Assessing the risks of pesticides to soil communities using terrestrial model ecosystems

Björn Scholz-Starke

Aachen 2013

Assessing the risks of pesticides to soil communities using terrestrial model ecosystems

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der RWTH Aachen University zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigte Dissertation

vorgelegt von

Diplom-Biologe

Björn Scholz-Starke

aus *Krefeld*

Berichter:

Universitätsprofessor Dr. Andreas Schäffer

apl. Professor Dr. Hans-Toni Ratte

Tag der mündlichen Prüfung: 16.07.2013

‘***Almost nothing important that ever happens to you happens because you engineer it***’

David Foster Wallace, Infinite Jest

Cover by Emma Grolms

Contents

I

Scope, aim and purpose of TME studies

Higher-tier multi-species test systems at a semi-field level, often called mesocosms, have been used in aquatic ecotoxicology for a long time. They were accepted as realistically depicting effects on communities and being suited to demonstrate the recovery of populations after the treatment within the study period. Recently, the concept was criticized for the lack of criteria for the acceptability of effects, theirs unexplored and uncertain detection limits, especially if multivariate techniques were applied (VAN DEN BRINK 2006). In the same period of time, a TME approach was applied to transmit the advantages of multi-species testing to the terrestrial environment (KNACKER *et al.* 2004). The Ecological Risk Assessment (ERA) obviously lacks methods to estimate the risk at higher tier levels for the soil compartment as well as an appropriate number of standardised single-species tests. The gap between laboratory and field tests could be probably bridged by semi-field tests like TME, because they contain a complex community of soil organisms, still interacting like under natural conditions. TME (often named mesocosms, microcosms, etc.) have been used to follow the fate and behaviour of xenobiotics for more than three decades. They have been used to answer ecological questions like inter-specific interactions, isolation effects or trophic relationships. They should replace cost intensive field studies and help to predict transport and degradation of mainly pesticides for different soil properties. Other approaches wanted to test the effects of chemicals on soil animals, namely collembolans, oribatid mites, earthworms as well as on different functional parameters, mainly mediated by the microbial communities of soils. MORGAN & KNACKER (1994) gave a classification of TME maintained under laboratory conditions. In the last few years, research was focused more on community-based approaches to assess the recovery potential of populations interacting with each other. Most of the approaches have been set up very artificially of sieved soil, while the animals have been introduced by hand and the systems have been tried to maintain under laboratory conditions (e.g. KRIEG *et al.* 2007).

We aimed to supplement recent approaches of soil ecotoxicological tests and designed a system consisting of soil taken from undisturbed grassland that is cored with minimum disturbance of the soil structure. Back to the year 2004, the development of a semi-field approach for the assessment of risks posed to soil organism communities by the use of pesticides was start-

ed at the RWTH Aachen University in a joint project with Bayer CropScience. For this purpose, Terrestrial Model Ecosystems have been designed and foreseen to address special concerns arising from the soil environmental risk assessment of plant protection products. The studies presented here were for the greater part funded by a series of subsequent projects and enabled the author to join the results to a coherent PhD-thesis that addresses most of the questions that arose during numerous discussions between the partners and in the scientific community. It follows from the mainly regulatory scope that the outcome of the studies should be well documented and presented to a broader public. For this reason, the approach was presented to the regulatory bodies during informal meetings (Registration authority of the Netherlands, CTB, Wageningen 2006, German authority for the Environmental Risk assessment of pesticides, UBA, Dessau 2010), and as poster and oral presentations at SETAC and other (more soil-ecological oriented) meetings and on workshops (SETAC-PERAS-workshop on the use of semi-field systems, Coimbra 2007). To outline the framework of present TME-studies, the first chapters describe the legal background and the historical use of semi-field methods. The ecology of soil organisms and the relevant features of soil ecosystems are sketched. To lead over to the results section, the conceptual approaches of the subsequent studies are outlined.

I-1 Conceptual approach of TME evaluation

The suitability of an experimental approach that adequately assesses the risk of pesticide use in the field depends on several criteria that have to be considered. The following chapter defines the basic concepts of the development of complex, ecologically intact and representative systems as TME are supposed to be. There are three main routes of critical evaluation. Figure I-1 summarises the conceptual approach of the TME studies and the systematic development of new test systems, namely the suitability of the TME methodology to depict effects of contaminants (i.e. here pesticides) to soil communities, the stability of systems over relatively long periods of tie of up to one year and the representativeness of the TME method regarding the extrapolation in an environmental risk assessment procedure. It was presumed essential in ecotoxicological studies to test on sensitive endpoints. The **effects** of tested stressors should be reliably detected within pre-defined limits. The limits of effect detection are determined by the system inherent variability, the time scale on which the system returns to its state prior

disturbance (resilience) and the resistance towards the factors under investigation (in the field of ecotoxicology: 'sensitivity towards toxicants').

Two semi-field experiments describe the effects of the model compound lindane on soil organism communities in great detail (chapters III and IV). The detection limits from a mere statistical viewpoint on the TME-method are analysed by chapter V-3. Research on the semi-field level is not always anticipated by pre-experiments that are considered essential for proper *planning of experiments*. In this work, the variability of microarthropods has been investigated on the designated coring area on different *scales*, down to TME 'micro-scale' The necessity of such pre-studies is stressed and its great usefulness is demonstrated by chapter V-1. The abundance and occurrence of microarthropods on the meadow of origin have been highlighted mainly under statistical viewpoints, as well as both species composition and species richness of collembolans has been described. The results of an intensive field study led us assume the coring area as suitable to core the TME-soil cores for effect studies. In particular, the predefinition of appropriate *sample sizes* to the actually expected level of variation, as

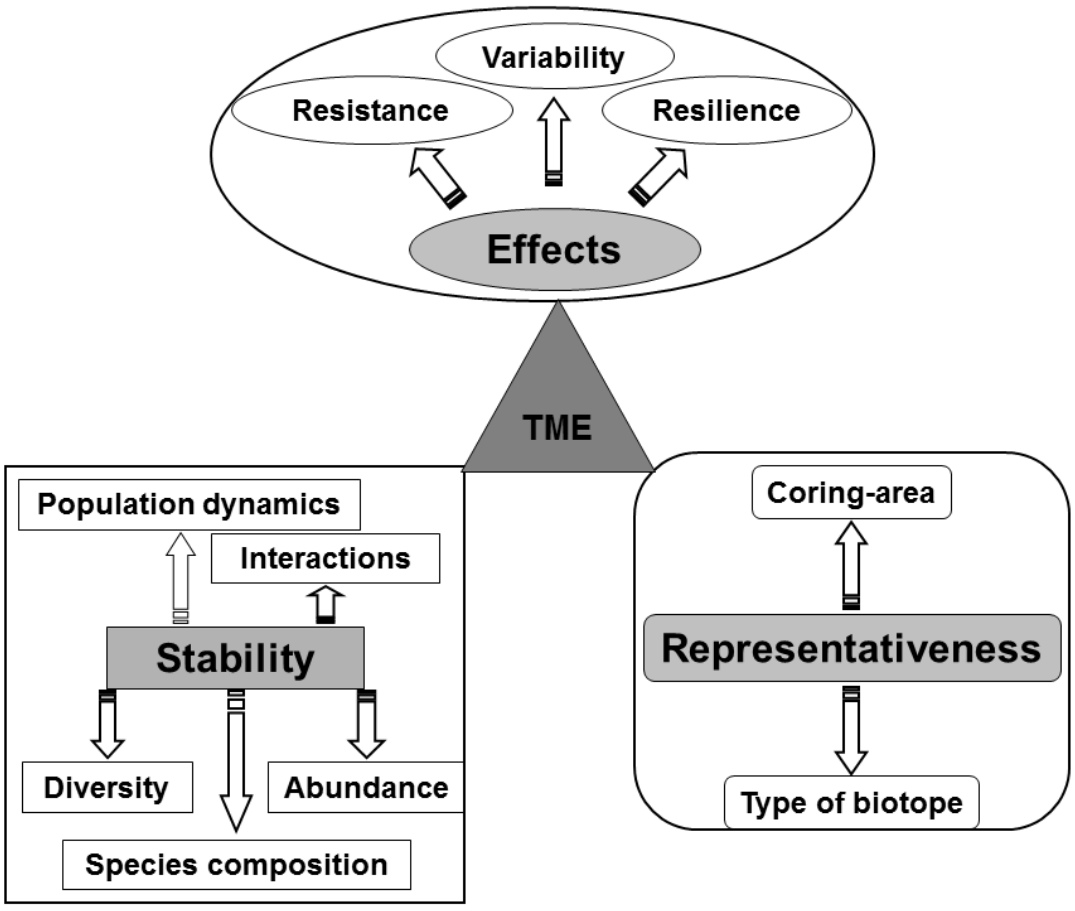


Figure I-1: Representation of the conceptual approach of the thesis at issue. The view is abstracted from the sequence of section in this thesis, but should reflect the general view of the author on the topic and the scientific problems to be solved.

well as to adjust the (sub-) sampling strategy is necessary (chapter V-3).

The **stability** of the test systems had to be assured for a sufficiently long period of time. It should be possible to test for long-term effects of e.g. persistent substances that fulfil the criteria laid down in the Terrestrial Guidance Document (EUROPEAN COMMISSION 2002a). Stable soil ecosystems are generally marked by few dominant and many rare species. If external stressors upset the dominance structure of a given site, it is known to shift towards highly dominating species, as HOPKIN (1997) exposed for the group of collembolans. It could be supposed that sensitive populations disappear for reasons of limitations of food supply or competition. This thesis examines the stability of a TME firstly concerning the communities of collembolans and oribatid mites as representatives of the most abundant groups of arthropods in soil. These groups play key roles in accelerating both degradation, and assimilation processes in soil (see chapter I-4.1 for the biology of soil organisms). Succeeding experiments involved further main groups of soil ecosystems will be included (enchytraeids, nematodes, soil fungi). The stability was tested against the three criteria of abundance, diversity and temporal integrity ('similarity') in chapter V-2.

Furthermore, the systems used should be **representative** at least for the area of soil core origin ('coring site'), which is partly a criterion of stability over time. This question is answered by analysing the community composition at the coring site compared to the TME community composition at a given point or during a certain period in time during a season (chapter V-4). But it is also important to have a system that can be regarded as representative for similar biotopes, to contain a typical number of species that are exemplary for the biotope on their part. The question if TME develop and react similar compared to original grassland communities (in the time-course of an experiment) is not the focus of risk assessors. Often, it is more importantly asked for the maintenance of a diverse and abundant community of whichever soil organisms. However, the degree of realism and the transferability of results trigger the interpretation of the data and the safety factors applied to the same. Anyhow, results have been analysed in the light of ecological caveats of a TME, concerning the influence of *isolation*, *disturbance* and *climatic changes*. In the present case, TME have been transported to an experimental facility under climatic conditions that deviate from the conditions at the coring site. The isolation of populations should not be a limiting factor for the reproduction of most soil taxa (as mentioned by SCHNEIDER *et al.* 2007).

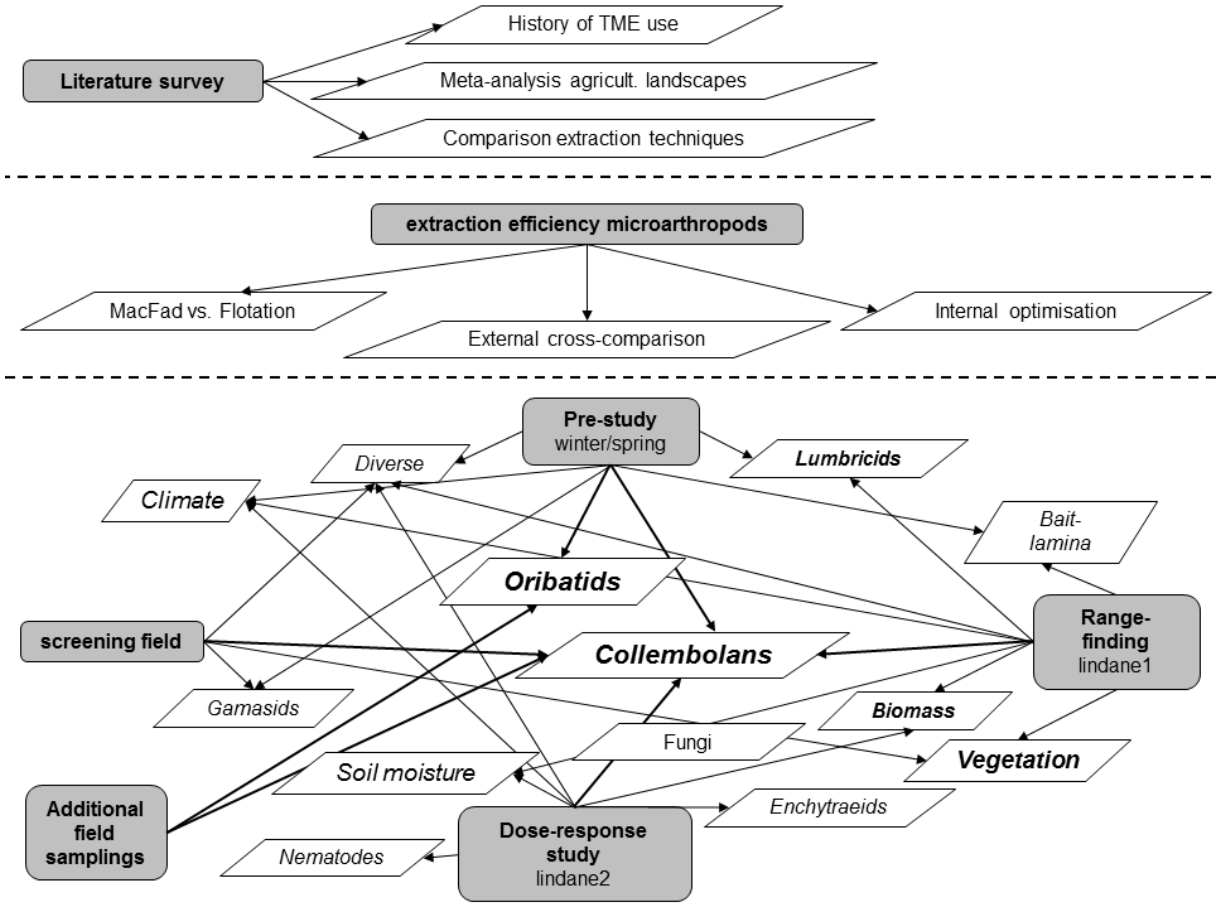


Figure I-2: Representation of the concept of the study at issue: database. The three levels of investigation are described in the text. Grey: Clearly defined work packages. Italics: Measurement endpoints during TME and field studies.

Figure I-2 gives an overview on the variety of endpoints and the mere intense effort to collect as much data as possible that was undertaken to evaluate the chosen approach. The process can be divided into three levels: Theory and literature research, general improvements of soil methodology at hand and experimental work to improve the TME approach. The **first** level was built by screening the available literature for data on the comparison of best-use extraction techniques of microarthropods on grassland, on expectable species composition and on the historical use of TME. The outcome has been a comprehensive literature survey (chapter I-3) and a meta-analysis of collembolan communities of agricultural landscapes (chapter V-4.2).

The **second** level was the improvement of the methodology, mainly for the extraction efficiency of microarthropods by systematic comparisons. The results of these experiments have not been objected in particular by this work but shown indirectly by the development of the variation observed in the course of the TME studies (chapter V-3.2.3).

The **third** level of investigation consists of consecutive TME experiments supplemented by field samplings to cover most of the aspects that could be criticized scientifically and from the

point of view of a risk assessor (chapters III and IV).

The strategy was oriented by a step-wise progress, triggering the next step by a positive evaluation of the pre-defined prerequisite for succeeding with the next, more complex step within the development of the methodology. The final aim was to 'stair-up' the initial system towards the testing of a commercially relevant test substance in a dose-response design that allows for the derivation of EC_x-values over a period of approximately one year.

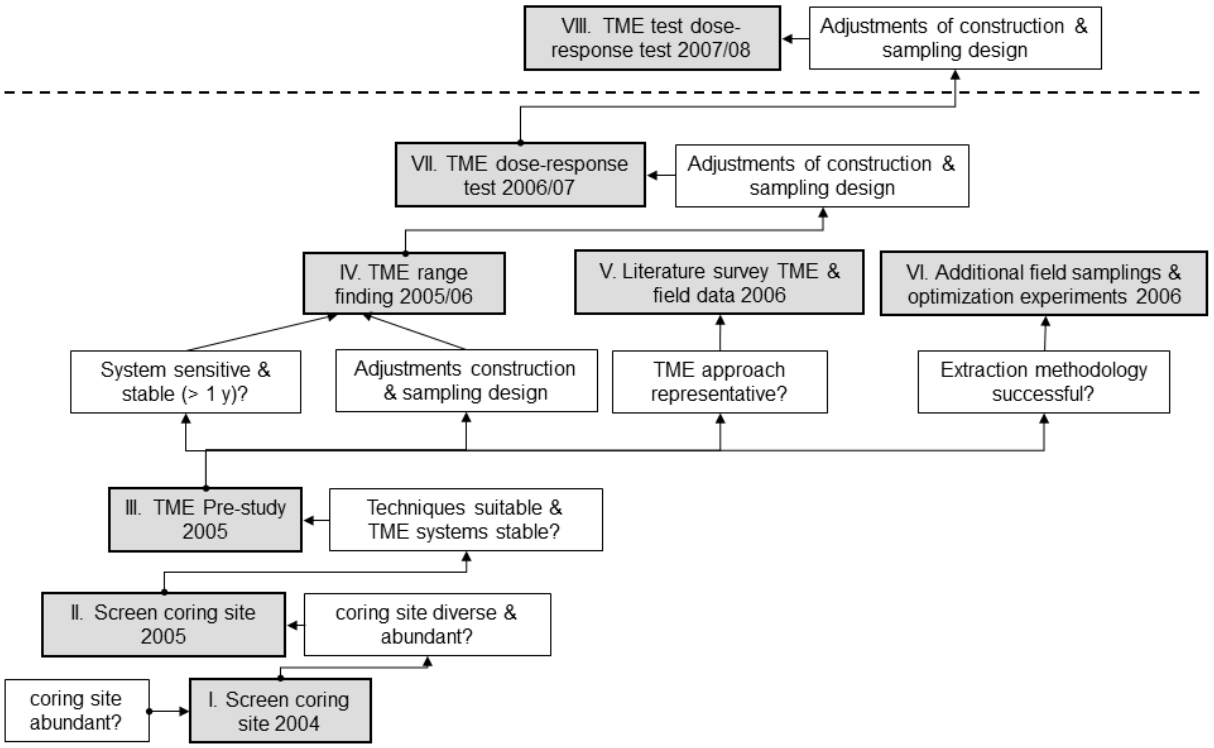


Figure I-3: Flowchart of the step-wise development of the TME-methodology.

I-2 Legal and regulatory background

I-2.1 Tiered approach

The environmental (or ecological) risk assessment (ERA) of pesticides within the EU according to Directive 91/414 is based on a tiered approach. On a first tier, single species toxicity tests have to be conducted, mainly on species that act as models for group specific sensitivity towards toxicants, namely *Eisenia fetida*, *Folsomia candida* and *Hypoaspis aculeifer*. The intrinsic uncertainties while extrapolating to the real world are addressed by (supposedly) protective safety factors that are applied on the effect data. Usually, the results of standard single species laboratory tests are extrapolated to the field situation and thus the geographical area that comes within the scope of a law, as anticipated by the eco-regions concept of the

EFSA (EUROPEAN FOOD SAFETY AUTHORITY 2010).The results of those studies may trigger litterbag tests or higher tier earthworm field studies if defined limits of ratios between measured toxicity and modelled exposure concentrations (toxicity-exposure-ratio TER) are not met or exceeded (EUROPEAN COMMISSION 2002a). TER values usually are considered safe between 5 and 10 for tier 1 assessments, according to the actual degree of uncertainty. The uncertainties implying the extrapolation are addressed by applying safety factors on the effect data that are assumed sufficiently protective. If the ratio of the assessed toxicity and the assumed exposure of the pesticide are lower than a defined threshold value, refined methods that are more realistic have to be applied. Standard tests with surrogate soil organisms are stipulated by current EU legislation if triggered by degradation halftimes longer than 100 days. Higher-tier semi-field systems that address structural end-points with standardized methods are lacking or little experience is available. In the field, many indirect effects after pesticide application may occur (FRAMPTON and VAN DEN BRINK 2007) cannot be adequately predicted by single species tests (WILES and FRAMPTON 1996; CAIRNS1984). Semi-field or field trials providing high taxonomic resolution can enhance both realism and certainty of the risk assessment and thus connect laboratory results with real world scenarios (TOSCHKI 2008).

I-2.2 Semi-field level

TME have been proposed an appropriate method to assess the risks of biocides, industrial chemicals and plant protection products (WEYERS *et al.* 2004). In 2007, a SETAC workshop ('Semi-field Methods for the Environmental Risk Assessment of Pesticides in Soil': PERAS, Coimbra, Portugal) identified TME as one suitable tool to assess effects on the structure of soil communities (SCHÄFFER *et al.* 2008, 2011; EUROPEAN COMMISSION 2002a). Several approaches were used in the past to address ecotoxicological (e.g. MORGAN and KNACKER 1994) and ecological (e.g. KAMPICHLER 2001) questions. Previously, TME were used in an extensive ring-test with four different European soils under grass- and arable-land, including a bulk of structural and functional endpoints. TME in these tests were exposed indoors under constant light-cycles, temperature and soil moisture (KNACKER *et al.* 2004). In the context of the new Regulation 1107/2009/EC of plant protection products in the EU (EUROPEAN COMMISSION 2009), the Terrestrial Guidance Document is under revision (expected to be finished until the year 2015, PSC 2012) and therefore BROWN *et al.* (2009) provided information on available higher-tier approaches on behalf of the European Food Safety Authority.

