	0.193 (0.031)	0.294 (0.166)
	Dryprop.		0.137 (0.063)	0.155 (0.061)	0.398 (0.243)

Table 4. Effects of dimethoate and drought on measured parameters (numbers of animals, soil ATP and nutrient concentrations, soil enzyme activities). Two-way ANOVA was applied for testing the differences. Statistical significances: (–), (+), (*) = p<0.10; – , +, * = p<0.05; – – , ++, ** = p<0.01; – – – , +++, *** = p<0.001. + = positive effect, – = negative effect, * = interaction effect.

	day 88			day 131			day 242		
parameter measured	dim	drought	dim x drought	dim	drought	dim x drought	dim	drought	dim x drought
Fol fim	–	n.s.	n.s.	– – –	n.s.	n.s.	n.s.	n.s.	n.s.
Tul mac	–	n.s.	n.s.	– –	– –	n.s.	n.s.	n.s.	n.s.
Iso ang	– 1	n.s.	n.s.	– – 1	n.s.	n.s.	n.s	n.s.	n.s.
Hyp acu	(–)	n.s.	(*)	– –	n.s.	n.s.	(–)	n.s.	n.s.
Enc sp.	n.s.	n.s.	n.s.	++	– – –	*	(+)	(+)	n.s.
Acr tri	n.s.	++	**	(–)	n.s	n.s.	n.s.	n.s.	n.s.
Cha ele	– 1	n.s.	n.s.	n.s.	– – 1	n.s.	n.s.	n.s.	n.s.
Aph ave	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Aph sap	n.s.	n.s.	(*)	+	n.s.	n.s	+++	–	**
ATP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Acid phos.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
Basic phos.	n.s.	n.s.	(*)	–	n.s.	*	n.s.	n.s.	n.s.
NH4	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NO3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
PO4				n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Plant bm							(+)	n.s.	n.s.

1 = Not statistically reliable due to not normally distributed variables

Table 5. Effects of propiconazole and drought on measured parameters (numbers of animals, soil ATP and nutrient concentrations, soil enzyme activities). Two-way ANOVA was applied for testing the differences. Statistical significances: (–), (+), (*) = p<0.10; – , +, * = p<0.05; – – , ++, ** = p<0.01; – – – , +++, *** = p<0.001. + = positive effect, – = negative effect, * = interaction effect.

	Day 88			Day 131			Day 242		
Parameter measured	Prop	Drought	Prop x Drought	Prop	Drought	Prop x Drought	Prop	Drought	Prop x Drought
Fol fim	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Tul mac	n.s.	–	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
Iso ang	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hyp acu	n.s.	n.s.	n.s.	n.s.	– –	(*)	n.s.	n.s.	n.s.
Enc sp.	n.s.	n.s.	n.s.	n.s.	– – –	n.s.	n.s.	++	n.s.
Acr tri	–	+	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cha ele	(+) 1	n.s.	n.s.	n.s.	– 1	n.s.	n.s.	n.s.	n.s.
Aph ave	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Aph sap	n.s.	+	n.s.	n.s.	n.s.	n.s.	– –	– –	*
ATP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Acid phos.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–	n.s.	n.s.
Basic phos.	n.s.	n.s.	n.s.	(–)	n.s.	n.s.	n.s.	n.s.	n.s.
NH4	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NO3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	(+)	n.s.	n.s.
PO4				n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Plant bm							n.s.	n.s.	n.s.

1 = Not statistically reliable due to not normally distributed variables

Table 6. Acute toxicity (survival) of dimethoate in single species tests to the arthropod species used in the experiment. Models used in the calculations and 95% confidence intervals in parentheses.

	NOEC	LOEC	LC_{10}	LC_{50}
I. anglicana (Exp. decay)	2.0	4.0	0.25 (0.15-0.36)	1.6 (0.96-2.27)
H. assimilis (Sigmoid)	6.0	8.0	5.2 (2.9-7.5)	9.8 (8.5-11.2)
F. fimetaria (ICp)	2.0	4.0	2.6 (2.0-3.3)	3.9 (2.9-5.9)
T. macrochaeta (ICp)	2.0	4.0	2.7 (2.3-3.5)	>10
H. aculeifer (ICp)	>20	>20	7.1 (-2.7-20.5)	>20

Table 7. Nutrient contents (µg/kg d.m.) of the soils in the microcosms. Means with S.E. (in parentheses). Statistical differences between the treatments, see Tables 4 and 5.