I-2.3 Protection goals

One pre-requisite and starting point for the revision is the definition of environmental protection goals for agricultural landscapes, which had been discussed during the EFSA 'Workshop on Protection Goals for Environmental Risk Assessment of Pesticides' in Parma, 2010. Once these protection goals have been defined, TME may be a suitable higher-tier method to address concerns raised by the out-come of lower-tier studies. It is assumed that off-crop grassland communities should be more sensitive towards disturbances through pesticides than communities that are adapted to usual cropping practice, which are under selective pressure in the long-term. There is still need for further research to confirm the latter assumption scientifically. Combined with an exposure pattern that aims to mimic good agricultural practice to test the principal suitability of the approach, our test design can be regarded as a realistic scenario to deduce protective thresholds for the terrestrial environment.

I-2.4 Perspectives under the new regulation 1107/2009/EC

The species structure of soil communities are widely accepted to be site-specifically related to soil properties (ETTEMA & WARDLE 2002, TOSCHKI 2008, TURBÉ *et al.* 2010, RÖMBKE *et al.* 2012). Indirect effects that occur in the field in any case (FRAMPTON & VAN DEN BRINK 2007) may not be accurately predicted by single species tests (CAIRNS 1984). The registration process of pesticidal substances was unified between the member states of the European Union. It was mainly guided by the clauses of the Directive 91/414/EEC of the COUNCIL OF THE EUROPEAN UNION (1991). The document was currently completely revised in the legal form of a regulation (No. 1107/2009/EC) and came into force at 14 June 2011 (EUROPEAN COMMISSION 2009). It demands for rather structural than functional endpoints in the tiered risk assessment scheme. This tackles especially the field of soil ecotoxicology since the ecological risk assessment procedures within the European Union under the new regulation 1107/2009/EC are currently subject to major revisions (EUROPEAN COMMISSION 2009). Firstly, the probability to conduct refined higher tier studies with soil macro-organisms increases. According to the yet unpublished annexes and terrestrial guidance documents to the new pesticide regulation, the trigger to conduct soil macro-organism studies (*Folsomia candida* and *Hypoaspis aculeifer* reproduction test) will be presumably altered. In the future, the mode of application (e.g. all preparations that are foreseen to be applied to bare soil) and the results of laboratory studies conducted with non-target arthropods (NTA), rather than the substances` degradation time (e.g. DT_{90}) will be decisive. This will cause a boost in laboratory testing. Secondly, there is lack of an accepted higher tier alternative to the field study using structural endpoints. How-

ever, the subject of future risk assessment will be rather structural than functional. Semi-field or field trials providing high taxonomic resolution can enhance both realism and certainty of the risk assessment and thus connect laboratory results with real world scenarios (TOSCHKI 2008, KNACKER *et al.* 2004). The European Food Safety Authority (EFSA) provides numerous opinions and reviews on suitable higher-tier approaches (e.g. BROWN *et al.* 2009). The definition of environmental protection goals for agricultural landscapes has been discussed during an International Workshop on Protection Goals for Environmental Risk Assessment of Pesticides (organized by EFSA in Parma, 2010). After the discussion of the workshop the EFSA formulated its decisive opinion on 'what to protect, where to protect it and over what period' (EFSA PANEL OF PLANT PROTECTION PRODUCTS AND THEIR RESIDUES 2010). These specific protection goals further refine the very general provisions of the regulation 1107/2009/EC and the scientific opinion of the EFSA points directly to the common tendency of a new guidance. The ecosystem services concept that is used by EFSA, however, has to be interpreted to protect the biodiversity, which is commonly agreed imperatively important for agricultural productivity. It was stated by the EFSA that a tiered approach should be maintained. The use of semi-field methods, inclusive Terrestrial Model Ecosystems, is considered since several decades as suitable to address questions of effects of various contaminants to soil communities (WEYERS *et al.* (2004) give an overview) as the key drivers for the provision of ecosystem services of soils, the communities of soil invertebrates and thus the structure of meta-populations have to be protected and assessed. TME were considered as a suitable tool to assess effects on the structure of soil communities during the SETAC workshop 'Semi-field Methods for the Environmental Risk Assessment of Pesticides in Soil' (SCHÄFFER *et al.* 2008, 2011).

Risk assessors wish to regulate on threshold concentrations for the community (No observed effect concentration [NOEC]); simultaneously, a quick and easy overview over the development of the treated communities compared to control in a time-series is demanded. The latter should give the possibility to define a 'time-to-recovery' of the communities after (chemical) stress indicated by non-significant deviations from the control level. As there is no guidance in the literature how to analyse TME data, the OECD 'Guidance document on simulated freshwater lentic field tests (outdoor microcosms and mesocosms' (OECD 2006) was proposed to consult. Data gathered in TME studies and in mesocosm experiments are of similar complexity and structure. It is stated that multivariate analyses are suitable both to describe the effects on community level and to detect particularly sensitive species. Those species identified to contribute considerably to the community response should then be further evalu-

ated by univariate methods. Recently MOSER *et al.* (2007) showed that PRC revealed very low NOECs for the community of enchytraeids compared with univariate comparisons of single populations. The first step to approach the complexity of semi-field studies was identified to be a comprehensive review of the available literature. It should serve as a source of information on available higher tier methods, particularly those characterised as Terrestrial Model Ecosystems.

I-3 Higher-tier multi-species testing in the soil and terrestrial environment

I-3.1 Standardisation of terrestrial and soil ecotoxicological test methods

The development of standardized ecotoxicological test procedures for the terrestrial environment has flashed back on a relative short history. In 1974, the 'International Organization for Biological and Integrated Control of Noxious Animals and Plants' invented a standardization of the methodology available. From 1974 until recent times a discussion about the suitable test methods started. The discussions led into a first EU Directive 91/414/EEC (COUNCIL OF THE EUROPEAN UNION 1991) which was supplemented at once in 1994 by the ESCORT 1 agreement (BARRETT *et al.* 1994). During the next 6 years, several methods were developed and standardised. This effort led to a modified ESCORT 2 document (CANDOLFI *et al.* 2000), which was highly influenced by an 'industrial pragmatism'. In 2002, the preliminary 'Terrestrial Guidance Document' (EUROPEAN COMMISSION 2002a) was published, which has been valid until today but currently under revision to be adjusted to the requirements of the new regulation 1107/2009/EC. A tiered approach is mandatory, which implies trigger target values concerning the relevant, sensitive endpoints tested. Dumbing the approach down, tests of higher tiers have to be conducted if the risk indicator falls short of the threshold (TER-concept). The above-mentioned guidance documents were written to set standards of the environmental risk assessment to regulate the registration process of pesticides. There is no general soil protection framework (e.g. to assess contaminated soils) comparable to the water framework directive; first sketches of EU-harmonized approaches (VAN WENSEM *et al.* 2008) failed on early stages for mainly political reasons. An overview of recently available ISO or OECD standard test methods for the terrestrial environment as well as suggestions for addi-

tional field monitoring, community based assessments and the concentration of test strategies gave RÖMBKE & KNACKER (2003) or RÖMBKE (2006). Nonetheless, there is still a need to develop advanced methods, particularly because the majority are simple laboratory tests with limited ecological meaning. They do not cover complex biotic interactions or effects on the community level (as e.g. KAMPICHLER *et al.* 1999 pointed out). The fact that risk assessment approaches for the toxicity of solid materials (like waste materials or soil from contaminated sites) use combinations of terrestrial and aquatic test systems, demonstrates the need for specialized methods for terrestrial ecosystems. An overview of methods to assess waste materials was given by DEVENTER *et al.* 2004, for soil remediation see WILKE & FLEISCHMANN 2004.

At the time of starting the project that should finally deliver the raw material of this thesis, there is no international standard test procedure for complex, multi-species higher tier-tests in soil under realistic exterior conditions than the laboratory standard conditions. Therefore, the development of a feasible methodology, which is covering several of the most important groups of soil animals and which is gathering reliable endpoints on population or community level was considered necessary. The variety of coexisting approaches and subsequent bad comparability has led to some effort to standardize tests with TME (called terrestrial soil-core microcosm test by ASTM 2004). The approach represents the only well described test protocol, which could be taken into account in the development of our test system as an existing 'standard'. Also in Europe, the legislation has tried to standardize terrestrial tests and there have been demands for further testing if a trigger value within the current risk assessment scheme has been induced. Usually, the risk is expressed as a 'toxicity-to-exposure-ratio' (TER) or as a 'Hazard Quotient' (HQ) which are both calculated as the relation between a modelled 'Predicted Environmental Concentration' (PEC) and an experimentally derived 'Predicted No Effect Concentration (PNEC) for the species that is representative and sensitive within the assessment area considered (EUROPEAN COMMISSION 2002b). A similar procedure was proposed by the US EPA (2006).

A possible approach could be the use of semi-field systems such as Terrestrial Model Ecosystems. The following sections contain detailed descriptions of a comprehensive selection of semi-field systems, in order to document the state of the art at the point in time of writing. The literature survey was conducted to decide about the suitable method for further developments; the focus was on community-based studies in soil. Many other aspects such as the suitability of extraction methods and the characterisation of mesofauna communities of different ecosystems were originally included in the survey but are not shown here in all the colourful details. All relevant information on TME studies has been incorporated in a comprehen-

sive access data base and can be queried by assigning the author. When considered reasonable, advantages and disadvantages of the approaches are discussed.

I-3.2 Definition and nomenclature of Terrestrial Model Ecosystems

During the last three decades (from the 1980ies until the year 2011), TME were widely used for various purposes. The variety of approaches was also reflected by numerous definitions and an alternating nomenclature. The term 'terrestrial' refers to the fact that the systems were not meant to be 'aquatic', it does not follow that e.g. terrestrial invertebrates only could be tested, excluding soil organisms. The following section gives a systematic survey of this diversity. The use of bounded systems, only partly permeable to their surroundings, was first proposed by ODUM (1984) for both ecological and ecotoxicological studies. He stressed the general advantages of such systems:

- They provide a high degree of realism, simplifying at the same time the real world and are therefore suitable to link laboratory and field tests.
- They are easily replicable.
- Parts (e.g. individual species) and wholes (e.g. populations, communities) can be investigated at the same time.

He described a set-up in the following called 'mesocosm', because a part of the 'real world' was experimentally manipulated indeed, but separated from the surrounding area without any removal (of soil or biota). He considered both aquatic and terrestrial systems.
For every terrestrial system that was artificially set up, that was removed from the study site, then kept under laboratory conditions, that consisted of sieved or intact soil and that included a natural or artificially composed fauna and flora, the term 'soil Microcosm' or more recently 'TME' should be applied in contrast to the term 'mesocosm' for field-born systems with boundaries. It is literally impossible to give a consensus definition of soil microcosms or TME but SHEPPARD (1997) defined exclusively systems, which were designed for the testing of toxic substances in soil. In his definition, a TME has to

- be a replicable, experimental unit containing soil,
- be a system in which the response of more than one biotic species can be measured and
- contain more than one biotic species larger than microbial organisms.

These prerequisites were not met by all of the approaches, which will be presented in the following chapters. There is no standard test including TME as semi-field test systems in the laboratory or outdoors. The first step to standardize a test system is a consistent nomenclature,

to avoid confusing communication. There have been some attempts to categorize TME, one of the more recent ones is the review of MORGAN & KNACKER 1994.

They postulate four different types of TME:

- Closed, homogenous TME (CHTME) were prevented from atmospheric exchange to measure the fate and the behaviour of xenobiotics. Shielding could be used to catch gaseous substances, but also to prevent immigration or emigration of organisms. The soil used in this systems was mostly sieved, sometimes artificially defaunated substrate, followed by a subsequent refaunation with a defined number of species.
- Open, homogenous TME (OHTME) were constructed very similar, but an exchange of the intrinsic gaseous phase with the atmosphere was allowed.
- Closed, intact TME (CITME) followed a different approach than the two set-ups mentioned before: they consisted of undisturbed soil columns, cored by means of special sampling techniques. They were often exposed under outdoor conditions and closed to prevent immigration of soil organisms or to avoid other disturbances.
- Open, intact model ecosystems (OITME) constituted the most realistic approach that was able to detect the recovery of populations. They often initially contained the natural community of the soil under concern, but there was much variation of abundance in the systems, so compared to the other three approaches presented above an increased number of replicates was deemed necessary by the authors.

It was apparently discovered that this classification was not valid for all studies using TME approaches. There were hybrid forms of these four approaches or studies dealt with combinations of field or semi-field studies and TME for reasons of cross-validation of the findings. It was not practicable to generate single datasets for every subunit of a study.

For this reason, a fifth category was introduced into the database, called **'combinations'**. An approach could be a combination of **two TME approaches** or of one TME approach and a **corresponding field study**. This definition covered all of the literature considered for the evaluation at hand.

In the past many different systems have been developed, ranging from small-scaled TME with controlled conditions to big-scale TME containing complex biotic and abiotic factors and interactions. The latter approach mostly ended up in higher imponderableness.

One of the most ambitioned experiments of this kind was the 'Ecotron Facility' in Silwood Park, USA. This facility was presented in a special issue of the journal 'Ecology' by LAWTON 1996, DAEHLER & STRONG (1996) made fundamental considerations; other results were included in the corresponding issue of the journal. In spite of the massive scale of their facility,

the workers had serious problems regarding stability and reproducibility of the systems. Prior to these experiments, it was commonly assumed that smaller systems were more difficultly maintained to build stable environments. Because only few specifically designed experiments or analyses were found on this topic, in the following chapters the problem of comparability of artificial systems under influence of chemicals will be stressed. The systems of concern in this study hardly ever reach the scale of the Ecotron experiment of several square meters and could therefore altogether be called 'medium-to-small-sized-systems'. They were mainly designed to supplement or replace field experiments and were aimed to be cost effective and to deliver predictive values for of the real environment. Many reviewers of TME tried to estimate the future use of them. They discussed the suitability partly with regard to concrete constructions or they thought about theoretical limits and the classification of obtained results from those systems (VAN STRAALEN *et al.* 1994). RÖMBKE & MOLTMANN (1996) described the advantages and disadvantages of laboratory and tests on field or semi-field level. Most of the publications have an academic background; the tremendous number of data from industry experiments for registration purposes was never published for reasons of competition between the market participants, or it was only available in the grey literature. In the following, the above-mentioned systems are reviewed with respect to the questions addressed in advance and to the reliability of the gathered information.

I-3.3 Quantitative analysis of literature

As shown by the results of this study, there was constant interest over the last decades in bridging the gap between laboratory- and field-testing of chemicals. Researchers tried to achieve the goal by means of numerous different constructions of TME. For investigations of the behaviour and the degradation of pesticides numerous examples of successful applications could be found, though results were often not directly transferable to the field situation without further considerations. In the author's eye, within EU legislative and consultant bodies (The Commission and the EFSA) a tendency towards an ecosystem based, more holistic environmental risk assessment and management approach became apparent and was expected to relate also to terrestrial systems. Summarizing the recent regulatory developments, APITZ *et al.* (2006) regard the inclusion of terrestrial habitats by gathering the 'habitats directive' of the European Union (COUNCIL OF THE EUROPEAN UNION 1992).

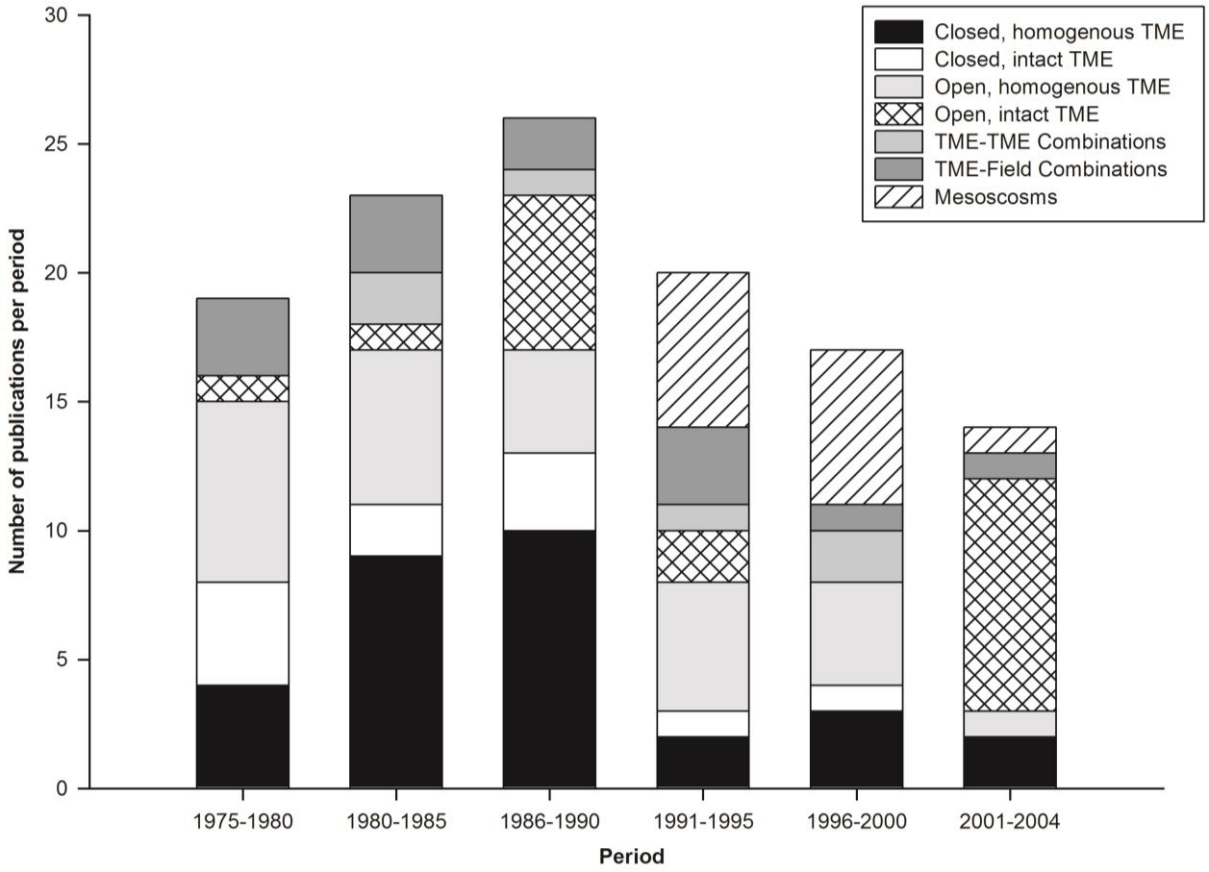


Figure I-4: Study approaches. Bar charts of 119 selected publications published in the last 30 years, divided by 5-year periods. Note: the extensive study of KNACKER *et al.* (2004) with nine sub-divisions in a special issue of the journal 'Ecotoxicology' was included to demonstrate the apparently increasing meaning of open TME approaches using intact soil cores.

Generally speaking, this will probably lead to a higher effort to develop methods to acquire information about whole populations of soil inhabiting animals. The following figures Figure I-4 and Figure I-5 were based on the outcome of the literature survey. The number of studies summarized by the categories 'approach of study' and 'goal of study' of the literature was plotted against the time of publication to show the development of TME-research during the last 25 years. Categories were slightly summarized compared to the detailed distinctions made in the database. Hundred-nineteen different studies were included. It has to be considered that the compilation could not be complete and the graphs are restricted to show a selection of the data available at the time of writing.

On inspection of Figure I-4, the meaning of closed systems decreased drastically in the last three five-year periods after a steady rise between 1975 and 1990. The number of open systems with homogenized soil was nearly constant over the considered period. The category 'TME combinations' (defined as a combinations of closed and open, just as intact and homogenous systems within one publication), was used at constant numbers over the period as well as 'TME-field combinations'. The use of open, homogenous TME was quite popular in

the late 90ies and could revive in recent times with the study of KNACKER *et al.* (2004) and related articles. The use of mesocosms as partly bounded field systems (for definition see chapter I-3.2) aroused 15 years ago and decreased again in the last five-year period (2001-2004).

Figure I-5 shows the main topics of investigation in the period from the year 1974 to the year 2004. Categories were summarized compared to the detailed categorisation in the database. Occasional categories were summarized as ‘other. Since the researchers of the late 70ies and 80ies focused the fate of chemicals, the use of TME to determine the transformation, the mobility and the accumulation of chemicals decreased in the following years and reached a minimum for the time being. The investigation of single species in TME was of minor interest in the decade from 1990 to 2000; in return, the study of whole communities was steadily growing. Because of relative simple methodology, the measurement of the mineralization function of mainly the soil microfauna (fungi and bacteria) was often included in the studies. Soil respiration was often measured additional to other endpoints. Ecological researchers had been using TME constantly over time, but the maximum number of publications dealing with ques-

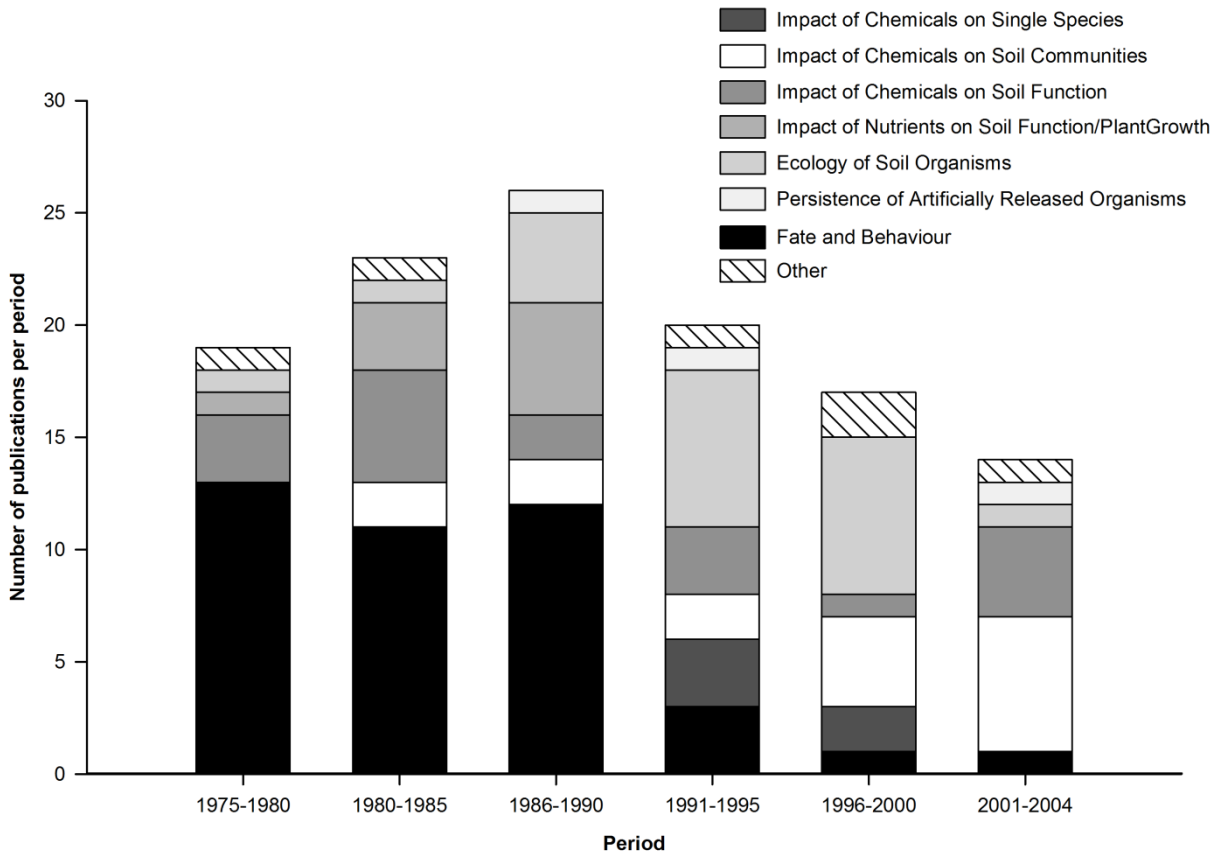


Figure I-5: Study goals. Bar charts of 119 selected publications that were published during the last 30 years, divided by 5 year periods. Note: the extensive studies of KNACKER *et al.* (2004) with nine sub-divisions in a special issue of the journal ‘Ecotoxicology’, dealing mainly with the impact of chemicals to soil communities were included as stand-alone counts to demonstrate the apparently increasing meaning of the approach in general.

tions of animal interactions or with the impact of the fauna on processes in soil were counted in the decade from 1990 to 2000. The research on the influence of nutrients or fertilization seemed to be finished until 1990 but the 'ecology section' summarized some publications, which deal with this topic apart from many other factors and could therefore not categorized specifically. In the future, the meaning of model systems for the release of genetically engineered organisms will probably grow but until recently there were only three publications found. In this study, TME were shown to be suitable tools for the assessment of the behaviour of agrochemicals under laboratory and outdoor conditions. Especially the use of radiolabelled pesticides was considered a powerful tool to understand the fate and persistence in the environment (RAGHU *et al.* 1996). Furthermore, TME could serve as models for the impact of chemicals on ecosystem level, providing the opportunity to investigate soil processes and soil organisms at one time and even to follow the fate of the substance at the same time (EDWARDS *et al.* 1996). There was no harmonized set-up for toxicity testing in TME beside the ('microcosm') approach of the American Society of Testing and Materials described above. SHEPPARD (1997) reviewed the potential benefits and failings of TME tests. Beneficial aspects of TME were based on the relative low cost in comparison to field tests. Single-species tests were not considered adequate to cover the range of sensitivities of multiple species, but TME offer the possibility of synchronous multi-species testing. TME offered information on impacts of toxic substances on the trophic structure and on the communities of soil organisms, as well as on indirect effects mediated by food web relations. They were more likely to contain information about ecological functions. In addition, in complex, but controlled systems, patterns and mechanisms occurred that would never have emerged in single-species tests. Sheppard exemplified some studies that brought out those endpoints measurable only in TME (and in the field) were more sensitive (e.g. life history changes, avoidance behaviour, population alterations) than crude mortality in single-species tests. On the other hand, TME had some substantial disadvantages in comparison to single-species tests. In a complex community there was assumed to be functional redundancy, so that the ecological function (e.g. mineralization rates, nutrient cycling) was maintained although several populations could be affected. Another reason to avoid TME-testing in Sheppard's opinion was a more indirect exposition. In a complex soil matrix, a proportion of each population could avoid exposition by taking refuge in protected soil pores. The sensitivity appeared thus lowered, compared to single-species tests. More disadvantages were hidden in an inappropriate scale for larger soil animals like earthworms, and in the rise of temporal variation in chronic exposure tests. Finally, it could be argued that a replacement of multiple single-species tests by one multi-species TME-test does

not match the (statistical) preconditions of independent samples in the calculation of extrapolated hazard concentrations (e.g. in order to arrive faster a statistically reliable HC_5 by applying the 'Species Sensitivity Distribution' approach). This approach is widely accepted to derive an acceptable concentration or dose to protect a major fraction of an ecosystem. Since CAIRNS (1984) emphasized, from an ecological point of view, that single-species tests were not adequate to predict the impact of chemicals on higher levels of organisation (population, community), a lot of research effort was done to improve TME approaches. He claimed the development of ecological sound toxicity tests by ecologists and stated that they do not need to be much more expensive, time-consuming or demanding for expert knowledge than successive single-species tests. From the literature compilation can be clearly concluded, that there is still a lack of realistic tests with natural soil communities, which would be suited to overcome the simplifications of artificial composed systems with sieved and defaunated soil.

I-3.4 Approaches using closed, homogenous TME

CTME were used extensively for the investigation of fate, behaviour and degradation of chemicals due to the high reproducibility of results, the avoidance of atmospheric exchange and the possibility of measuring gas concentrations in the air. CHTME frequently consisted of artificially composed food chains, and of both terrestrial and aquatic compartments to link the effects of pesticides or other chemicals to the 'total' environment. The chemicals applied were usually radiolabelled to track their fate independently of medium-specific extraction efficiencies and to estimate the amount of non-extractable residues. Those studies were conducted from the early 1970ies on to the late 1980ies and that by using generally similar approaches (e.g. METCALF *et al.* 1971, COLE *et al.* 1976 A, B, NASH *et al.* 1977, FIGGE *et al.* 1983, SCHUPHAN 1986a, SCHUPHAN *et al.* 1987). The work of GILLETT and GILE exemplifies a successful and continuous work on the topic, using so-called 'terrestrial microcosm chambers'. A series of publications can be consulted: GILLETT and GILE 1976, GILE *et al.* 1980, GILE and GILLETT 1981, GILE *et al.* 1982 and GILE 1983. They especially measured residues of insecticides and fungicides in or on plants, in soils and in the air (for that purpose the systems were closed), as well as in vertebrate and invertebrate animals. The scientists detected the parent compounds and their comparably toxic metabolites in biota in such concentrations that were suitable to adversely affect the efficiency of decomposition. Beside this, the chemicals were often bound to the upper soil layers, and thus were not detoxified further. For this reason, the risk of applying them in the environment was deemed high. The pesticide formulations belonged mostly to the group of foliar sprays and included of insecticides of high concern like parathion,

methylparathion, aldrin, dieldrin, methoxychlor, nitrophenol, DDT, fonofos or toxaphene. 2,4-D, dieldrin or aldrin served as a reference compound in some cases. Many of them were banned from the use in agriculture for their unwanted degradation characteristics or ecotoxicological profiles. Not least because of the results of the semi-field TME studies, the regulatory decisions were made. The results were presented as measured concentrations in the compartments of consideration or as metabolisation rates (half-lives, half-concentration times) of the compounds. Each of the authors proposed their systems as appropriate for testing the behaviour of pesticides and other chemicals or as well as to assess the impact on ecosystems. They concluded that it would be possible to differentiate the hazardous from non-hazardous chemicals by means of their individual experimental set-ups. The distribution and amounts of the compounds distributed in soils, plants, air and animal tissues were measured with great significance, but effects on the ecosystem parts of consideration (e.g. acute effects on the survival of voles) were rare and weak. Hence, it could be concluded that the chosen systems or endpoints were not very sensitive towards the chemicals of concern, supposable for statistical and further reasons concerning issues of experimental design. Besides the great variety of intensive research activities to understand the distribution of chemicals within the process of pre-registration, other studies dealt with effects of chemicals on the soil function, the structure of soil communities or the interaction of numerous environmental variables in closed, homogenous TME. Soil biota were mostly not present in their natural composition but artificially added to defaunated soil. Some research was done on the influence of chemicals on soil function and on how to measure it in model ecosystems. In particular, ANDERSON & INESON (1982) described systems, which allowed for the measurement of gas exchanges and leaching losses together. Thus, they were in the position for conclusions on respiration rates and nutrient cycling processes. HADERLEIN *et al.* 1999 pointed out the need of sufficient aeration in mineralization studies and described the set-up of systems assuring it. Studies like those were mainly interested in decomposition processes that were mediated by microorganisms (or large macro-decomposers) under the influence of chemicals or chemical mixtures. The measurement endpoints were carbon or nitrogen flux rates, enzyme activities or concentrations of micro-nutrients (DOUGHERTY& LANZA 1989, SETÄLÄ & HUHTA 1990, VAN WENSEM *et al.* 1991, VAN WENSEM & ADEMA 1991). The last-mentioned authors used organic pollutants like phenothiazine, chloronaphtalene, polychlorinated biphenyl, fentine hydroxide together with inorganic reference compounds like potassium fluoride in one study. It was found that the endpoint 'microbial respiration' reacted insensitive compared to fluxes of nitrogen and phosphorous towards the treatment with different toxicants. In some cases, it was not possible to de-

tect differences because of the high variability of microbial responses due to successional processes that antagonised statistical significance. Throughout the 1980ies, the investigation of fly-ash amendments (a by-product of coal combustion processes) was intensified. It was aimed to support the decision on industrial waste disposal with or without further treatment. At this time, it was a common topic of TME-research. Only small effects could be found, due to heavy metals, which occurred in toxicologically relevant concentrations regarding plants and the microbial activity at very high deposition levels of fly-ash (ARTHUR *et al.* 1984). BLAIR *et al.* 1989 investigated the impact of naphthalene on the microbial respiration, the abundance of fungi, bacteria and the nitrogen pool. Naphthalene was often used as a biocide to exclude the microarthropod fauna in field studies assuming a specific effect on this group and no influence on non-target species. They found significant alterations of the above endpoints indicating highly biased results and interpretation difficulties in field studies. The closed systems were used under laboratory conditions to study the impact of chemicals on single species (e.g. by ADDISON & HOLMES 1995) as well as to soil communities; BORN *et al.* 1989 used soil micro- and mesofauna like nematodes, enchytraeids, rotifers and microarthropods in previously defaunated soil. ADDISON & HOLMES (1995) expected negative effects of *Bacillus thuringiensis*-based insecticides on the survival of collembolan species *Folsomia candida*. They examined a decrease of population densities but this effect was solely attributed to the oil based formulation of a commercial product. Interactions between two or more species were investigated by GILLETT & GILE (1983). They added a gravid vole to a TME-chamber containing a certain number of crickets and observed both negative and positive effects of four different wood preservatives on the predation intensity. Fewer studies were driven by pure ecological not ecotoxicological questions. The interactions between different groups of soil organisms were investigated by BRIMECOMBE *et al.* 2001. Others investigated the role of soil fauna in processes of nutrient cycling or decomposition of soil organic matter. SULKAVA & HUHTA 1998 connected the soil fauna to the habitat structure measured as litter patchiness, while SETÄLÄ *et al.* 1988 and SETÄLÄ & HUHTA 1990 related decomposition processes to the diversity of soil fauna. The authors found that a patchy environment enhanced the diversity of microarthropods; however, their productivity was better maintained in more homogenous environments. That was hypothetically due to a determining scale of millimetres in homogenous environments that was more suited for microarthropods, while soil fungi could bridge greater gaps by building up connections of some centimetres length in patchy litter layers. A diverse community consisting of collembolans, mites, enchytraeids and nematodes enhanced significantly the activity of soil microbes, thus accelerating the total decom-

position process. The promoting effects of soil fauna were stronger in TME with birch or spruce litter than in humus amended TME. It was reported that soil animals themselves enhanced the humification of litter accompanied by a retarded decomposition. The authors doubted the value of measuring mass loss in open systems that serve as an indicator of decomposition, because it could be due to mere transport out of the system. This problem would be solved by using closed model systems allowing simultaneous measurements of respiration. Vice versa, the impact of nutrient regimes on soil fauna or vegetation was investigated. The influence of an enrichment of the atmosphere with CO_2 on plant growth was examined by OVERDIECK & REINIG 1986 a, b and OVERDIECK 1986. They found that almost all parts of the examined plants (white clover and perennial ryegrass) increased in length, weight or abundance with increasing carbon dioxide concentration. FAIRBANKS *et al.* 1984 tried to take the amount of ATP in soil as a measure for microbial activity or microbial biomass. As a result, it turned out that ATP concentrations could not be assumed as representative for microbial biomass because of the high influence of moisture, nutrient or predation regime.

I-3.5 Approaches using closed, intact TME

Similar to CHTME, the usage of intact soil cores was focused by most of the academic effort to the fate and behaviour of various xenobiotics. General considerations regarding systems established with model chemicals were described in CHECKAI *et al.* (1993), SCHEUNERT *et al.* (1996), PIWONI *et al.* (1986) and HEISE *et al.* (1988). There are numerous prerequisites to bear in mind when setting-up such systems to mimic the field situation realistically, concerning e.g. water tension, recommendations to core undisturbed soil cores and characteristic of the materials of boundaries and others. Among the chemicals investigated, not only agricultural pesticides, (i.e. insecticides by WINKELMANN & KLAINE (1991) and NASH & BEALL (1980B), fungicides by NASH & BEALL (1980A)) were tested. In addition, mixtures of toxicants like in sewage sludge were used by FIGGE & SCHÖBERL (1989); organic and inorganic pollutants were investigated by JACKSON *et al.* 1978A and SCHUPHAN 1986b. The behaviour of potentially toxic trace elements (e.g. arsenic in JACKSON & LEVIN 1979) or nutrients (BENGTSSON & ANNADOTER 1979) was examined in closed systems to follow explicitly the volatilization of the substances. The suitability of these systems, as a screening tool, and for tracking the chosen chemicals was considered well by the authors. To estimate the comparability of the systems, sometimes accompanying field or semi-field studies were conducted (e.g. by J JACKSON *et al.* 1978A, JACKSON & LEVIN 1979, NASH & BEALL 1980B and SCHUPHAN 1986b). Especially photolytic processes under natural light conditions were assumed not to be analysable

in the laboratory and therefore an adjustment by natural light regimes was deemed necessary. CITMEs, which were validated by field studies, were assumed to represent the field situation in terms of chemical transport. Researchers using the CITME-approach investigated the influence of soil fauna on nutrient cycling or decomposition with or without pollutant influence. Publications on this topic were written for example by JACKSON *et al.* 1977, JACKSON *et al.* 1978B, MITCHELL *et al.* 1982 and BILLINGS *et al.* 1984. The latter authors proposed their systems as suitable to elucidate mechanisms interacting between the biotic and abiotic environment. Little work was done on modelling biotic soil processes by conducting specifically designed experiments (SHIRAZI *et al.* 1984). Most of these experiments were insufficiently replicated, disregarding the high variability of biotic endpoints measurements (BENGTSSON & ANNADOTER 1989). Comparing the chemical parameters and biological parameters (e.g. JACKSON *et al.* 1977) it was found that for instance the endpoint 'nutrient losses from a soil through leaching to the groundwater' answered more sensitive to stressors than population parameters. The last mentioned authors recommended the use of TME as an early warning system in the frame of process based soil monitoring.

I-3.6 Approaches using open, homogenous TME

Similar to the closed systems containing homogenized soil, this category of model systems was mainly used in fate studies since the late 1970ies. Among various chemicals mainly pesticides were tested. Most of the chemicals act as insecticides and are related to different substance classes. The following researchers investigated the behaviour and the metabolism of insecticides in different terrestrial compartments: LICHTENSTEIN *et al.* 1974, COLE & METCALF 1980, LICHTENSTEIN 1980, BRANHAM *et al.* 1985, LICHTENSTEIN & LIANG 1987. To a much less extent, fungicides, nematicides and herbicides were tested (COLE & METCALF 1980, see also BIGGAR *et al.* 1984 or O´CONNOR *et al.* 1980). In summary, there was an apparent lack in research effort on the substance classes 'fungicides' and 'herbicides'. This does not reflect the industrial turnover of these substances in the last few years. Concretely, about 10-15-fold the amount of herbicides and fungicides in comparison to insecticides were sold, resulting in obviously differing environmental concentrations. Investigations on the mobility and behaviour of nutrients like phosphorous (NAGPAL 1986) or pesticide fate under the influence of nutrients (SMITH & WILLIS 1985) were carried out infrequently. The set-up of model systems for the investigation of the fate and behaviour of contaminants was partly terrestrial, partly the TME were containing an additional aquatic compartment to simulate the runoff and drainage of water into reservoirs or into sediments. These approaches were maintained under laboratory

conditions, where the temperature, light-dark regime and irrigation could be controlled. Using radiolabelled compounds, these studies showed that the mass balance was almost closed and that chemical concentrations can be measured exactly in each of the compartments chosen. However, the use of TME over a long period seemed to hold the risk of unpredictable changes of soil properties, as O´CONNOR *et al.* 1980 observed after several irrigations. Some authors thought that the results obtained in the laboratory test systems should be confirmed in corresponding field studies to check their reliability. Indeed, this evaluation in corresponding field studies sometimes took place (WILSON *et al.* 1987) or results were compared to results derived from closed systems (LICHTENSTEIN *et al.* 1982, 1983; for further details refer to chapter I-3.8). The impact of chemicals to soil communities or functions shifted into the focus of researchers little later than fate and behaviour of pesticides. Different herbicides were applied (MALANCHUK & JOICE 1983, PARKER *et al.* 1985), as well as insecticides (i.a. SEASTEDT & CROSSLEY 1983) or fungicides (i.a. FÖRSTER *et al.* 1996, BURROWS & EDWARDS 2004). The use of combinations of two or more chemicals was invented to test mixture toxicity. VINK & VAN STRAALEN (1999) interrupted both the microflora activity and the arthropod feeding by the combined application of the fungicide benomyl and the insecticide diazinon. PARMELEE *et al.* 1993 applied copper as well as the explosives p-nitrophenol and trinitrotoluol to TME. They aimed to investigate the effects on soil fauna communities. They counted the total numbers of nematodes, which increased at medium concentration levels. This was due to a change in community structure; the predators within nematodes and microarthropods were more sensitive than their prey. They concluded that patterns of indirect toxicant effects could only emerge in complex test systems. Further different environmental toxicants emitted by industrial processes (heavy metals and hydrocarbons in PARMELEE *et al.* 1997) were examined in TME. The latter authors suggested that nematodes and microarthropods were both mandatory to investigate while testing the impact of chemicals on complex soil communities, due to their strongly differing sensitivity profiles. A number of publications dealt with functional or structural items not directly related to the fate or effects of chemicals. MALANCHUK *et al.* (1980) investigated the influence of micro-nutrient supply on plant growth. They looked at the potential beneficial effects of fly-ash amendment as a fertilizer on the nutrient uptake by plants and the nutrient losses through leaching in agricultural soils. LARKIN & KELLY (1988) investigated the influence of enhanced concentrations of sulphur on the respiration rate of two forest soils; one with background sulphur concentration, another with sulphur concentrations elevated for a period of 25 years. They found minimum threshold values for enhanced respiration; above the threshold the degradation performance decreased. Low and medium additions of nutrients

had no effect on the decomposition rates of the soils. ELLIOTT *et al.* (1980) examined the impact of the soil fauna on decomposition processes, precisely the trophic interrelationships within the microfauna and its correlation to soil structure. It turned out that a complex food web, containing amongst others bacteria, nematodes and amoebae, was superior to simple food chains in terms of respiration rates and nutrient mobilisation, especially in fine textured soils. Amoeba transported the nutrients from inaccessible pores to the nematodes, emerging as a food resource to them. NAEEM *et al.* 1995 researched the influence of soil fauna diversity on the performance of terrestrial ecosystems. HAMILTON & DINDAL 1989 worked on ecological interactions in TME. They discovered that activities of large earthworms (mainly represented by the species *Lumbricus terrestris*) positively affected the aggregate structure of a soil and its organic matter content. However, the interaction with another earthworm species (*Eisenia fetida*) veiled these effects and decreased the survival rate of *L. terrestris* significantly. HUHTAA *et al.* (1998a) reviewed many experiments dealing with the influence of soil fauna diversity on the ecosystem function; particularly on mineralization rates had been focused. It could be concluded from the reviewed ecological studies that the influence of the soil macrofauna on the soil structure and conditions is evident. However, the contribution of the soil microfauna to soil appeared to be low and unpredictable. Enhanced mineralization rates were observed in cases of raising 'trophic group diversity' raised. The influence of increased nutrient supply on respiration rates of soil microorganisms was rather low down to not detectable. Most recently, an approach called 'small-scale terrestrial ecosystem' (STEM, SANTOS *et al.* 2011) had been used to test the mixture toxicity of the acaricide spirodiclofen and the insecticide dimethoate on non-target plants (*Brassica rapa*) and on earthworms (*Eisenia fetida*). They found some antagonistic effects on endpoints in both species at recommended field rates.