		day 60	day 88	day 131	day 242
	Moist	*	*	0.033 (0.002)	0.015 (0.005)
	Dim.		*	0.032 (0.005)	0.017 (0.003)
PO4-P	Prop.		*	0.040 (0.013)	0.010 (0.003)
	Dry		*	0.039 (0.016)	0.012 (0.002)
	Drydim.		*	0.028 (0.003)	0.016 (0.005)
	Dryprop.		*	0.033 (0.006)	0.012 (0.001)
	Moist	7.623 (1.416)	1.229 (0.363)	1.019 (0.186)	0.200 (0.017)
	Dim.		2.507 (0.494)	0.774 (0.219)	0.186 (0.013)
NH4-N	Prop.		2.644 (0.915)	0.761 (0.137)	0.201 (0.022)
	Dry		1.875 (0.701)	0.969 (0.110)	0.185 (0.028)
	Drydim.		2.412 (0.827)	0.843 (0.132)	0.221 (0.018)
	Dryprop.		2.014 (0.458)	0.953 (0.074)	0.205 (0.231)
	Moist	0.717 (0.195)	0.266 (0.010)	0.230 (0.037)	0.192 (0.015)
	Dim.		0.298 (0.012)	0.223 (0.027)	0.169 (0.007)
NO3-N	Prop.		0.285 (0.019)	0.274 (0.031)	0.253 (0.025)
	Dry		0.260 (0.012)	0.270 (0.033)	0.177 (0.007)
	Drydim.		0.259 (0.022)	0.177 (0.015)	0.219 (0.023)
	Dryprop.		0.296 (0.024)	0.296 (0.034)	0.199 (0.032)

* under detection limit

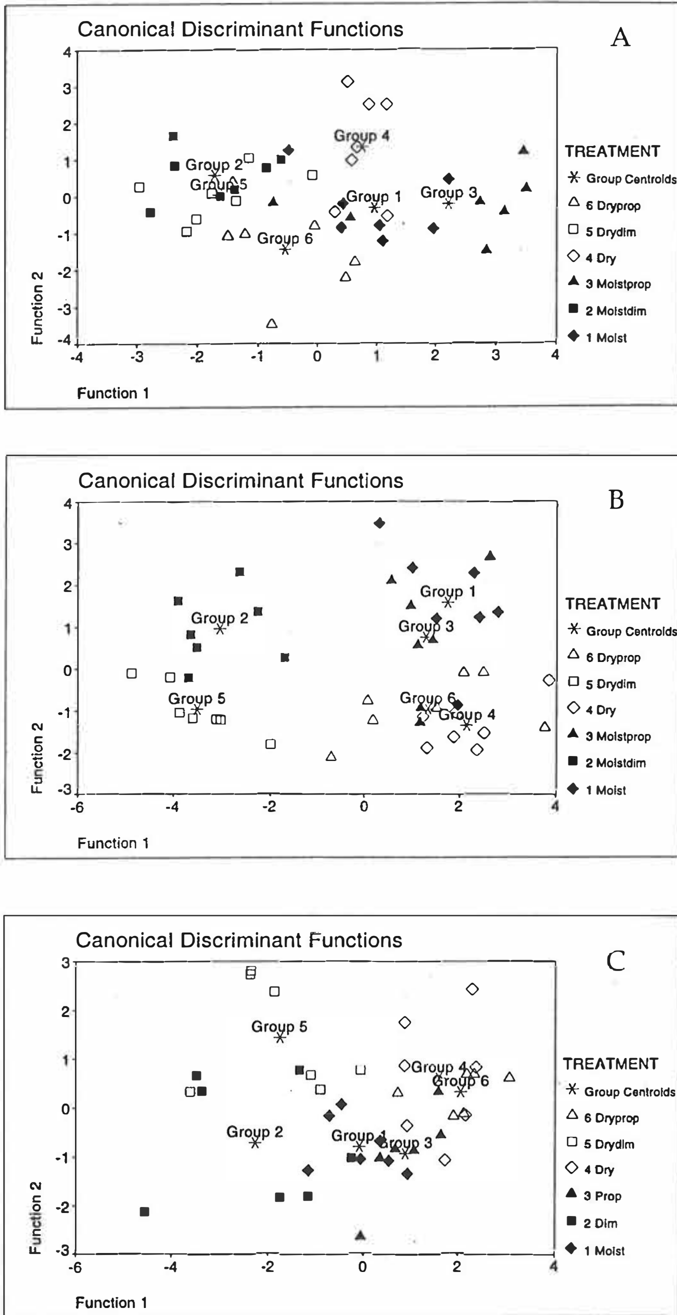

FIGURE 1 CDA-figures of soil animal communities after the first (A) and the second (B) treatment procedure, days 88 and 131, respectively, and after the recovery period (C), day 242.