I-3.7 Approaches using open, intact TME

The studies using this category of a TME approach focused on the fate and behaviour of chemicals. The leaching and distribution of nitrogen and nickel in nickel-amended TME was investigated by DE CATANZARO & HUTCHINSON (1985), KNACKER *et al.* (1989) analysed the residues of lindane and pentachlorophenol in plants, soil and leachates, and FERMANICH *et al.* (1991) measured the mobility of bromide, carbofuran and chlorpyrifos. All of these authors considered their systems appropriate to determine the fate of the chemicals regarded. The systems were assumed to reflect the field situation appropriately, especially when the TME were kept under greenhouse conditions, simulating the temperature and water regimes of natural

climatic conditions. The influence of environmental biotic and abiotic parameters on the behaviour of nutrients was examined by BULDGEN & REMACLE 1988 and CRONAN 1980 in open systems, consisting of intact soil cores. As an outcome, BULDGEN & REMACLE observed that the temperature, the quality of rain and the type of humus were the predominant factors acting on the leaching of nutrients from forest soils. With regard to the complexity of the systems and the time-varying biotransformation, processes of the humus layer had to be taken into account as well. CRONAN found similar interactions between the environmental factors. However, he advised to bear in mind that there were predictable differences between TME and the field situation, especially in the case of observing the leaching of anions, which was strongly controlled by plant uptake. TOLLE *et al.* 1990 tested, on behalf of the US military, two smoke aerosols that were deployed to hide troops while transporting them to another theatre of war. Apart from moralistic doubts on the use of such materials, they found no negative effects, neither on plants considering the biomass yield, nor on trace element uptake, nor on nutrient cycling at concentrations near the predicted battlefield level. Some studies tried to use TME to investigate the effect of chemicals or other kinds of environmental stressors on different soil biota. HAMILTON *et al.* (1988) dealt with two earthworm species in sewage sludge amended TME with regard to their decomposition ability. WRIGHT & COLEMAN (1988) observed the effect of fertilization in combination with different treatment combinations of three biocides (captane, carbofurane, naphthalene) on soil biota and plant nutrition. Some authors described experiments with genetically modified microorganisms released in the 'model environment' (FREDRICKSON *et al.* 1989, ANGLE *et al.* 1995, GAGLIARDI *et al.* 2001). The former authors found that mutant *Pseudomonas spec*., which no longer produces root-growth inhibiting toxicants, could survive in the rhizosphere for a long period. Coincidentally, they were dispersed in the leachate and in the gut of earthworms. Both the two latter publications described very similar results. They recommend the use of TME prior to field-testing. The most recent, complex and demanding study using open, intact TME was a ring test conducted by using different soils of four countries in Europe. This large project funded by the European Union, was developed to support the progress in standardizing terrestrial model ecosystems. It was published in a special issue of the journal 'Ecotoxicology' in the year 2004. The results of this study are of special interest in the recent context of this thesis, so the following description is somewhat more detailed. KNACKER *et al.* (2004) presented the conceptual approach, the test design and the summarized results in an introductory paper. After two years of method development, during which suitable test sites were identified and range-finding tests of exposure concentrations were conducted, a ring test and a parallel field validation study were per-

formed. At four different sites in Germany, The Netherlands, Portugal and The United Kingdom TME were cored and the results were validated in field studies. A wide range of structural and functional endpoints was measured. The consortium used the fungicide carbendazim (in an EC formulation Derosal) in a dose-response design to determine effect levels. Various endpoints as the fate and behaviour of the model chemical, the number and diversity of microarthropods, nematodes, enchytraeids, earthworms and microbes, and the effects on nutrient cycling were investigated. It could be shown that statistically evident results were very difficult to gather, due to the very high variability of numbers, especially in the case of arthropod abundances. The fate of the model chemical in plants (roots and shoots), soil and leachate was also investigated (JONES *et al.* 2004). The TME were concluded as useful to predict the fate of a chemical in the environment. Further, the results could be successfully extrapolated to the field situation. The recovery after treatment with carbendazim was higher in TME than in the corresponding field study. The impact on soil microbial parameters was observed by measuring substrate-induced respiration (SIR), dehydrogenase activity (DHA), thymidine incorporation (TI) and phosphatase activity (PA) to detect differences between the treatments of TME on the one hand, and the field studies with the different soils on the other. Results showed that the effects in both studies were comparable, even though they were weak and occurred at the highest applied dosages exclusively (SOUSA *et al.* 2004). A lot of work was done on the assessment of effects on functional and structural organismic endpoints. Investigations were made concerning the abundance, trophic structure, the number of families and the maturity index (MI, as a measure of disturbance) of nematode communities. For this group of organisms, the sampling took place 16 weeks after application of the pesticide at the point of the termination of the experiment (MOSER *et al.* 2004A). For the calculation of the MI, nematode families were ranked on a five-part scale from early colonisers (equivalent to r-strategists) to stable stage persisters (equivalent to K-strategists). Low values of the MI indicate a high degree or frequency of disturbances while high values were indicating a relatively stable environment. The MI decreased with increasing treatment level, but differences were significant at the highest treatment only. Due to a rather high variation of all endpoints for the group of nematodes, the NOEC values were higher than the highest treatment level and could therefore not be calculated. The most sensitive endpoint was the relative abundance of omnivores, while the overall abundance of nematodes was not affected. For this group, the EC_{50}-value was slightly higher, but in the range of laboratory single species tests. The treatment related shifts in species composition of microarthropod communities were determined by extracting collembolans and soil mites. For those two groups of organisms only few less significant ef-

fects regarding the endpoints considered as the overall abundance, Shannon's diversity index and the community structure, as indicated by Principal Response Curves (PRC) were seen. Results of TME-tests and field studies were comparable, so the authors concluded that TME would be suitable to replace field studies (KOOLHAAS *et al.* 2004). They guess that effects were masked by the very large variation in abundance; however, they assumed that some species like *Isotoma viridis* would be more sensitive than others. On the other hand, some deep-living species were promoted; this was because of the test substance was bounded to the top soil layer. Another important group of the soil mesofauna were considered enchytraeids. They reacted in a similar way like the soil microarthropods to the model chemical, showing a very high variation of abundance. For this reason, it was often not possible to calculate EC_{50} values; NOEC values ranged from the lowest to the highest treatment level. Only in the highest concentrations, significant differences of enchytraeid numbers were observed. One genus (*Fridericia*) reacted very sensitive to the application of carbendazim. In terms of the effects on enchytraeids, the system was considered as suitable to reflect the field situation; the authors concluded from the study at hand and from earlier studies that in the field significant differences of abundance could be explained by normal seasonal population changes (MOSER *et al.* 2004B). Because of low population densities in TME, effects on earthworms were generally weak. However, some species seemed to be more sensitive towards carbendazim than others (especially *Lumbricus terrestris* and *Lumbricus rubellus*). No NOEC values could be determined for most of the endpoints for statistical reasons, but EC_{50}-values indicated similar effect levels independently of the soil. Both abundance and biomass of the earthworms decreased constantly from lowest to highest treatment level and were occasionally significantly different from the control level (RÖMBKE *et al.* 2004). Beside the numerous structural endpoints, functional endpoints were measured as sum parameters of the activities of soil fauna and microflora. The effects of the model chemical on nutrient cycling (sulphate, phosphate, nitrate, ammonium concentrations), pH and soil moisture content were measured by VAN GESTEL *et al.* 2004. In this study, the breakdown of organic matter by using cellulose filter papers and the feeding activity by means of the bait-lamina test after VON TÖRNE (1990) was determined. No significant effects could be detected for the abiotic endpoints (nutrients concentrations), indicating not to be sensitive endpoints. For this reason, some of them were excluded from the measurements after pre-testing, (FÖRSTER *et al.* 2004). Both the organic matter breakdown and the feeding activity decreased with increasing chemical treatment and showed a dose-response relationship. Bait-lamina consumption appeared to be more variable than cellulose paper decomposition. Some correlations between the two functional endpoints

and the abundance of earthworms and enchytraeids were found, whereas the decomposition was known to be highly dependent on soil moisture content. The residual part of decrease could be explained neither by the soil fauna´s feeding activity nor by the microbial activity, which were described in the other papers of this special issue (SOUSA *et al.* 2004). All tests and endpoints of the ring-test indicated a good comparability of field and TME measurements, so the system was assumed suitable to replace or complement field tests. However, this was a standardized, stereotype statement at the end of each of the nine papers of this special issue, it remains sometimes unclear to what degree TME and field results were conducted or analysed in parallel. To sum up, almost all the authors using open, intact TME concluded that their approach was useful for answering their special questions, as the fate and degradation of the model substance, and the effects on various groups of organisms or on ecosystem level. It was often expressed that there is further demand for refining the systems, to improve constructions or measurements, and to find better endpoints to evaluate the given questions.

I-3.8 Combinations of TME approaches

The fate of chemicals was examined in combinations of open and closed TME with homogenous soil (LICHTENSTEIN *et al.* 1982, LICHTENSTEIN *et al.* 1983). Some of the combined approaches represented very extensive studies, funded by national authorities. HEISE *et al.* (1988) were instructed by the Federal Biological Office of Germany, the work of MIETH *et al.* (1993) was funded by the German Federal Environment Agency, dealing with combinations of closed, disturbed and intact soil columns. It became obvious that these studies included a great variety of ecotoxicological, microclimatic, chemical, biological and ecological endpoints; the combination of several different approaches should serve as an estimate of the representativeness of different environmental conditions, vegetation units or soil properties. EDWARDS *et al.* (1998) described very similar systems containing sieved soil and reduced fauna and flora, as well as systems consisting of intact soil cores with diverse flora and fauna to assess the impact of chemicals more realistically. They proposed a battery of different functional and structural measurements and concluded the systems as suitable for use in a tiered approach to assess the risk of new pesticides. WEYERS & SCHUPHAN (1998) examined systems that were especially designed to calculate the variability of endpoints expressed as the coefficient of variance in artificially composed closed and open model ‘ecosystems’ equipped with an artificially introduced combination of grass species, arthropods and microorganisms. They concluded that the apparently high variation could be lowered with more experience of the experimenters and that effect levels of 10-20 % could be observed in such systems. There was

less attention on field studies in this survey but some of the model ecosystem studies were accompanied by field studies or the results were compared to field studies. Some authors that used combinations of different TME approaches paid special attention to the question whether TME could predict the field or semi-field situation. Others only wanted to measure the degradation products which occurred to less extent in the laboratory than in the field (e.g. by photolytic reactions that could not occur in glass chambers that filter the UV-light, compare NASH & BEALL 1980b). JACKSON & LEVIN (1979), JACKSON *et al.* (1979), TOLLE *et al.* (1983) or KLOSKOWSKY *et al.* (1981) calibrated the results of experiments with open or closed TME by a corresponding field study. They concluded that TME could represent the field situation appropriately and would offer several of the following advantages compared to field studies. That is the contaminated soil could be controlled and separated, and therefore pollution of the environment could be avoided. The mass balance of the test item could be calculated more precisely. Relatively small systems would allow for a centralized testing of several ecosystems and would be cost-effective. Unfortunately, it seemed not to be possible to predict the long-term behaviour of chemicals. The pollutants were ranked in terms of the intensity of observed effects. A combination of open TME with sieved soil and intact soil cores compared to field studies was used to determine the survival of artificially released microorganisms in the environment. Intact soil cores provided better conditions for the survival of *Pseudomonas aureofaciens* than TME consisting of sieved and dried soil. The former systems maintained stable populations of microorganisms for up to three months use of intact TME (ANGLE *et al.* 1995). In the opinion of ANGLE *et al.*, the intact TME may reflect the field situation sufficiently (these finding was later confirmed by further experiments of GAGLIARDI *et al.* (2001) with another species *Pseudomonas chlororaphis*. The extrapolation to the field situation may be restricted to studies on chemical fate, to single species systems or to very realistic approaches. However, often the soil structure was disturbed, the soil was maintained under laboratory conditions, and was therefore not comparable to normal outdoor circumstances, or there was only a small, isolated part of natural communities introduced in the experiments. This could lead to overemphasize the role of a few, less representative species or one could disregard the complex mutual interrelationships between biotic and abiotic factors. LAAKSO *et al.* (1995) conducted several 'ecological' experiments on the topic 'community structuring factors'. Some of the above authors had to put their former results into perspective while repeating the experiments including more endpoints or more advanced set-ups and obtaining inconsistencies. For example, TOLLE *et al.* 1983 found their TME very predictive in terms of trace element uptake by plants, but when including the comparison of dose-effect relationships (TOLLE

et al. 1985), they detected only a poor accuracy in predicting plant responses in the field. However, they concluded that corrections on the experimental design would be necessary. WILSON *et al.* (1987) obtained results that overestimated the biotransformation of bromoform and carbon tetrachloride while the behaviour of tetrachloroethylene and hexachloroethane was simulated accurately using their TME approach. Following the authors conclusions, this was due to natural variability and poor replication. As mentioned by many authors, the natural variation of biological results has to be taken into account if one aims to conduct well-controlled and reproducible experiments.

I-3.9 Mesocosms – outdoor enclosures

In contrast to TME, that are meant to be soil cores usually transported to and stored in an experimental environment after coring, mesocosms were defined as enclosed outdoor systems partially permeable to their surroundings, according to ODUM (1984). They should contain the full complexity of biotic and abiotic components of natural systems and provide replicable units. They are invented to overcome the simplicity of many small-scaled TME (in other words 'microcosms'), based on the prerequisite that realism is seen as a crucial feature of mesocosms (KAMPICHLER *et al.* 1999). For the purposes of ecotoxicological test systems, mesocosms were designed to validate the predictions from standard laboratory tests in a semi-field situation (SVENDSEN & WEEKS 1997). The category 'mesocosm' was subdivided into two classes of mesocosm studies, which were frequently found in the literature. On the one hand, a more realistic approach dealt with intact soil mainly replanted undisturbed after manipulation directly at the study site. It accepted a relatively high natural variability of soil conditions and an inhomogeneous distribution pattern of the soil fauna. Some experiments were conducted with homogenized soil; this feature defines here the second class of mesocosms. In the following, the setup, objectives of research and some results of exemplified studies are presented.

I-3.9.1 Homogenised mesocosms

The first division of this category was marked by a relative homogenous quality of the soil. Homogenous conditions were introduced by sieving (and occasionally defaunating through deep freezing) of the soil before the start of the experiments. SMIT *et al.* (2002) cross-inoculated their mesocosms additionally with living nematodes from fresh soil to further improve the homogeneity of the distribution of organisms between the mesocosm-experimental units. They found a high persistence of total numbers after application of zinc while analysing various community related parameters and the community as a whole (analysed by *Principal*

Response Curves) was the most sensitive endpoint. GREVILLE & MORGAN (1991) conducted another experiment concerning the effects of metals on various biotas. They transplanted unloaded slug species to a heavily polluted, former Pb/Zn mine site and compared the bioaccumulation of heavy metals with residential slugs. It became obvious that the adapted slugs built up a metal tolerance or a phenotypically reduction of accumulation, which could lead to misinterpretations of monitoring measurements. Experimenters using mesocosms asked often non-ecotoxicological questions. TEUBEN (1991) examined the functional roles of the fungivorous collembolan *Tomocerus minor* and the detritivorous isopod *Philoscia muscorum* during the decomposition of *Pinus nigra* needles. They concluded a new mechanism from their study: A buffering effect on the microbial activity of soil was attributed to both species. In cases where the control abundances of the soil fauna were high, the microbial activities and decomposition rates decreased and vice versa.

I-3.9.2 Intact mesocosms

In the previous chapter, semi-field enclosures with sieved soil were focused. Historically, much more effort was put into the development of systems containing intact soil cores that were replanted to the field. KAMPICHLER and his working group at the Federal Forest Office in Austria conducted many of the studies that are available in the literature. The most important aspects of theirs and other approaches and the related results are presented in the following. FRAMPTON & WRATTEN (2000) applied systemic fungicides (propiconazole, carbendazim and triadimenol) on open and barrier-enclosed wheat-crop plots. They found no effects on the activity of surface-dwelling collembolans, but negative effects were found on the overall abundance, using two different sampling methods (pitfall traps and suction sampling). The ecology of soil organisms was investigated by NIEMINEN & SETÄLÄ (1998). They concluded by the results of their experiments with enclosed and open natural ecosystems, which they compared with the field situation that the diversity of microarthropods was not affected by reducing the space for a single growing season. However, in a long term, the decomposer food web structure would be changed. BRUCKNER *et al.* (1993) first described the above-cited construction of mesocosms. They examined the re-immigration of microarthropods in previously defaunated soil cores, exposed under natural outdoor conditions. In further experiments, KAMPICHLER *et al.* (1995), BRUCKNER *et al.* (1995) and KAMPICHLER *et al.* (1999) evaluated the side effects occurring in intact soil monoliths in the field by treatments of deep-freezing, wrapping in nets of different mesh-sizes and replanting the soil cores into the field. Most groups of the soil fauna investigated (ciliates, collembolans, tardigrades, nematodes) were not affected by the treatments, but oribatid mites were reduced by 30 % of

the control abundance, six month after incubation. In order to overcome the low recovery potential of oribatid mites, the authors recommended an artificially reintroduction of this group into the defaunated soil monoliths. ZECHMEISTER-BOLTENSTERN *et al.* (1998) used an identical approach as KAMPICHLER to investigate the effects of meso- and macrofauna on the nutrient availability in forest soils. They inoculated fauna of different complexity into defaunated soil and detected an increase of exchangeable base cations and a decrease of exchangeable acidic cations with increasing complexity of fauna. They explained these effects with an indirect enhancement of humification processes in the presence of fauna, causing a rise of ion-binding sites in the soil. KANDELER *et al.* (1999), coming from the same group of soil ecologists, supplemented these findings by detecting an enhancing effect of the mesofauna on the microbial activity. This result was in contrast to the findings of SOUSA *et al.* (2004) who could not explain statistically the changes of microbial activity by the feeding activity of the soil fauna.

I-4 Ecological traits of soil organisms and the structure of soil ecosystems

Communities of soil invertebrates are particularly important to maintain ecosystem functions of all types of temperate biotopes. Collembolans and oribatids as well as nematodes, annelids and microbes are mainly involved in the decomposition of organic matter. The former enhance the primary production by exerting grazing pressure on soil micro-flora and in maintaining soils microstructure (e.g. RUSEK 1998; COLEMAN 2008) assuring soil fertility over long periods. They interact in complex food webs (SCHEU 2002). Most abundant organism groups in soil important for the maintenance of ecosystem functions belong to the annelid order Nematoda, to the oligochaete family of Enchytraeidae, and to the Collembola and Oribatida, two orders of the phylum Arthropoda (BECKER 1991). Dominance and species structure of soil communities are widely accepted to be site-specific (TOSCHKI 2008, RÖMBKE *et al.* 2012). Diverse soil communities are agreed to be crucial for the maintenance of beneficial processes such as soil fertility or coherence (NEHER 1999). Long-lasting alterations in community structures and diversity indicate disturbances (e.g. through climate, chemicals, invasive species, fertilizers) that can lead to irreversible and adverse shifts of ecosystem functions (SCHEFFER *et al.* 2001; KRYAZHIMSKII & BOLSHAKOV 2008). Furthermore, community related endpoints integrate indirect effects e.g. food web relations. Diverse communities are expected

to be more resistant against disturbances caused by e.g. removing soil during sub-sampling procedures in untreated model ecosystems and are thus assumed to be stable over time (SCHNEIDER *et al.* 2007).

Table I-1: Size range and abundance per area for the most dominant soil taxa of meso- and macrofauna. (SRU 1985)

Size range [mm]	Taxonomic group	Mean abundance [m²]
0.1-1	Rotatoria	10.000-25.000
0.2-2	Nematoda	1.000.000-3.000.000
0.2-3	Acari	70.000-100.000
0.4-3	Collembola	50.000-100.000
1-40	Enchytraeidae	30.000-100.000
1-80	Diptera (larvae)	100-300
1-80	Coleoptera (larvae)	300-800
2-80	Gastropoda	50
3-80	Diplopoda	50
3-80	Chilopoda	30
20-150	Lumbricidae	100-450

The soils of temperate woods and grasslands host many highly specialised organisms. The diversity of soil ecosystems is greatly astonishing, so that USHER *et al.* (1982) named it 'a poor man's rainforest'. The investigation at hand focuses on the most abundant taxa of soil organisms: The biocoenoses under investigation comprises the groups of collembolans, oribatid mites, enchytraeids and nematodes. This is mainly for reasons of practicability; representativeness and statistical evaluation because those are known to be the most abundant groups of soil organisms (see Table I-1). They realise a number of key functions as decomposers, herbivores, algivores, fungivores and predators in soil (DUNGER & FIEDLER 1997). They represent different modes of reproduction (sexual reproducers as well as parthenogenetic species), they accomplish a variety of locomotory and mobility behaviours, and they belong to different taxonomical groups. Communities of soil organisms such as lumbricids, enchytraeids, collembolans, nematodes, fungi and oribatids are considered important to maintain numerous ecosystem services. They are the main driving forces for the decomposition of organic matter and thus providing the cycle of nutrients (MULDER *et al.* 2011). They interact in complex food webs (SCHEU 2002) and provide great biodiversity, which is often seen as an ecosystem service itself. Diverse soil communities are agreed to be crucial for the maintenance of beneficial processes such as soil fertility or coherence (NEHER 1999). TME-approaches offer the opportunity to investigate direct effects of contaminations as well as long lasting, indirect or sublethal effects on parts or the complete complex food web structure.