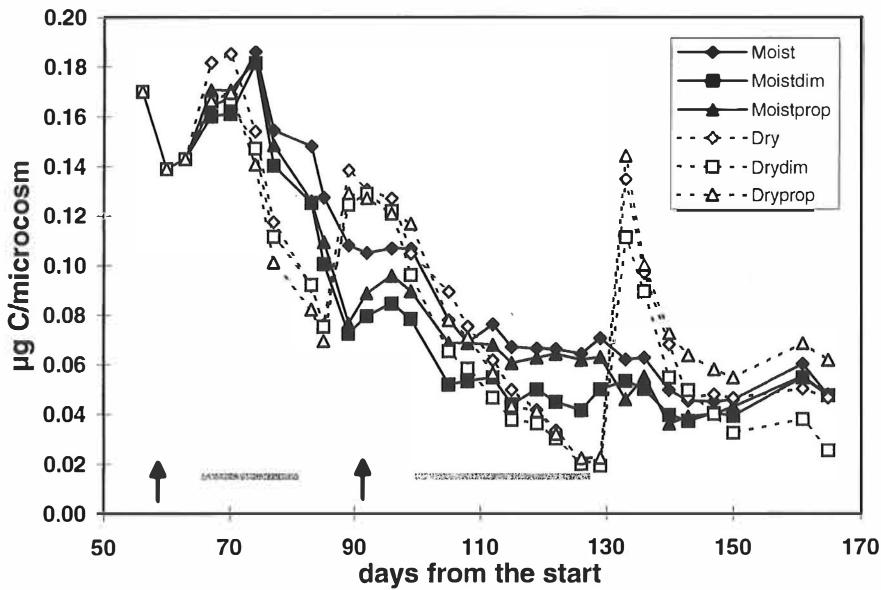

FIGURE 2 Soil respiration in the microcosms during the experiment. Statistical differences, see Table 2. Arrows indicate pesticide applications and horizontal lines indicate drying periods.

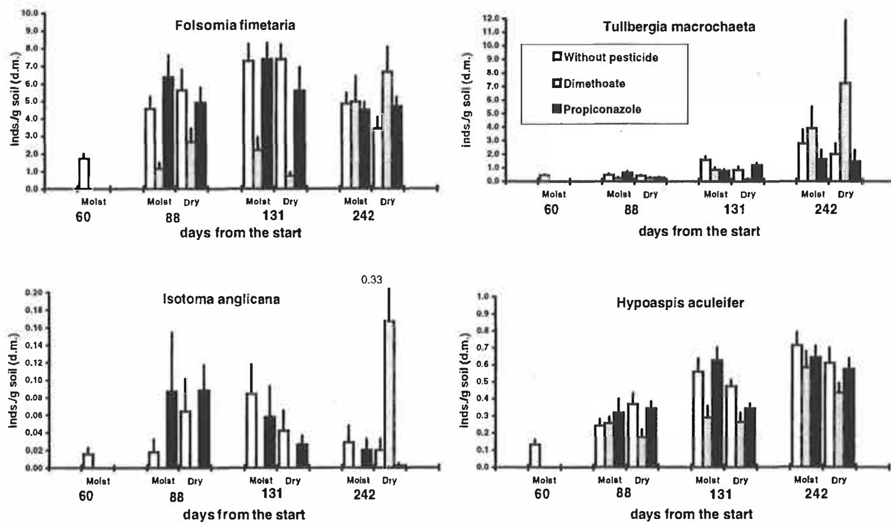

FIGURE 3 Numbers of three collembolans and one predatory mite in the microcosms during the experiment. Means with S.E., statistical differences between the treatments, see Tables 4 and 5.