I-4.1 Variability in soil

The variation of biotic and abiotic variables in soil is commonly and amongst soil ecologists perceived as extraordinary high. It is further widely recognized but scarcely investigated that the variability of soil organism counts depends grossly on the spatial scale that is considered

relevant and on the spatial habitat heterogeneity (ETTEMA & WARDLE 2002). In this thesis as in the before mentioned comprehensive review different definitions of variability have been used. The term variability means the change of a given properties such as population abundances and has been mainly used to describe the limits of pesticide effect detection (chapter V-3). The terms heterogeneity, patchiness and autocorrelation refer to the geostatistical description of soil communities, which is here investigated by chapter V-1. It was found by semi-variographical methods (method see chapter II-4.7) that the spatial heterogeneity of e.g. collembolans was maximized in horizontal direction on a scale between 1 and a few meters (RUSSELL & BLÜMEL 2003). The horizontal and vertical variability in food web and community structures in soil could be widened to temporal components of variation, such as seasonal dynamics. The taxonomic resolution of a study highly affects the characteristics of the variability. In this respect, the functional group composition is much more stable over time than the abundance of single species (BERG & BENGTSSON 2007, refer to sections V-2.1, V-2.2, V-2.3). Very often in soil, the species diversity increases with increasing microhabitat diversity and so does the variability of the soil communities (shown for oribatids by HANSEN & COLEMAN 1998).

Generally in ecotoxicology and in particular in connection with the topics of this thesis, highly variable effect data could hamper the detection limits, as it blurs the knowledge of ecologists. In the following, it is taken as a guiding principle of the investigations at hand that the variability in soil is not extraordinarily far outside the typical variation of other aquatic or terrestrial habitats.

I-4.2 Biology of soil organisms

I-4.2.1 Collembolans

HOPKIN contributed a comprehensive and detailed description of the biology of springtails. If not denoted aberrantly, all information on the biology of springtails could be found in his standard textbook (HOPKIN 1997).

Taxonomy and systematics

The precise position of springtails within the taxonomic classification changed grossly over time and the taxonomists subject the topic to discussions until today (ANDRÉ *et al.* 1994). Formerly belonging to the class of insects, they have been currently regarded as a separate class of the phylum Arthropoda. More than 6500 species have been already described worldwide. In Germany, 416 species have been recorded (SCHULZ *et al.* 2003). Biogeographical patterns of the local taxonomic diversity have been recognized poorly described and cannot be

concluded yet (RUSEK 1998). Species richness has been highest at places providing many niches. In temperate woods up to 60 species could be found; grasslands usually appeared to be less diverse (RÖMBKE *et al.* 2012).

Occurrence and distribution

Springtails are amongst the most abundant taxa in terrestrial habitats. They occur in virtually all climates. Densities reach 60.000 individuals per square-meter. On average, they occur in densities between 1.000 and 10.000 specimens per square meter, which is rather constant within one order of magnitude. Collembolans tend to exhibit mass reproduction events in case of favourable moisture and temperature conditions. In particular, after those events, their small-scale distribution is highly aggregated; therefore, counts of springtails use to be very variable.

Ecology and ecotoxicology

Despite the comparably small portion that collembolans contribute to the total biomass in soil, they play important roles in the decomposer food web (compare chapter I-4.3) and in nutrient cycling processes. This contribution is mainly attributed to the consumption of fungal hyphens and soil borne algae. However, collembolans are named food generalists, their diet contains of a mixture of detritus, fungi, algae and bacteria. Grazing is known to stimulate the growth of mycorrhizae (under certain circumstances); parasitic fungi can be significantly reduced by collembolans. The structure of collembolan communities is shaped by a high dominance of few species while most species are rare (contributing less than 1 % of the total abundance). Springtails are generally susceptible to chemicals, as for metals and pesticides, but appeared to be less sensitive than other groups of soil organisms in single species laboratory tests (FRAMPTON *et al.* 2006, JÄNSCH *et al.* 2006). One test with the isotomid species *Folsomia candida* was internationally standardized and stipulated to be applied for the risk assessment of plant protection products, if triggered by intrinsic properties of the test substance or by preceding tests as stipulated by the Terrestrial Guidance Document. The use of *F. candida* was based on ISO 11267 (ISO 2001) and was comprehensively reviewed by FOUNTAIN & HOPKIN 2005. Laboratory studies test for direct toxic effects; in field studies, indirect effects resulting from disturbed predator-prey relationships or competition occur. FILSER (1994) found an example of an indirect effect as an association between the use of a fungicide, the reduction of the hyphal biomass and the abundance of fungal feeding collembolans.

I-4.2.2 Oribatids

Taxonomy and systematics

The class Arachnida comprises the sub-order Oribatida (superorder Acariformes). The taxon-

omy is very complex because of many polyphyletic lineages and was revised intensively in the last decades. Recently, WEIGMANN (2006) revised the oribatids of Germany and integrated them into the paraphyletic (i.e. not all derivatives of the principal form could be included into the cladistic group) group of Prostigmata. Oribatids were then considered as a very basal taxonomic group; oldest fossils were known from devonic sediments of 380 million years of age. Worldwide, approximately 9.000 species were described; conservative extrapolations suggest numbers of up to 50.000 (maybe 100.000) species in total (MARAUN *et al.* 2004). In Germany, about 520 species are known (WEIGMANN 2006), in Mid Europe about 800-1000 species could be expected (RÖMBKE *et al.* 1997).

Occurrence and distribution patterns

Oribatids are similarly ubiquitous in soil and widespread distributed over all habitats as the collembolans. They live mainly in the uppermost organic layers of a soil at all altitudes and latitudes but also enter deeper inorganic layers (ANDRÉ *et al.* 1994). Most abundant and diverse habitats are coniferous woods, followed by deciduous forests and moors (density up to 400 thousands/m^2, about 50-80 species) (MARAUN & SCHEU 2000). Grassland and arable land are much less species rich and comprise significantly lower numbers as the former habitats (10-40 thousand individuals per square-meter, 20-30 species). RÖMBKE *et al.* (1997) stated that no direct correlation between the occurrence of oribatids and the type of vegetation as the only factor could be found. However, the thickness of litter and humus layer, the soil moisture or anthropogenic factors was assumed crucial parameters in triggering the distribution and species spectra of soil mites. TOSCHKI (2008) proved for Germany that the oribatid distribution depends beside the before-mentioned factors also on the geographical position and thus on climatic and other large-scaled factors.

Ecology and ecotoxicology

The feeding type of oribatids is phytophagous; some exceptional species feed on carrion or even hunt for prey (RÖMBKE *et al.* 1997). The population biology of oribatids is marked by the longevity of adults (often more than one year). Consequently, thereof, oribatids show a relative low fecundity, high densities and stable community structures. They are commonly described as K-strategists with slow life cycles and low metabolic rates. A surprisingly high portion of species reproduces obligatorily or in cases of need parthenogenetic. For ecotoxicological studies, the possibility of changing between reproductive modes has to be taken into account, as well as a 'no-effect' answer because of reduced male fertility that was masked by fast parthenogenetic reproduction (LEBRUN & VAN STRAALEN 1995). Oribatids show a great diversity of life cycle traits as well as morphological and ecological adaptations to the condi-

tions found in soil. The knowledge on the sensitivity of oribatids in ecotoxicological laboratory studies is limited. A few trials were executed with *Plathynothrus peltifer* exposed to heavy metals. These experiments indicated that oribatids were able to accumulate cadmium as described by LEBRUN & VAN STRAALEN (1995). For the effects of insecticides on different oribatid species, no studies on field or lab scale were at hand. LEBRUN & VAN STRAALEN further stated that due to their sedentary life style and direct exposure to contaminants oribatids were expected to react particularly sensitive towards insecticides.

I-4.2.3 Enchytraeids

Taxonomy and systematics

Worms of the family Enchytraeidae belong to the phylum Annelida and the class Oligochaetes, which are the closest relatives of lumbricids. NIELSEN & CHRISTENSEN described in 1959 112 European species of enchytraeids, classified into 16 genera. Since then, many new species have been described or will likely be described in the future; for example SCHMELZ revised the genus *Fridericia* in 2003. Between 200 and 300 species were expected for Central Europe (RÖMBKE *et al.* 1997), 50-100 species are known from agricultural ecosytems in Central Europe (RÖMBKE *et al.* 2012). Worldwide 600 species were described.

Occurrence and distribution patterns

Enchytraeids exhibit a clustered spatial distribution and a high temporal variation in abundance, like most of soil inhabiting organisms (CHALUPSKY & LEPS 1985). They were found on all continents and in all possible habitats but data were collected mainly occasionally during faunistic studies. Temporal consistency and habitat relatedness was often lacking (DIDDEN 1993). Population sizes underlie large variations between and within habitats. Moors have on average the most abundant populations, followed by deciduous and coniferous forests of temperate regions. On German grassland, comparably low numbers between 2700 and 40000 individuals per square-meter have been reported (compiled by DIDDEN 1993). The lowest numbers were observed on arable land, indicating a definite dependency of enchytraeids on intact humus layers. As for the two latter groups, a patchy distribution applies for enchytraeids as well. It is very likely to be due to microclimatic heterogeneities. Estimates available in the literature of small-scale distribution of single species presume a cluster size of 100-1000 cm^2 (DIDDEN 1993).

Ecology and ecotoxicology

Similar to lumbricids, enchytraeids are described as saprophytic feeders and of great importance in forming humic and fulvic acids in soil, giving major contribution to soil formation processes. Although providing less biomass than lumbricids, they excel them in the realloca-

tion of organic materials through respiration. As all oligochaetes, enchytraeids are hermaphrodites, while most species reproduce sexually (MOSER 2004). CHRISTENSEN (1959) described asexual reproduction through fragmentation or parthenogenesis. Relatively much information is available on the reaction of enchytraeids towards chemical stress. Enchytraeid laboratory testing looks back to a comparably long history of standardisation and harmonisation. Recently, ISO, ASTM and OECD guidelines for standard tests with *Enchytraeus albidus* in artificial soils have been developed (in JÄNSCH *et al.* 2005). Several species of enchytraeids were sensitive towards a variety of chemicals and pesticides of different modes of action, strongly related to the bioavailability of the substances evolving of the chemical properties of both the chemical and the test substrate (DIDDEN & RÖMBKE 2001). Compared to other groups of soil animals, enchytraeids (generally speaking oligochaetes) were less sensitive in laboratory tests than arthropods (FRAMPTON *et al.* 2006).

I-4.2.4 Nematodes

Taxonomy and systematics

Nematoda (roundworms) represent an own phylum within the animal kingdom. Thus, it is a very species rich group. The position in the taxonomic hierarchy of Nematodes was subject of long controversies. Nowadays, they are ranked in the superphylum Ecdysozoa, together with e.g. the group of Rotatoria (BURDA *et al.* 2008).

Occurrence and distribution patterns

Nematodes have been colonizing almost all terrestrial and aquatic habitats, often in very high numbers and densities (STORCH & WELSCH 2002). It has to be distinguished between free-living and parasitic forms. In soil ecotoxicology and in this thesis, one refers to the free-living species only. The free-living species of terrestrial nematodes usually occur in densities of several thousand per square-meter, and extreme values reach up to some billions of individuals on a single square-meter on mineral soils of grassland. In our studies, the absolute maxima were about 1000 individuals per sample of 1.7 cm in diameter, which equals densities up to 4.4 Mio. individuals/m². On agricultural soils, the highest densities of several typical species were recorded for no-tillage ridges, the lowest for spring and fall ploughed systems (THOMAS 1978).

Ecology and ecotoxicology

WARWICK (1975) classified the feeding habits of terrestrial and aquatic Nematodes according to Yeates by their main food source and the method of feeding. The food source could be roughly distinguished into bacteria, fungi, algae and higher plants feeders, as well as predators that *ingest the whole* prey or *pierce and suck* on dead and living animals, which describes the

main methods of ingestion of the whole group (YEATES 2003). It is a very difficult task to determine the group to the taxonomic level of species. For the analysis of the results of ecotoxicological studies often the maturity index is used to describe the current status of disturbance of the community. It ranks the nematodes on a 5-parts scale between pure colonizers (*r-strategists s.l.)* and persisters (*K-strategists s.l.)* (BONGERS 1990).

I-4.2.5 Fungi

Taxonomy and systematics

Worldwide about 100.000 species of fungi have been currently described. Projections of the total species diversity assume that more than 1 million species exist (HAWKSWORTH 2004). Particularly about soil fungi, little is known. The detection of the species diversity of soil fungi is hampered by basic methodological problems. To begin with the morphological description, the fungi have to be cultured under selective conditions that prefer fast growing generalists rather than specialised species. It is not possible for more than 17 % of the species to bring them in culture under laboratory conditions (HAWKSWORTH 1991). Nowadays, the taxonomic group of fungi is defined as the 'higher fungi', such as Ascomycetes ('sac fungi'), Basidiomyetes ('mushrooms') and Deuteromycetes ('fungi imperfecti') (SCHLEGEL 1992). The group of the lower fungi, such as Myxomycetes, Acrasiomycetes and Phycomycetes are no longer included in the kingdom of fungi.

Occurrence and distribution patterns

Fungi provide the largest portion to the total biomass of soil micro-organisms (ANDERSON & DOMSCH 1975). Spores of bacteria and fungi are ubiquitous and colonised dead organic materials very fast (r-strategists). The so-called 'sugar fungi' and bacteria compete intensively for easily accessible carbon hydrates. The competitive advantage of fungal species becomes evident in the second phase of decomposition when indigestive components like lignin ('white rot' fungi) and cellulose ('blight' fungi) are available (BEGON *et al.* 1998).

Ecology and ecotoxicology

Fungi fulfil essential functions in the recirculation processes of dead organic materials into nutrients. They outclass bacteria by far in cases where cellulose has to be decomposed and the pH-value of a soil is low (SCHLEGEL 1992). In soil, saprophytic life forms dominate. The most important ecological function of fungi is the ability to coalesce in symbiotic interaction to higher plants. More than 80 % of all higher plants are assumed associated with vesicular-arbuscular mycorrhiza, which intercede minerals through an increased root surface to the plant and gain nutrients like carbon hydrates from the plant on their part (TAIZ & ZEIGER 1998). Many fungi appear as plant pathogens and pests for agricultural products. Therefore,

they are subject of intense use of plant protection products before and after harvesting. The amount of fungicides applied ranks amongst the most frequent and abundant used pesticides (compare FRAMPTON & WRATTEN 2000 for Great Britain).

I-4.3 The structure of food webs in soil

I-4.3.1 Interactions, dynamics and structure

For a reasonable discussion the topic for the data set at hand, it is necessary to link the ecotoxicological results with the result of the food web analysis. Soil food webs usually consist of many and omnivorous species and the groups of organisms distinguish by a high degree of interconnectedness. Down to the present day, the scientific understanding of food webs in soil is limited and general food web theories have been rarely applied, compared to the detailed knowledge of aquatic (i.e. limnic) ecosystem interactions (BENGTSSON *et al.* 1996). In ecotoxicology, a deeper understanding not only of the energy and material flows but also the interspecies interactions (predator-prey relationships) is essential for the interpretation of the complex results of large-scaled field or semi-field studies. At least two different mechanisms act upon the species relation.

- Two or more species are competitors for food resources (intra-species competition)
- Two or more species have a consumer-producer or prey-predator relationship (interspecies relationship)

The first mechanisms of interaction acts mainly within trophic levels, the second is more effective between different trophic levels. If one knows about the direction and the strength of the interaction it is possible to judge whether indirect effects of e.g. plant protection products occurred in a given test system. There are many examples of different concepts how to build the soil food web. SCHEU (2002) reviewed the current understanding of soil food webs and gave an outlook how to improve this knowledge in the future. Commonly accepted is the idea of two main channels of energy and material flow in soil: the primary decomposer channel uses directly plant residues while in the secondary decomposer channel the residues are utilized in a stepwise process by fungi and bacteria; the secondary decomposers consume on her part the microorganisms or detritus. The spectrum of food web concepts that can be found in the literature reaches from very complex concepts to extremely complicated models (for examples see DE RUITER *et al.* 1998). Common characteristics of soil food webs are their dependence on dead plant materials and roots (decomposer food webs) featuring complex interactions, partly reaching over several trophic levels. On the other hand, the simplified view of linear ‘channels of energy flow’ is generally questioned for most food webs. In reality, it ap-

plies that donor-controlled life history or mutualistic omnivory is rather the rule than the exception in a dynamic food web (POLIS & STRONG 1996). By means of ecotoxicological semi-field effect studies, no topological representation of the food web energy flow can be built, but our approach allows for the description of manifold interactions that still act on the intact soil communities after coring of TME. In the context of the thesis at hand, these interactions were not measured or observed directly. However, it becomes directly evident that the connectedness can be described and visualised by statistical methods.

I-4.3.2 A hypothetical TME food web

The TME method was tested and verified using the soil of only one site (chapter II-1.5). Hence, it was worthwhile to sketch the food web structure beyond the general assumptions and concepts described above. An attempt was made of hypothesizing a food web based on species level relationships. A statistics-based approach was used to get a first image of the specific food web of the TME, as used for the dose-response study. In a step-wise procedure, a pictorial representation of the hypothetical food web of the TME was produced by statistical correlation analysis and a priori knowledge. It is often tried to simplify natural complexity by summing up the different species into taxa groups and thus lacking explanatory power (BENGTSSON 1998), and so it was done here (Figure I-6). The representation will not be discussed in further detail in this thesis, but one single point has to be made: the oribatids reveal only weak links to the predatory guild and are thus not assumed to underlie the usual competitive mechanisms in the soil food web.

- The control abundance of oribatids, collembolans, enchytraeids and nematodes at days -1, day 26 and day 149 was averaged for each species or family, dependent on the respective determination level. The way of compiling the data assured a closed dataset, because study the design was imbalanced regarding the sampled organism groups per date (0).
- The correlation between each species (family) was calculated using the Pearson product-moment correlation coefficient. It was tested if the two variables are linearly, significantly dependent of each other (one-sided alpha < 0.1) and gives a measure of the extent of the correlation (Pearson 1896). The one-sided form of the test was applied to have an indication of the direction of interaction between the two species. It was assumed that variables are distributed bivariate normal (SPSS Inc. 2005). The result of the analysis is a matrix of correlation coefficients, corresponding significance levels and covariance values.
- The matrix was then filtered and arranged by using the following criteria.

- Negative correlations were chosen to indicate a competitive or food-donor relationship between the taxa considered. I.e. the more individuals of the one species, the less of the other species occurred, and vice versa. The correlations observed were all weak; the coefficients of correlation were between 20 and 30 %.
- Only significant correlations were further used, the criterion for consideration was a significance level alpha less than 10 %.
- Correlations within trophic levels were interpreted as competitive, correlations between trophic levels as consumptive 'predation'.
- Each of the taxa was assigned to trophic guilds according to the classification to be found in the conceptual model of Scheu (2002).
- Food preferences were compiled from Bongers (1988) for Nematodes, from i.a. Rusek (1998) for collembolans, from e.g. Schneider (2005) for oribatids and from Didden (1993) and others for the family of enchytraeids, and therein. In general, the flow of energy in soil food webs is based on either bacteria or fungi and changes in a characteristic and predictable manner after disturbance (Hedlund *et al.* 2004).

A theoretical approach can help to build hypotheses and, in an ecotoxicological context to understand indirect effects of toxic substances, which is a challenging task indeed. So-called 'modelling approaches' are suitable to safe much effort of measuring parameters that could not be estimated with adequate precision. Especially the promotion of certain species under influence of the test item can be better explained while making ecological presumptions about their ecological traits regarding trophic niches or food preferences or parasitic relations.

The approach of building a hypothetic food web was adopted by numerous different approaches in the literature. MOORE *et al.* (1996) stated that food web models can be derived by controlled microcosm experiments by using knowledge gained of field experiments or theoretical models (e.g. community models like Lotka-Volterra population dynamics and process oriented ecosystem models), link them together at equilibrium state of the community and establish measures of interaction strength from it. The way aims not at building a completely mechanistic picture of soil community but on generating hypotheses about possible interactions.

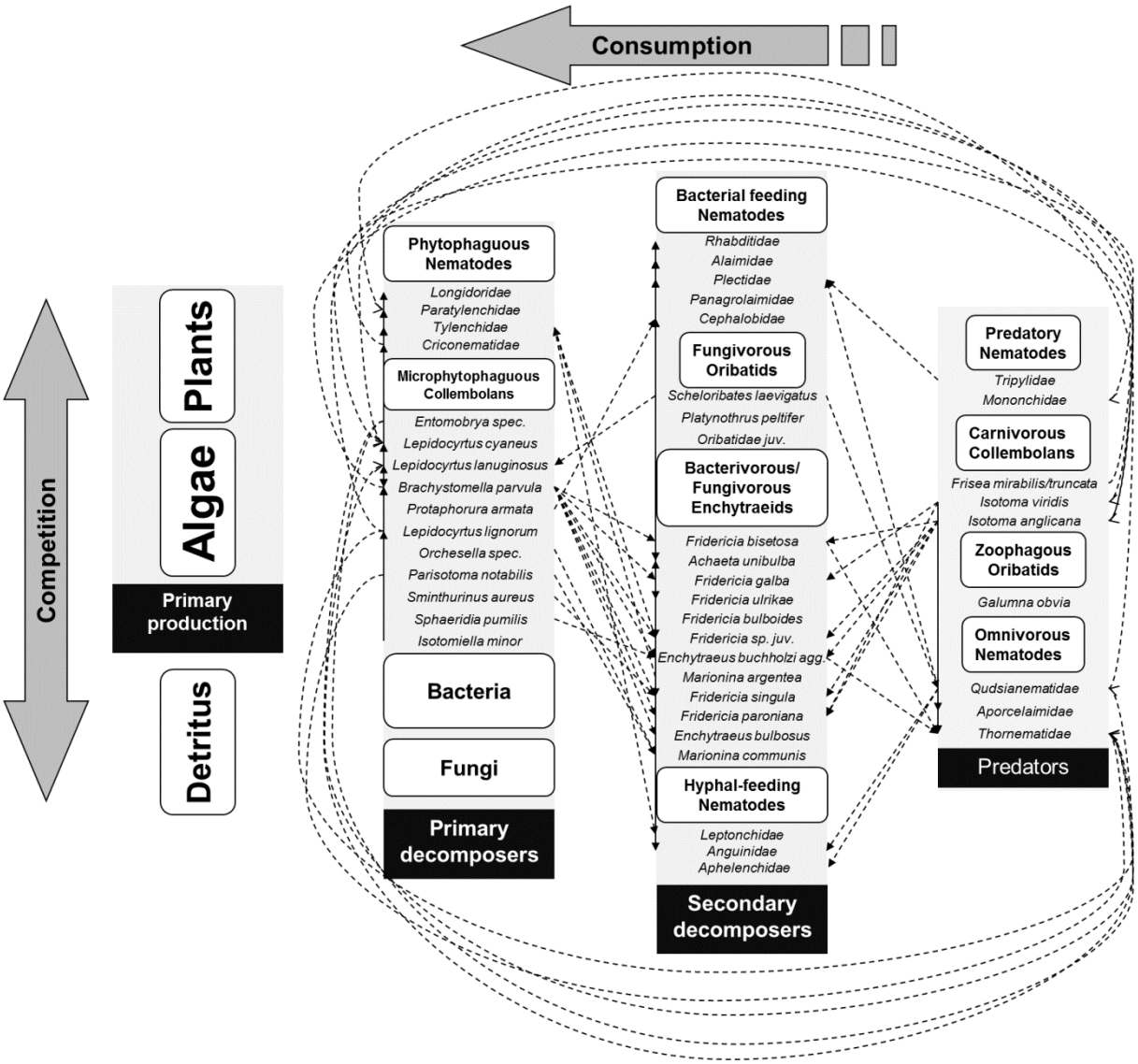


Figure I-6: Hypothetical food web of the TME biocoenosis. The structure was obtained by combining literature information on the trophic levels of a grassland soil food web and the assignment of taxa to feeding guilds, as well as the results of the data analysis of TME experiments. Only negative correlations, which indicate prey-predator relationships or intra-guild competition, were included. Straight and curved broken arrows: 'consumption'; vertical arrows: 'competition'.

Those are probably suited to explain some of promoting, adverse effects of insecticides to soil communities. For the readers' convenience and to judge the results of the TME studies that follow in the course of the thesis, the results of the food web analysis are prefixed.

I-4.4 Concepts of ecosystem stability

The general approach of investigating the stability of TME communities was to consider them as isolated patches of the grassland biotope of their origin. Referring to the ecological island theory, the isolation of populations should lead to decreasing densities and diversity (in BEGON *et al.* 1998). This can be either due to a lack of possibilities to avoid competition or due

to the restriction of resources. Most of the knowledge on the effects of isolation originates from the field of conservation biology, which has been mainly interested in the fragmentation of habitats. The findings were often obtained from the observation of large mammals and birds rather than from arthropods (DEBINSKI & HOLT 2000). In the case of TME communities, only little influence of isolation was expected. This was because the area of a single TME was supposed to exceed the critical minimum area of microarthropods by far, which is known to be a few centimetres. A basic association in community ecology is the species-area relationship, which says that there is a positive relation between a habitable area and the species richness (in LINDO & WINCHESTER 2007 discussed for microarthropods). Quite the contrary, there was evidence from recent experiments of SCHNEIDER *et al.* 2007 that isolation could promote the total number of collembolans and oribatids because of the exclusion of main predators (gamasid mites). It is subject of in-deep analyses described in the chapter 'Temporal stability of TME' to which extent TME provide enough habitable space to maintain viable and diverse communities over a long period. IVES & CARPENTER (2007) reviewed the recent models of ecosystem stability and discriminated distinct concepts of stability depending on inherent system dynamics and the type of disturbance that the system experienced. It is commonly agreed between ecologists but also contradictory evidence exists that diverse communities enhance the stability of ecosystems; hence, there is a positive diversity-stability relationship. IVES & CARPENTER also found the stability as well as the diversity of ecosystems affected by anthropogenic activities, so they could hardly discriminate between the two mechanisms. Since TME studies have been foreseen to be used in ERA, it was necessary neither to affect the stability nor the diversity by the mere experimental assembly. In general, two stabilizing mechanisms were assumed to explain the stability of population abundances or the composition of biocoenoses: The time to recovery from disturbance (*resilience*) and the persistence towards disturbances (degree of deviation from the equilibrium position or *resistance*). The variables that could be indicators of stability in semi-field studies could be the species richness or species diversity (measured as the combination of species richness and evenness in chapter V-2.2) and the connectedness or intra- and inter-species interactions (chapter I-4.3.2) and its actual characteristic strengths. The basic concepts were reviewed and criticized for their confusing variety by PIMM (1984) and GRIMM & WISSEL (1997). Very important for the application of most concepts is the assumption of a system in an equilibrium state. In ecotoxicology and in the context of soil semi-field studies, this need could be dropped because of its experimental character with a comparison between treatment and control groups. Soil communities recover comparable slow from disturbances; the time to recovery in a given study is expected

to be long and could reach up to few decades for the group of earthworms with relatively long reproductive cycles (BENGTSSON 2002). While talking about and assessing the stability of ecosystems a certain and defined concept of stability is necessary as several indicators of stability were developed. This thesis picks up the relevant concepts for the demands of the development and recommendations for a sound set-up of TME studies, which are natural soil systems isolated by boundaries and exposed outdoors under unchanged climatic conditions. The special criteria for stability of soil model ecosystems are described in the chapter 'V-2 Temporal stability of TME'.

II

Methodology

II-1 Experimental design

II-1.1 Overview on the experimental design of all studies

The data and the hypotheses of this thesis were based and developed based on several consecutive TME studies. The conceptual difference of the main datasets is described as '*field samples*' and '*TME samples*'. Field samples were either taken prior to a TME study in a '*screening*', or in parallel to a TME study to check for the comparability of the TME situation to the field situation as a '*validation*'. Several samples have been taken to improve the methodology of extracting the microarthropods from soil samples: '*extraction efficiency*'. From August 2006 onwards, the feasibility of the integration the groups of enchytraeids and nematodes in the assessment of the effects of the model compound lindane was proofed. Table II-1 gives an overview over the total sample number that constitutes the data basis of this thesis. Figure II-1 serves as a pictorial representation of the exact locations where on the coring site the small screening soil cores and the large TME soil cores have been taken. This is because the right side was reserved for other studies and the upper part has been identified as somewhat more homogenous than the part below. Detailed descriptions of the sampling schemes are given in the following sections. As a basic principle, TME were sampled sequentially, that means the soil cores representing the experimental units (replicates) were not sacrificed at each sampling date. The communities of soil organisms were monitored as a continuous time-series in one system over the period of one year, avoiding the problem of temporal pseudo-replication (HURLBERT 1984). This strategy results due to the destructive sampling method in a loss of soil surface that is assumed not to be substantial for the integrity of the system. It has been described in the literature (e.g. SCHNEIDER *et al.* 2007) and own additional studies suggested that reduced surface area does not influence the soil communities of micro-arthropods (THEIßEN *et al.* 2010). In the light of these assumptions, a maximum of seven sub-samples were cored in a TME. Total area of a TME is 0.7 m^2. After taking the last sub-sample 19.4 % of total area was removed.

Table II-1: Overview of the experimental design of the studies that built the base of this thesis. Crosses: Taxon was sampled at the specific date.

Study	Date	Location	Main purpose	No. samples	Collembolans	Oribatids	Enchytraeids	Nematodes
Field survey	2004-08-30	field	screening	20	x	x		
Field survey	2004-10-13	field	screening/gradients	189	x	x		
Pre-study	2005-01-21	TME	stability	44	x	x		
Pre-study	2005-03-10	TME	stability	16	x	x		
Pre-study	2005-03-22	TME	stability	44	x	x		
Field validation	2005-04-05	field	validation comparability TME-field	15	x	x		
Range-finding	2005-05-09	TME	effect detection	40	x	x		
Range-finding	2005-06-07	TME	effect detection	40	x	x		
Field validation	2005-06-13	field	validation comparability TME-field	15	x	x		
Range-finding	2005-07-20	TME	effect detection	40	x	x		
Range-finding	2005-08-16	TME	effect detection	40	x	x		
Range-finding	2005-09-13	TME	effect detection	39	x	x		
Range-finding	2005-10-12	TME	effect detection	40	x	x		
Extraction efficiency	2006-04-06	field	methodology improvement	72	x	x		
Range-finding	2006-04-19	TME	effect detection	40	x	x		
Dose-response test	2006-05-17	TME	effect detection	42	x	x		
Field validation	2006-05-17	field	validation comparability TME-field	24	x	x		
Dose-response test	2006-06-14	TME	effect detection	42	x	x		
Field validation	2006-06-14	field	validation comparability TME-field	24	x	x		
Dose-response test	2006-08-16	TME	effect detection	42	x	x	x	x
Field validation	2006-08-16	field	validation comparability TME-field	24	x	x		
Dose-response test	2006-10-16	TME	effect detection	42	x	x	x	x
Extraction efficiency	2006-10-16	field	methodology improvement	59	x	x		
Field validation	2006-10-16	field	validation comparability TME-field	23	x	x		
Extraction efficiency	2006-12-14	field	methodology improvement	80	x	x		
Dose-response test	2007-05-08	TME	effect detection	42	x	x	x	x

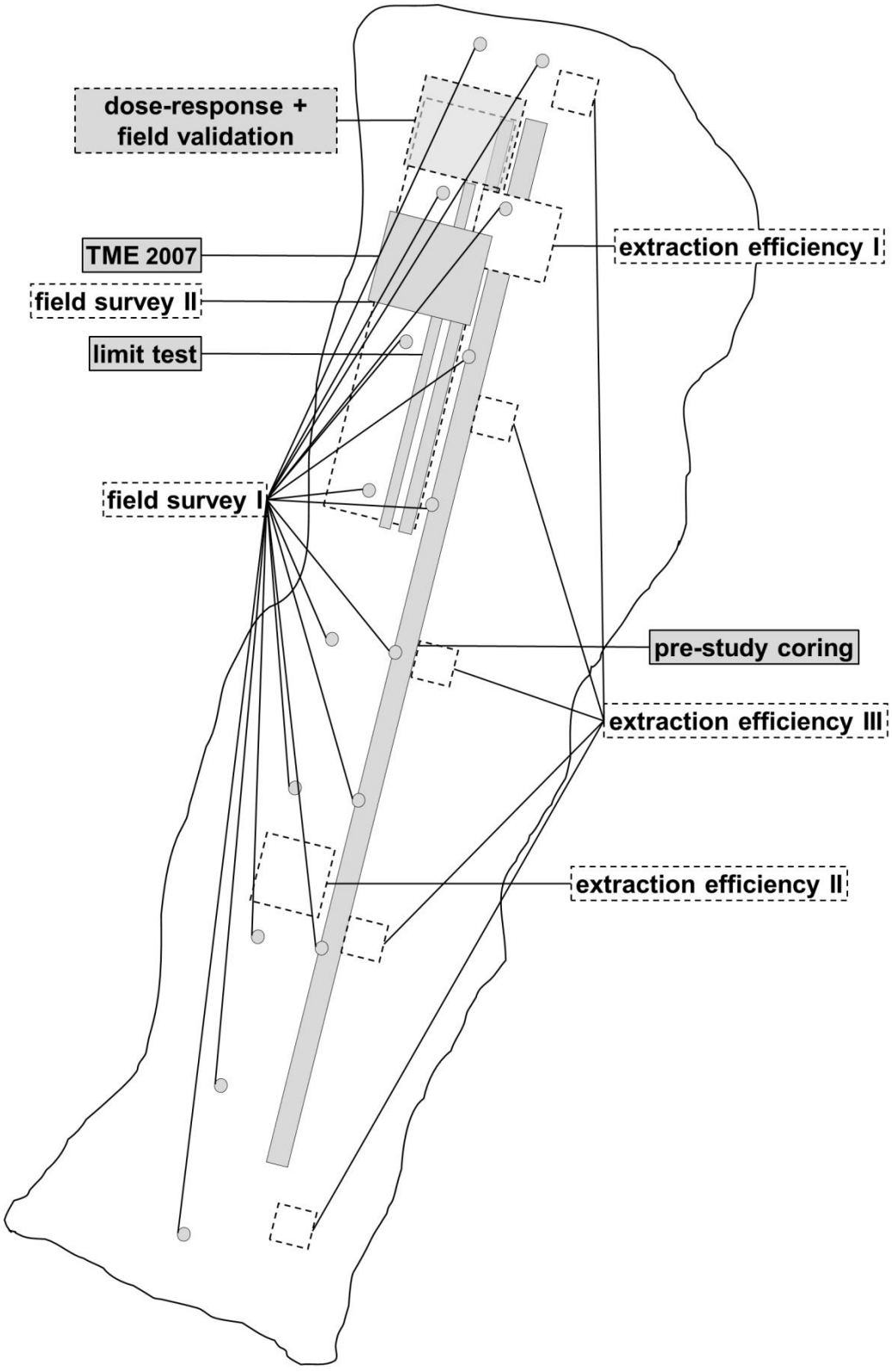


Figure II-1: Sampling and coring locations on the coring site near Monheim a. Rhein, Germany. Grey shaded areas with full lines: TME soil coring; empty areas with dotted lines: field samples; combinations of grey areas and dotted lines: both TME were cored as well as field samples were taken. Scale: Maximum width 40 meters, maximum length 120 meters.

II-1.2 Construction of TME cylinders

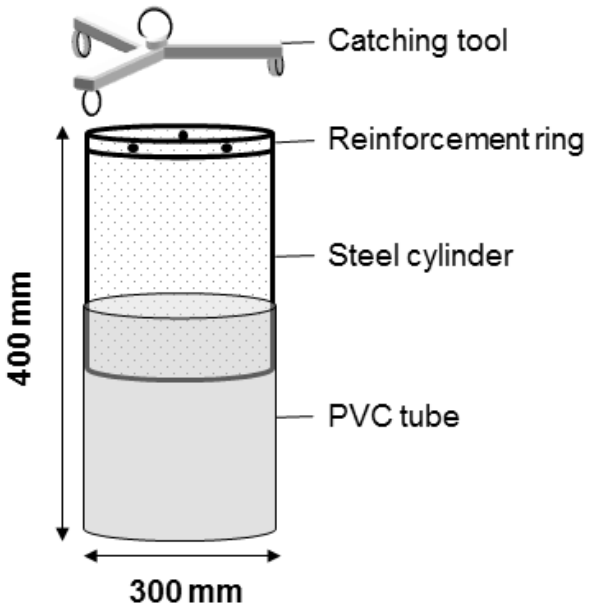


Figure II-2: Construction of the TME and pull-out device.

Figure II-3: Soil coring by means of a hydraulic pile pusher.

TME were made of VA-steel sheets of 2 mm thickness. They were rolled to cylinders of 400 mm in height and 300 mm in diameter, resulting in a volume of 28.3 litres. A ring of steel (30 mm width, 5 mm thick) was weld at the top to strengthen the construction. Three holes of 16 mm in diameter were bored in regular intervals of 120 degree at the top. This was to fix a pull-out triangle move the TME into or out of the facility. The triangle was made of three square steel pieces weld together in ternary fission, as well. An eye was fixed at the top to connect to hydraulic pile pusher or handles. The construction assured minimum wrapping of the steel cylinders (schematics shown by Figure II-2). A TME is completed after coring an undisturbed soil core of approx. 65 kg weight from the coring area.

II-1.3 Coring and preparation of TME

Prior the **pre-study**, 20 TME were cored by means of a modified hydraulic pile pusher in November 2004. The pile pusher was fixed on a tractor while assuring minimum disturbance of soil cores due to strictly vertical coring (see Figure II-3: Soil coring by means of a hydraulic pile pusher). TME were placed at patches providing as homogenous vegetation cover as possible and arranged in the middle of the coring area. TME were cored on a

transect of 80 meters length in mean distances between 4 meters. Having pushed the cylinders into the soil, soil cores could be pulled out containing an undisturbed soil core with natural vegetation cover and a relatively flat bottom. The TME were immediately transported in a lorry to the facility in Aachen. Styrofoam sheets of 5 cm thickness absorbed vibrations. The weight of the TME was approximately 65 kg, depending on actual water saturation. Before putting TME into the facility, the bottom of each of the soil cores was flattened to the level of cylinder bottom to bring it in contact with the soil-sand layer of the facility. That assures sufficient water suction from deeper layers. Gauze of 125 µm mesh size was placed under the TME to avoid immigration of earthworms and other allochthonous organisms. Until the first sampling was conducted in late January (until then the soil was continuously frozen), the TME left undisturbed for 80 days.

For the **range-finding study**, forty-five TME were cored on a longitudinal transect on 5 April 2005. The use of a hydraulic pile pusher assured minimum disturbance (Figure II-3). TME were immediately transported to the experimental site. The bottom of each soil core was flattened to guarantee a close base contact and sufficient water suction. Gauze with a 125 lm mesh size was placed on the bottom of TME to avoid immigration and emigration of soil organisms living in deep soil. TME in the **dose-response-study** were cored on a squared patch of 5 m edge-length. Forty-five TME (VA-steel cylinders, height 400 mm, diameter 300 mm) were cored at the coring site - an untreated tall oat grass meadow on sandy, alluvial soil near Monheim, Germany- on April 21, 2006. They were transported to the test facility and maintained for 26 days under natural site conditions until the application of the test item.

Figure II-4: Experimental field station of the RWTH Aachen University. The yellow square marks the position of the TME facilities. Adjacent areas: 30 aquatic mesocosms (right), abandoned maize field (above), grove (left below), meadow (right below). Source: Google Maps (Accessed 2012, picture taken 3/2006).

II-1.4 Construction of the experimental facilities

The TME facility held 45 TME and was located at the experimental site of the Institute for Environmental Research of the RWTH Aachen University (Figure II-4). A plot of 3.3 m length, 2.7 m width and 1 m deep was excavated, resulting in a volume of approx. 18 m^3. A 30 cm soil-sand-layer, manufactured by homogenizing four parts of the excavated material and 1 part of washed sand with a grain size of 0-2 mm, was set up on a 30 cm drainage layer of gravel (grain size 8-16 mm). On the surface of the soil-sand-layer, 45 PVC-tubes (450 mm height, 300 mm inner diameter) were placed in regular intervals as placeholders for the TME. The space between the TME was filled with a passable gravel layer of 40 cm thickness. A fence around the experimental facility protected the test facility from disturbances by humans and local wildlife. TME were exposed outdoors to normal sunlight, temperature, rainfall, and enable therefore natural fluctuations of populations of soil organisms. In the TME **pre-study**, 20 positions for soil cores were available; for further tests the facility was expanded to 45 positions which could hold test systems.

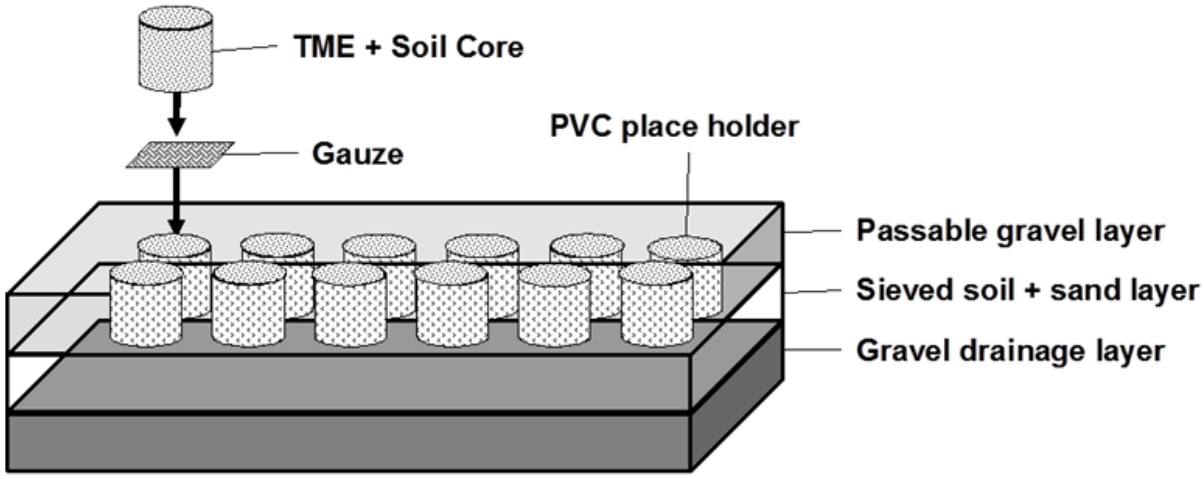


Figure II-5: Schematic view on the facility.

II-1.5 Characteristics of the coring site

TME were cored on a tall Oat Grass meadow on alluvial soil, situated 2.2 km linear distance from the river Rhine at the city of Monheim, Germany. No pesticides or fertilizers had been applied at this site within the last 20 years. The area was mown regularly while grass was left on the site. The soil contained 48.2%, sand, 40.4% silt and 11.4% clay. Organic matter content was 1.6% and pH was 4.9. Vegetation cover was dominated by *Bromus hordeaceus*, *Holcus lanatus*, *Taraxacum officinale* and *Arrhenatherum elatius*. The phytocoenis was addressed a tall oatgrass meadow according to its characteristic weed species A. *elatius*. Those meadows

Table II-2: Environmental conditions and soil properties of the coring site (Monheim a. Rh., Germany) and the experimental field site (Aachen, Germany). Data sources: Deutscher Wetterdienst (DWD), Bayer CropScience (BCS); Geoserver North-Rhine Westfalia (NRW), Institute for Environmental Research (UBC), Google Earth (GE), n.a. = not applied

	Coring area Altjudenhof,		Experimental facility RWTH Aachen	
Altitude above sea level [m]	41	*BCS*	197	*GE*
Cartesian coordinates	N 51°04´	*NRW*	N 50°46´	*GE*
	E 6°55´	*NRW*	E 6°02´	*GE*
Long term average precipitation (mm/a)	781.3	*DWD*	828.3	*DWD*
Long term average temperature (°C/a)	10.8	*DWD*	9.7	*DWD*
Long term sunshine duration (h/a)	1426.8	*DWD*	1552.0	*DWD*
Soil classification	typical parabrown earth, in places alluvial brown earth	*NRW*	typical colluvium, pseudogley	*NRW*
Soil texture	sandy, clayey loam	*NRW*	silty loam	*NRW*
pH [1N KCl, H_2O]	4.85	*BCS*	7.45	*UBC*
Water holding capacity [%]	56.6	*BCS*	n.a.	
Cation exchange capacity [meq/100g]	9.6	*BCS*	n.a.	
C org. [%]	1.6	*BCS*	3.5	*UBC*
N [%]	0.2	*BCS*	0.3	*UBC*
mg microbial C/kg dry weight soil	550	*BCS*	n.a.	
microbial biomass in % of $C_{org.}$	3.4	*BCS*	n.a.	
pore volume soil [%]	n.a.		54	*UBC*

can be found predominantly in all agricultural landscapes in Central Europe, often representing the only vegetation unit outside the cropped areas (ROß-NICKOLL *et al.* 2004). Fifteen plant species were found, mainly of the Poaceae-family. The field site was slightly sloping towards a small ditch surrounded by arable land.