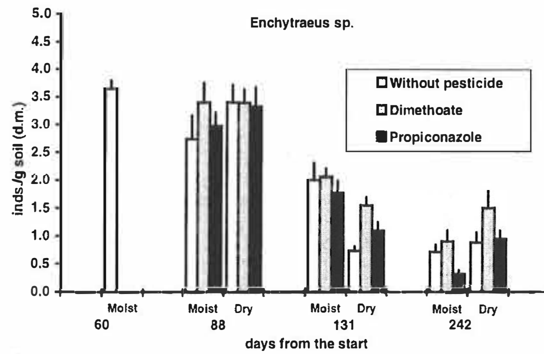

FIGURE 4 Numbers of an enchytraeid worm *Enchytraeus* sp. in the microcosms during the experiment. Means with S.E. Statistical differences between the treatments, see Tables 4 and 5.

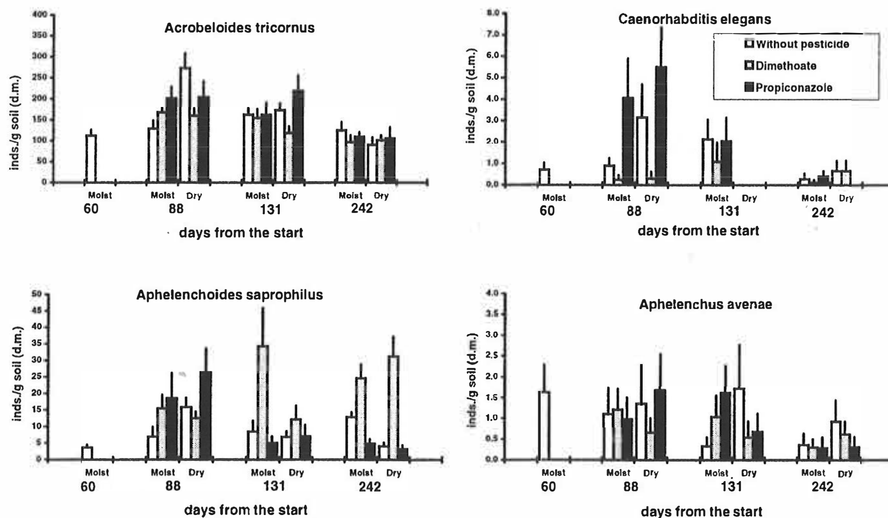

FIGURE 5 Numbers of a bacterial (above) and fungal (below) feeding nematodes in the microcosms during the experiment. Means with S.E., statistical differences between the treatments, see Tables 4 and 5.

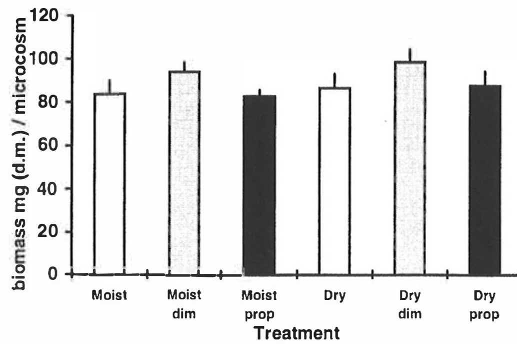

FIGURE 6 Grass weed *Poa annua* biomass in the microcosms at the end of the experiment. Means with S.E., statistical differences between the treatments, see Tables 4 and 5.

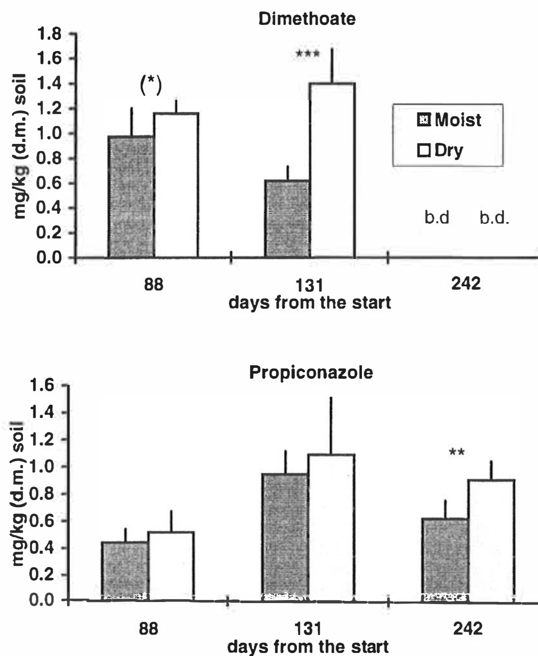

FIGURE 7 Dimethoate and propiconazole concentrations in the microcosms during the experiment. Means with S.E., statistical differences between the treatments: (*) = $p < 0.10$, ** = $p < 0.01$, *** = $p < 0.001$, b.d. = below detection limit.