II-1.6 Sampling schemes

II-1.6.1 Field surveys

To assess species numbers, species diversity and distribution of certain taxa over the coring area, a thorough screening was conducted before TME-coring. For this, an area of 10 m width and 40 m length was marked and a grid of 10.000 virtual sampling points was created. The distance of the centre of each sampling point to another was 20 cm. This design results in 200 sampling points per column and 50 sampling points per row. At two locations, squared 7 x 7 sampling grids were arbitrary chosen and served as *small-scale estimators* of species distribution. The two squares had an edge length of 1.4 m and included 49 samples (5 cm height, 5 cm diameter, taken by means of a steel soil corer with PVC inlay tubes). Another 94 samples were randomly distributed over the whole area, excluding the small-scale squares. They served as *big-scale estimators* of species distribution. In sum, 192 samples were taken on this area (identifiable to species level 189 samples, overview gives chapter II-1.1).

In the course of the consecutive projects that aimed at developing the TME methodology, several additional field samples were taken. Table II-1 and Figure II-1 give an overview over the field screening activities. Especially during the dose-response experiment of the year 2006, each TME sampling at the experimental facilities in Aachen was accompanied with corresponding field samples. These were taken very closely to the exact locations where the TME soil cores were cut. Figure II-1 indicates the samples as 'dose-response study and field validation experiment'. Two samples per each of the 12 control TME soil cores have been taken and extracted simultaneously to the samples from the dose-response study and then pooled for determination. Further samples served merely the comparison and evaluate the extraction efficiency of the MacFadyen high-gradient extractor; those are also traceable referring to Table II-1.

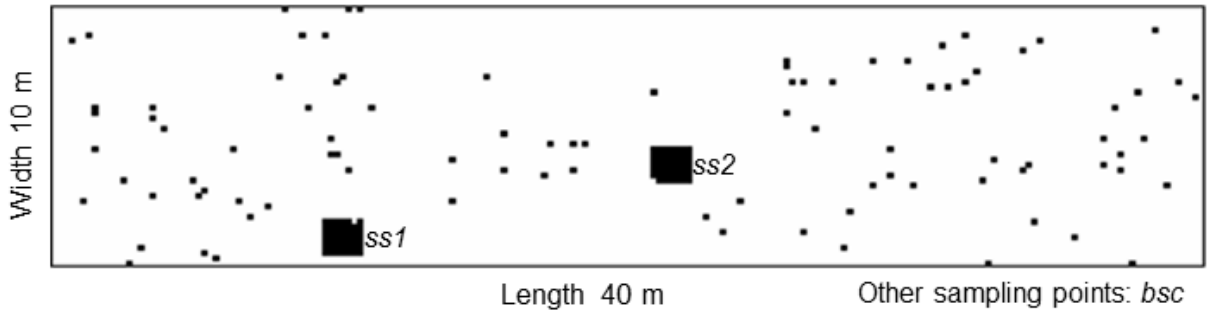


Figure II-6: Sampling grid field-survey 2004. Crosses represent sampling points on the coring area. The samples served to assess the large- and small-scale distribution of microarthropods total abundance and collembolan population densities before the first TME soil coring.

II-1.6.2 Pre-study

Sixteen TME were used during the 2004/2005 pre-study. Four systems were kept in reserve. The positions of the sub-samples were found by means of a calibration disc with seven equidistant openings for the soil corer. One hole was located in the centre, surrounded by six further in circle fission (Figure II-10). At two out of three sampling dates, the seven possible subsamples were taken from four TME to assess the variability inside a single TME ('full sampling'). To assess the stability of the biocoenoses and the variability between TME, two sub-samples per sampling date were taken out of the remaining eight TME at three sampling dates ('partial sampling'). The position of the respective subsamples on the calibration disc was randomly chosen out of the seven possible positions, whereas the last one was discarded. Additionally, the two samples were used to calculate the 'within-TME' variability (results used by chapter V-2). At three sampling-dates, seven samples were taken out of each TME. To compare the variability 'within'- and 'between'-TME, the mean of the two subsamples of the 'between-TME' was calculated. The number of replicates for the assessment of the within

TME variability was seven (sub-samples per TME), for the between TME variability replicate number was eight TME (Figure II-7 for illustration and study plan). Sampling took place from January to March 2005 (80-159 days after soil coring). In total, 104 samples were taken from 16 TME.

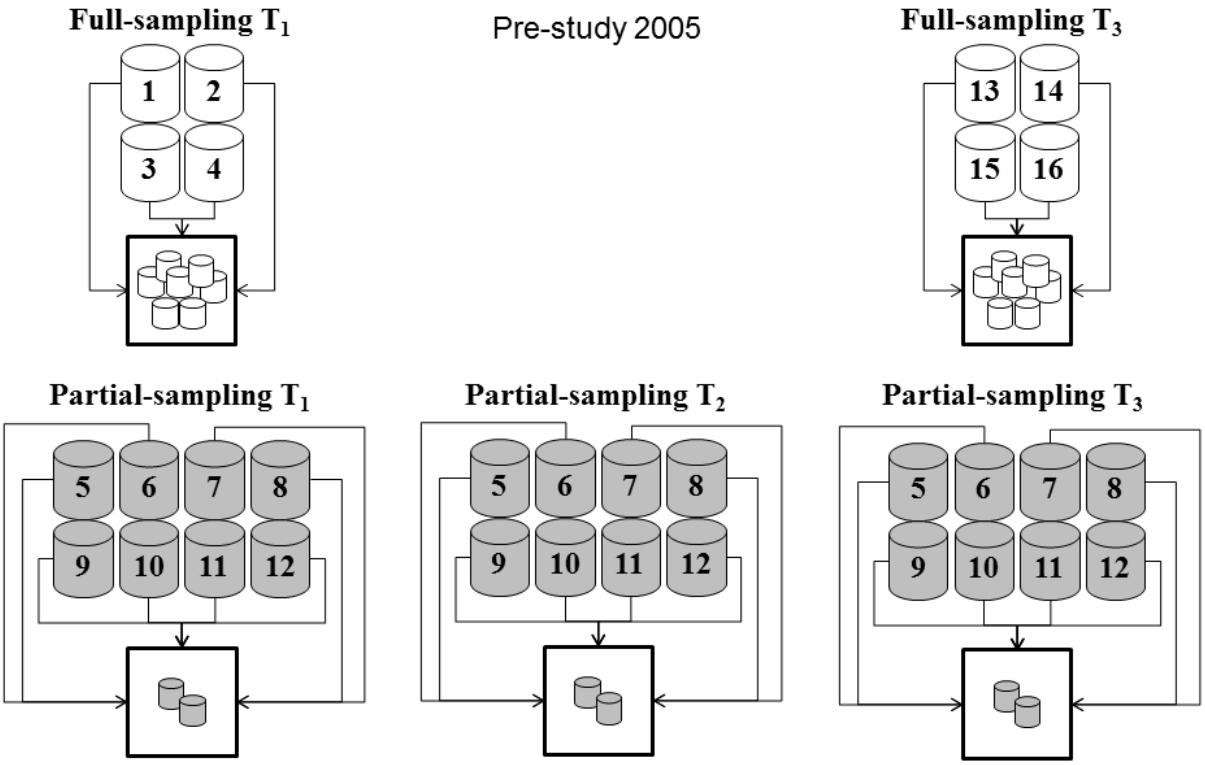


Figure II-7: Sampling scheme of TME pre-study. T_1: 80, T_2: 128, T_3: 140 days after TME coring. In the full sampling design 28 samples were taken per date, whereas in the partial sampling 16 samples per date were cored. The sum of sub-samples was 104. Numbered cylinders: TME, empty cylinders: sub-samples.

After taking the maximum number of subsamples, the soil cores were removed from steel cylinders in order to reuse the TME. Additionally the number and identity of earthworms was determined. The soil cores were divided into four layers of equal thickness of 100 mm and animals were sorted by hand according to ISO 2003a.

II-1.6.3 Range-finding study

TME were maintained for 4 weeks after coring under test site conditions before the first sampling. After the pre-application date in May 2005 (day 0—05/09/2005), six post-application samples were collected until mid-April 2006 (also Figure II-8: day 28-06/07/2005, day 71-07/20/2005, day 97-08/16/2005, day 124-09/13/2005, day 153-10/12/2005, and day 340-04/19/2006). Bait-lamina sticks were removed at day 14.TME were sampled sequentially (‘sub-sampling’), i.e. TME replicates were not sacrificed at each sampling date by using the total area available (Figure II-8). The communities of soil organisms were monitored in a 1-year time-series in which a maximum of seven 5 cm-sub-samples was cored in each TME.

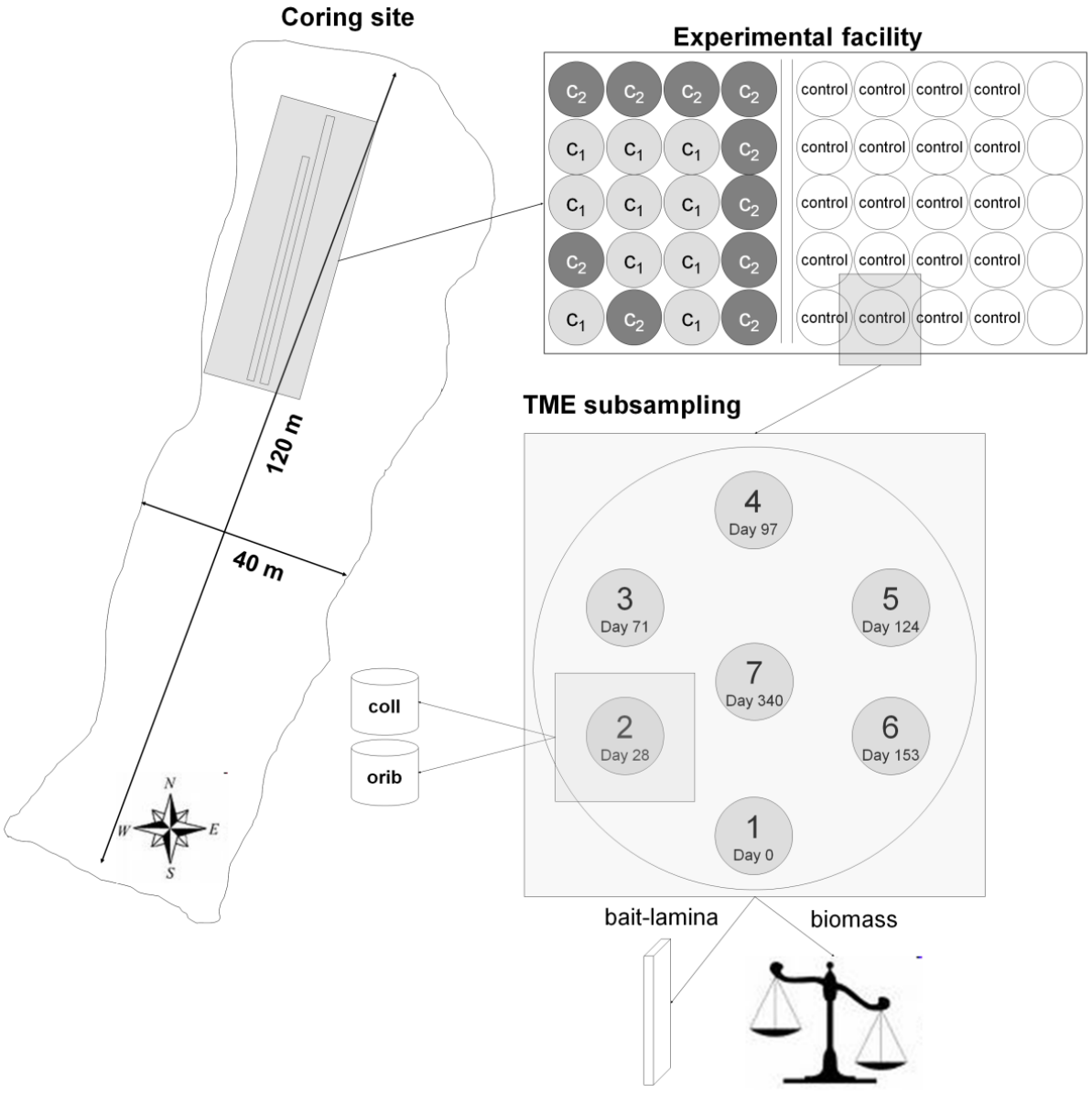


Figure II-8: Dimensions are described in the text. TME were cored on the grey shaded area on the sampling site and transported to the experimental facility. c_1 = 10 mg active ingredient (a.i.)/kg soil, c_2 = 100 mg a.i./soil. From each TME consecutive sub-samples as soil cores (numbers 1-7) were taken. Coll + orib: extraction of collembolans and oribatids from the same soil core. Out of five spare TME (empty circles in the upper right), two were used for measurements and three were kept as substitutes.

The total area of a TME covered 0.7 m^2 of which 17 % was removed after taking the seven sub-samples. Twenty TME were randomly chosen to be stored at the right part of the experimental facility and served as untreated controls. Out of the twenty TME on one side of the facility, each 10 were randomly assigned to one of the treatment groups c_1 (10 mg a.i./kg soil) and c_2 (100 mg a.i./kg soil), respectively. A further two systems were used to monitor the water holding capacity and soil temperature. Three TME were kept as substitutes (Figure II-8).

II-1.6.4 Dose-response study

Based on the experiences of the 2005 range-finding study, a dose-response study was designed to detect effects below the lowest concentration in the previous experiment of 10 mg lindane/kg dry weight soil. Concentration is referred to the amount of nominal active compound in the upper five centimetre-layer of the test soil. Each of the TME was placed in a random design with unequal replication using the Edgar II software (BROWN 2004) and assigned either to the control group (c_0 = 12 TME-replicates) or to one of five treatment groups (c_1-c_5, 6 replicates each = 30 TME-units).

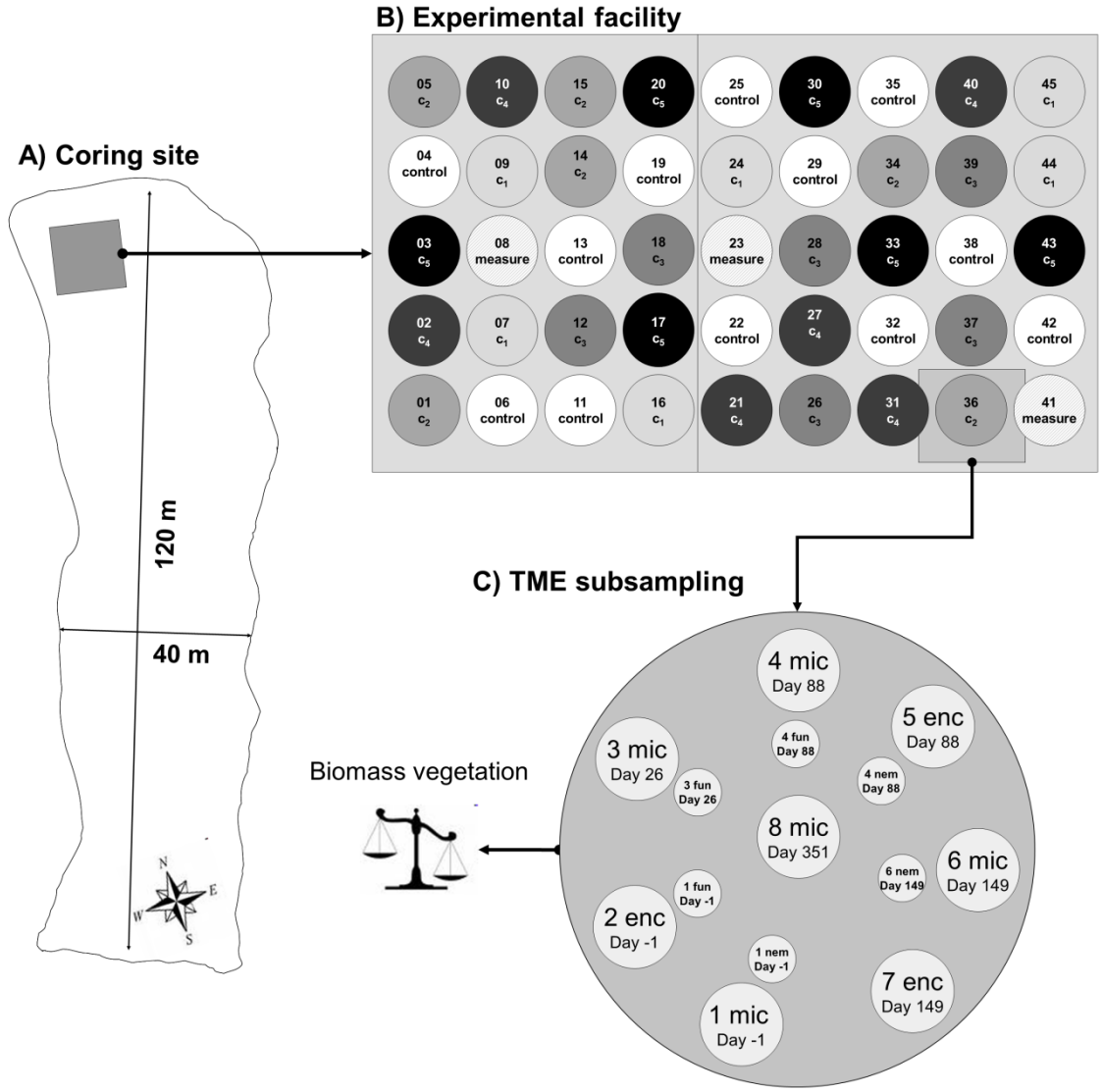


Figure II-9: Study design of the TME dose-response experiment. Clockwise from the left: A) Position of the soil cores at the coring site B) Random storage of the TME at the experimental facility C) Sampling slots on the TME surface/calibration disc. mic = microarthropods, enc = enchytraeids, nem = nematodes, fun = fungi

Three further TME were maintained for measurements of soil moisture and soil temperature (Figure II-9). Grass cover was cut, dried and weighed prior to the sampling of the soil organisms. This delivered an additional endpoint on the one hand; on the other hand, it facilitated the sub-sampling of TME. *Microarthropods* were sampled at five occasions during the one-year experimental period by means of a soil corer (5 cm height and 5 cm diameter). The application of the test item was done on day 0 (2006-05-18). Sampling was done one day before application (2006-05-17; day -1), one month after application (2006-06-14; day 26) three months (2006-08-16; day 88), five months (2006-10-16; day 149) and one year after application (2007-05-08; day 351). *Nematodes* and *enchytraeids* were sampled at day -1, day 26 and day 149. Within this study, the technical feasibility as well as the detectability of effects was assessed. Soil cores for the extraction of enchytraeids were gained with the same soil corer as used for the microarthropods. Nematodes were sampled with a steel corer (diameter 1.7 cm; height of 5 cm). The soil was removed with a plunger, pressed into a crimp top glass vial of 50 ml volume, and stored in a separate icebox for transport. *Soil fungi* were characterized by applying sum parameters of fungal biomass (ergosterol content) and by means of molecular fingerprinting techniques to evaluate the potential to record communities of soil fungi. Nine TME (three replicates of c_0, c_3, c_5, respectively) were sampled using cylinders of 1.7 cm in diameter and 5 cm in height. Sampling took place at day -1, day 26 and day 88. All of the analyses that refer to the sub-set of samples until day 149 are denoted as '*set 5*'. The sub-set until 351 days after treatment is referred to as '*set 12*'. The application procedure is described in chapter II-3.2.

The knowledge gained during the development projects led in the year 2007 to a TME study in a further refined design using a test substance relevant for registration. The experimental setup is not described in this thesis. Since the outcome of this study is confidential, only control data is used to demonstrate the improvements in the course of the methodological developments in parts of chapter V. It is then referred as **'TME-2007'** study.

II-2 Endpoints

The soil is a study object difficult to investigate. It could be observed only from the surface where only a part of the complex diversity can be found. For this they have to be extracted from the soil by suitable and on the distinct properties adapted methods (e.g. KRELL *et al.* 2005). In the literature, a great number of methods can be found and virtually every author modified the well-known techniques for his special purposes. As an unpleasant side effect, the

results of many studies in the soil are very difficult to compare, so that an estimate of the true population size is not possible. A method that fits well for one group could be less suited to sample or extract another group of soil inhabiting invertebrates. EDWARDS (1991) gives a detailed review of different methods and recommendations for different taxa groups and soil properties. A good reference textbook is written by DUNGER & FIEDLER (1997). Furthermore, an ecosystem consists of many abiotic and biotic factors. A complete description and understanding is only possible if all chemical (organic and inorganic) properties, all environmental factors, all autotrophic and heterotrophic biota are known and measured. The main characteristic of model ecosystems is to reduce the large number of components to a manageable amount. We focused on the main abiotic properties as common soil parameters (e.g. pH, texture, geogenic origin), climatic variables (e.g. temperature, rainfall, radiation energy), the actual humidity (matrix potential) and the actual soil temperature as well as on the documentation of nominal application rates. The functions of an ecosystem were investigated by recording the overall feeding activity of soil organisms by applying the bait-lamina test and the plant biomass gain between two sampling dates. Fate and microbial functional endpoints have not been under consideration. In the following, a detailed description of the concepts to measure besides functional endpoints mainly structural measures of disturbances by the model compound lindane is provided.

II-2.1 Environmental conditions

The soil matrix potential was measured by means of two equitensiometers (EQ3505, Meier-NT, Germany) to monitor the moisture of the top 5 cm soil layers of two spare TME continuously. The soil temperature was measured and logged (sensor ‘BT02’, ‘Advanced Datalogger’, Meier-NT, Germany). Data on precipitation and air temperature were provided by a weather station nearby the experimental site. The data were used to schedule sampling dates with respect to the actual weather forecast and to design an irrigation concept in case of long-lasting drought. However, irrigation was not necessary during the studies.

II-2.2 Biomass of vegetation and plant diversity

Before application and each sampling in the **range-finding study**, the grass cover was cut and the plant biomass was determined (from day 97 until the end of the study). Weed and herb species were identified with reference to ROTHMALER (2002a, b). The proportional cover of each species was estimated. During the **dose-response study**, the biomass was determined before the application and immediately after each sampling from day -1 to day 251. The

growth rates are the increase in biomass between the date of application and each sampling date, respectively. Five growth rates could be calculated.

II-2.3 Feeding activity in the TME pre-study and the range-finding study

The feeding activity of soil organisms was measured using the bait-lamina method (VON TÖRNE 1990, KRATZ 1998). Sticks were filled with powdered, dried nettle leaves. Before taking the first soil cores during the *TME pre-study*, sixteen bait lamina sticks per TME were exposed to measure the natural variability of the overall feeding activity. This was done to test the applicability of the method for the use in our TME. After 14 days, one stick per TME was controlled and because of low activity, the exposure duration was prolonged until 42 days after exposure of the sticks. During the range-finding study, seven bait-lamina sticks (PVC, 130 mm long, 6 mm width, 1 mm thick, Terra Protecta, Germany) were equipped with 16 holes 5 mm apart. The perforation was filled with powdered, dried nettle leaves. The sticks were exposed for 14 days. In both experiments, the number of open holes per bait-lamina stick was determined at the end of the exposure period.

II-2.4 Communities of microarthropods

II-2.4.1 Reviewing extraction methods

Various extraction methods of the soil mesofauna have been established since ecologists and taxonomists work on the topic. For larger invertebrates, it could be possible but very time consuming to count them directly in the field by hand sorting. That is suitable for earthworms, millipedes, centipedes, larvae of butterflies or beetles and molluscs. For very active species, different kinds of trapping techniques are available (e.g. pitfall traps). It is then very difficult to estimate the real populations because the abundances depend on species-specific activity. However, it could be calculated after in mark-and-recapture experiments. It is mainly applied to beetles or spiders, but it could also lead to high numbers of surface dwelling microarthropods like collembolans or mites. A suitable method to collect medium-sized surface-dwelling collembolans is the use of suction-samplers. More commonly, microarthropods are captured by coring soil samples by means of soil corers. Those are often modified by the user's demands depending on the soil properties. A soil corer consists of tubes of steel. The core sizes range from 2.5 cm to 1 meter in diameter and from 5 to 50 cm in depth. Very often, the 0-10 cm top soil layers are sampled because the population density of soil living invertebrates decreases rapidly with increasing penetration depth. A sufficient size for soil cores to assess the

mesofauna density is 5 cm in diameter (information compiled from EDWARDS 1991, ANDRÉ *et al.* 2002 or DUNGER & FIEDLER 1997). For faunistic or ecological studies, a sampling period of at least one year is recommended. Otherwise, large differences in abundance due to seasonal population dynamics could be overlooked.

Behavioural and mechanical methods

In principle, there are two different ways to extract microarthropods from the soil, setting aside the very time-consuming hand-sorting methods or direct field sampling by suction samplers.

- Behavioural or dynamic methods, which yield only living, intact stages of the organisms. This review focused on MacFadyen-type high-gradient methods.
- Mechanical methods, which yield both living and dead stages of animals. Here, flotation-type techniques will be described only.

Most widely spread and economically sense behavioural methods are Berlese-Tullgren funnels (TULLGREN 1918). There are or numerous modifications of the original method, e.g. Merchant-Crossley type extractors as developed by NORTON 1986 and GODDARD 1979. A very basic version was described by MERCHANT & CROSSLEY 1970, which was further modified by SEASTEDT & CROSSLEY 1978. They usually consist of a heat source, that are light bulbs or fan heaters above the soil core. Soil cores often have been oriented upside down and equipped at the bottom of the soil sample with a funnel and a collecting vial that is filled with preservation liquid like alcohol, benzoic acid or simply. The soil core then dries out for an extraction period of a few days up to two weeks. In principle, active and thus living stages of the organisms avoid dry, warm, light conditions and tend to leave the sample in direction collecting vial (negative Hygro-, Thermo- and Phototaxis). Kempson-MacFadyen type high-gradient extractors represent advanced versions of this principle (MACFADYEN 1961, KEMPSON *et al.* 1963). They have been trying to create a steeper gradient of temperature and humidity between the hot top and the cool bottom of the sample by means of an additional cooling device. Both methods only yield active, living individuals and are biased towards surface dwelling taxa. Mechanical methods and especially flotation techniques are marked by a high demand of labour. It is possible to enhance the efficiency by repeating the procedure consecutively. Passive methods are based on the physicochemical properties of the cuticle of invertebrates, which tend to be soluble the hydrophobic phase of a mixture of water and e.g. alkanes. A soil sample is treated with an aqueous solution of heptane, dibromoethane or carbon tetrachlorides. The mixture is gently stirred or shaken, and after the phases have been separated, the supernatant containing the animals can be removed with a pipette (DUCARMÉ *et al.* 1998,

WALTER *et al.* 1987).

Efficiency

The International Standardization Organization recommends for the extraction of living stages of microarthropods the high-gradient method of MacFadyen and its modifications (ISO 2003B). These methods could be summarized as 'Tullgren-type-extractors'. The efficiency of this method for adult oribatid mites is pretended by ISO to be about 75 %. However, this refers to a publication that deals with a single species of the high arctic region without a structured soil matrix (SOVIK & LEINAAS 2002). Other referenced experiments were conducted by BLOCK (1966). He introduced mites into the soil artificially so that the organisms were not able to penetrate the soil matrix completely during three-day experimental period. In sum, the referenced literature in the ISO-guideline is poor concerning experiments dealing with the extraction efficiency of Acari, especially for oribatid mites. For Collembolans there is much more evidence in the literature to estimate the efficiency of different extraction methods. VAN STRAALEN & RIJNINKS (1982) described their specific method and its efficiency, and they reviewed all available literature. The same method was also used in the TME ring-test of KNACKER *et al.* (2004) to extract both the collembolans and the mites of all suborders. In addition, EDWARDS 1991 reviewed the literature. Both authors summarize that the efficiency can be tested by three consecutive methods after using a Tullgren or MacFadyen like extractor. They list the following:

- Subsequent or parallel flotation
- Introduction of a known number of individuals.
- Hand sorting of the remaining individual organisms from extracted samples or hand-sorting of reference samples.

Each of the methods has specific biases, depending on the experimental set-up. If a flotation technique is used on intact soil, both living and dead stages are counted and the efficiency of the method is not known. This could lead to an under- or overestimation of the actual population size. If the organisms are artificially introduced and are not able to penetrate the soil completely, they could be extracted easier. This results in overestimated population sizes. Moreover, hand-sorting is relatively ineffective. It is highly time consuming and biased towards personal accuracy. Furthermore, the densities of especially smaller species are undervalued (TUF & TVARDIK 2005). Very few people did it with Oribatid mites. As SOVIK & LEINAAS (2002) pointed out, there is no representative experiment for a variety of mineral soils and for a natural community of mite species because they differ significantly in their activity, their quiescence behaviour and the soil layer they live in. Other authors that gathered

detailed data (ANDRÉ *et al.* 2002, TAKEDA 1979) are not sure that it is sufficiently known how efficient the cited extraction methods really are.

It is known that high-gradient methods perform much better in extracting oribatid mites than normal Tullgren-type methods, which cannot achieve a steep gradient of humidity within a soil core (NEF 1971). There is evidence that the efficiency of extraction could alter during time. LEINAAS (1978) found that there is an artificial winter maximum of collembolan populations. It could not be assigned to fluctuating reproduction rates because the age structure was constant over the year. He assumed that a higher efficiency due to less soil compaction in winter could be the reason for an increase of the collembolan numbers. However, by using mechanical methods there is a bias towards counting animals, which were already dead before extraction. WALTER *et al.* (1987) recommend only counting the intact individuals especially of hard covered oribatid mites. When they lost their legs, the inner organs or many of the hairs and trichobothria they are assumed dead already before extraction.

The biases of mechanical and behavioural in different directions suggest the application consecutive extraction methods. This would assure that all organisms, even those living in deep soil layers species, are extracted. The efficiency of different consecutive methods is described by SNIDER & SNIDER (1997). It is often suspected that active methods are more efficient than flotation methods, but more recently, WALTER *et al.* (1987) found twice the number of species and four times the number of individuals in sandy soils with low organic content by applying a heptane flotation method. Especially for heat extracting methods, the extraction efficiency

Table II-3: Overview of the extraction efficiencies of different Tullgren-funnel-like methods (combined by VAN STRAALEN & RIJNINKS 1982 and EDWARDS 1991). A more recent and comprehensive compilation of studies dealing with extraction efficiencies is given by ANDRÉ *et al.* 2002. The efficiency is given as the percentage of the individuals originally introduced to a soil sample.

Reference method	Taxonomic Group	Efficiency (%)	Authors
Flotation	*Acari, Collembola*	15-70	SNIDER & SNIDER 1997
	Acari, Collembola	93-97	BIERI *et al*. 1978
	Acari, Collembola	80-90	PETERSEN 1978
	Arthropoda	66	SULEMAN *et al.* 1979
Introduced organisms	*Acari, Collembola*	85	TUF & TVARDIK 2005
	Collembola	76-83	JOOSSE 1970
	Collembola	90	NIIJMA 1971
	Collembola	82	VAN STRAALEN & RIJNINKS 1982
	Acari, Collembola	73-98	MARSHALL 1972
Hand Sorting	*Collembola*	23-25	TANAKA 1970
	Collembola	16	TAMURA 1976
	Acari, Collembola, Protura, Diplura	58-95	LUSSENHOP 1971
	Collembola	28-88	TAKEDA 1979

varied clearly between sampling dates, different taxa and within single samples. Some endogeic species have been extracted by (sugar) flotation methods only. For this reason (especially due to the great variation), it is not possible to introduce correction factors to calculate the real population size as postulated by EDWARDS 1991 (SNIDER & SNIDER 1997). SULEMAN *et al.* (1979) found that mechanical methods yield 1.24 times more arthropods than behavioural methods. Only Collembolans of the upper soil layer are better extracted by behavioural methods. For Acari, the behavioural method was inferior for all layers. By summarizing all of the different opposed aspects, it was concluded that the most exhaustive extraction would be a combination of an active with a passive method. This is regardless the practically limited possibilities for economic reasons and time constraints. In biocoenotic as well as in ecotoxicological studies dealing with natural soils and communities of animals, it is of major importance to choose the appropriate methods of sampling and extraction.

II-2.4.2 Methodology in TME and field studies

Sampling of soil cores

TME in the **pre-study** and the **range-finding study** were sampled by aid of steel tubes of 5 cm in diameter and 6 cm height. They were driven into the soil 1 cm below the top and caught up with a wire. Positions in TME were found by using a calibration disc. The surface area of a TME of about 700 cm^2 allowed for a maximum of seven sub-samples for microarthropods to be taken. As a result, 19.44 % of the whole area was lost after the last sampling. **In the field**, samples were taken by means of a soil corer with PVC-inlay tubes of each five centimetres height and diameter (Figure II-10). There are no principal differences between the two sampling methods, but steel tubes have sharper edges, presumably causing less compaction and thus disturbance of the soil cores. **In the TME dose-response study,** a modified soil corer was used. The plastic inlay and the much-sharped edge allowed a more disturbance-free sampling and an easier handling

Figure II-10: Sampling equipment for TME (pre-study and range-finding study) (right side) and in the field (left side). Tools were made by the University`s own facilities. Calibration disc used for the TME pre-study, the range-finding study and the dose-response study. The modified soil corer with plastic inlays was used was used from the dose-response onwards.

samples (Figure II-10).

MacFadyen-Extraction

In all studies, collembolans and oribatid mites were extracted using a modified high-gradient canister according to MACFADYEN (1961). During the pre-study and the first field studies including the second field validation study in June 2005, the soil samples, regardless if cored in steel or PVC tubes were put upside-down in plastic flowerpots that were equipped with a sieve of 2 mm mesh size at the bottom. The flowerpots were put into beaker glasses containing benzoic acid as a fixation solvent. At the start of the extraction process, the canister temperature canister was about 20°C. Within 14 days, the temperature was increased in 3°C-steps up to 59°C. The fixation solvent was then sieved over a Schauermann funnel over gauze of 125 µm mesh size and the microarthropods were fixed. In the course of an intensive methodological validation- and optimization process, the efficiency of the MacFadyen extraction was verified. For this purpose, the heptane flotation method of WALTER *et al.* (1987) was applied. The soil cores were floated twice after the high gradient extraction, gaining the remainder of dead and living soil organisms. The results were analysed and served as a rationale for further modifications of the high-gradient extraction process (not shown in this thesis).

Sorting, preparation and identification of microarthropods

After extraction, all microarthropods were sorted and counted under a stereomicroscope (minimum magnification 10-fold, maximum 40 fold of original) to groups of collembolans, oribatid mites, gamasid mites and others. Oribatids, gamasids and others were stored in small vials under 70 %-alcohol as fixation solvent. Collembolans were prepared for microscopy on object slides under a drop of lactic acid to clear the pigmentation. Slide cover glasses were equipped with edges of paraffin, to protect the larger animals from being crushed. Collembolans were determined to species level using a differential contrast microscope (Leitz Dialux 20EB, maximum magnification 400-fold). Collembolans were examined to species level with reference to FJELLBERG (1998), POTAPOV (2001), BRETFELD (1999), THIBAUD *et al.* (2004), ZIMDARS & DUNGER (1994), FJELLBERG (1980), STACH (1960, 1963) and oribatids with reference to WEIGMANN (2006), WILLMANN (1931), STRENZKE (1952). Beside the fact, the taxonomical work on this group is still in progress. Juvenile stages could only be determined to genus level because identifying characteristics were developed not till then being well sclerotized adults (MARAUN & SCHEU 2000). BECK & WOAS pointed out that the effort of taxonomic determination of this group is rather high (in: BUNDESVERBAND BODEN 2005). The oribatids were determined by means of a stereomicroscope (Nikon SMZ 1500, max. magnification 170-fold) and a microscope (Olympus BX51, max. magnification 600 x).

II-2.5 Communities of lumbricids - pre-study and range-finding study

After the last sampling date of the **TME pre-study**, the soil was removed from TME and divided into four layers of 10 cm thickness. Earthworms were picked up by hand and preserved in 70 % alcohol for determination. In the range-finding study, the Lumbricids that came up to the soil surface immediately after treatment and died within the next two days were counted. At the end of the study, lumbricids were extracted by applying an appropriate volume of 0.2% formaldehyde solution to the soil surface (ISO 2003a). Earthworms were fixed in 4 % formaldehyde solution and then finally preserved in 70 % ethanol. The individuals of both tests mentioned were counted, the adults were identified to species level and the juveniles to genus level (with reference to CHRISTIAN & ZICSI 2005).

II-2.6 Communities of micro-annelids – dose-response study

Enchytraeids (and one species of tubificids) were extracted from the soil samples by the wet extraction method according to GRAEFE (in DUNGER & FIEDLER 1997). This procedure is in accordance with the ISO guideline 23611-3 on sampling and soil extraction of enchytraeids (ISO 2003c). Soil samples were rinsed for 48 hours, water was changed once after 24 h. Animals were identified using the keys by NIELSEN & CHRISTENSEN (1959) and SCHMELZ (2003).

II-2.7 Communities of nematodes – dose-response study

Nematodes were extracted according to the modified sieving and decanting method of Cobb (COBB 1918, SOUTHEY 1986, VAN BEZOOIJEN 2006). After extraction and fixation, nematodes were determined on family level according to BONGERS (1988). A hundred individuals of each sample were determined to keep the effort within a limit. In order to gain more information to facilitate and improve the interpretation of results, families were attributed subsequently to distinct trophic groups and to a maturity indication system (BONGERS 1990).

II-2.8 Communities and biomasses of soil fungi – dose-response study

Immediately after sampling, the soil samples were frozen at -20°C and then lyophilized in order to facilitate sieving of soil particles to grain sizes of less than 2 mm in diameter. Each soil sample was divided in two parts and treated separately in the following to determine first-

ly the ergosterol content as an indicator of fungal biomass and secondly to characterize the fungal communities by means of the Denaturing Gradient Gel Electrophoresis (DGGE) method.

II-2.8.1 Ergosterol-extraction and high-performance liquid chromatography (HPLC)

The extraction was conducted for three sub-samples of a soil sample in parallel. Each replicate was treated two times with methanol and KOH at 85°C, according to the protocol of EASH *et al.* (1996). After centrifugation, the supernatant was cleaned up three times by using hexane liquid-liquid extraction. After vaporization, the cleaned residue was solved in methanol, transferred into HPLC-vials and analysed using a diode array detector.

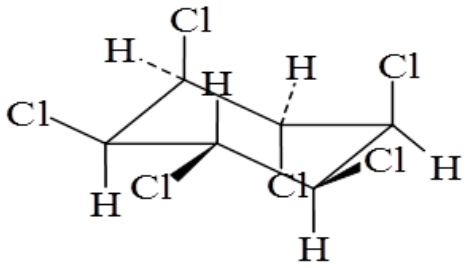
II-2.8.2 DNA-extraction, Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE)

Extraction of the fungal DNA from soil samples was done using the 'FastDNA spin kit for soil' (Qbiogene) according to the manufacturer's instructions with minor deviations from standard protocol (RUMPLER 2007). After extraction, a 350bp fragment of the 18S-rDNA was amplified in a polymerase chain reaction (PCR). The primer-system used was the unspecific primer NS1, based on a conserved region of the *Sacharomyces cerevisae* genome and the fungal-specific primer *fung*, a construct based on a sequence that was found conserved in 50 different fungal species but different to the same region in plants (MAY *et al.* 2001). A 40bp guanine/cytosine-oligonucleotide ('G/C-clamp') was added to primer fung to prevent complete degradation of the amplification product during following DGGE. After successful PCR-amplification, products were used in DGGE analyses to separate fragments of different sequence. The basics of the DGGE-principle were described by MUYZER *et al.* (1993) and by KENNEDY & CLIPSON (2003). A DCode Universal Mutation Detection System (Biorad) served as a tool for DGGE. A gradient of formamide/urea-concentration between 18 % and 28 % was identified to assure an optimal separation. The method is described in detail by BULAWA (2004). After the runs, gels were stained with silver and scanned (ScanMaker I 900 Mikrotek). The fragments can be regarded as 'pseudo-species' or 'phylotypes' and are used to describe the communities of soil fungi on a rather high taxonomic level.

II-3 Properties of the model compound lindane

The insecticide lindane (γ-hexachlorocyclohexane) was chosen as the model substance in order to examine the performance of the test system in general and to evaluate the study design. Properties of this compound are shown in Table II-4. It belongs to the class of organochlorines. HCH is synthesised by additive photo-chlorination (by means of ultraviolet light) of benzene. During this process, eight possible isomers are formed, differing in the alignment of Cl-atoms and in relative portions of total amount. All isomers are persistent, but exclusively the gamma isomer showed high insecticidal activity. It has to be isolated in a stepwise crystallisation procedure. The 99.9 % pure product acts as a strong toxic agent and inhibits the acetylcholinesterase of insects (EUROPEAN COMMISSION 2000). Fate studies identified the substance as persistent in the environment and led to its ban in European countries and worldwide restrictions of use (DUTTA & SCHAFER 2003).

Table II-4: Chemical and ecotoxicological characterization of gamma-HCH (lindane). Data taken from LUBW 1993, HERBST & VAN ESCH 1991. Chemical structure taken from http://www.alanwood.net/pesticides/lindane.html

IUPAC Name: 1,2,3,4,5,6-hexachlorocyclohexane Chemical formula: $C_6H_6Cl_6$	
	
Log P_{OW} (pH 7, 20°C)	3.69
K_{OC} [ml/g] (slightly mobile)	1100
Water solubility [mg/l] (20°C)	7.3-8.5
Vapour pressure [mPa] (25°C)	4.34
DT_{50} [days]	40-70
Application rates [kg a.i./ha] (soil treatment)	0.75-1.5

Lindane is an insecticide with a high toxic potential for soil invertebrates based on standard toxicity testing (e.g. LOCK *et al.* 2002) and is highly persistent in soil. It was designated as a

potential candidate as the reference substance in future standardized TME. Our approach was meant to deliver long-term data on long-lasting effects, which are expected from relatively persistent and toxic substances. MONFORTS (2006) described difficulties of persistence classification in different EU member-states, but lindane is generally described as a non-degradable compound. In the EU risk assessment scheme, higher-tier test options are triggered either by substance properties or by the outcome of tier-1 risk assessments using lower tier risk indicators. For lindane, the degradation half-life after spray application directly to bare soil is reported to be 4-6 weeks, hence showing a fast photo-degradation at a DT_{90} of 30-40 weeks in the dark (HERBST & VAN ESCH 1991). After mixing the substance into the soil though, 50 % of the substance was still found after 15-20 weeks in the upper soil-layer (0-10 cm); the DT_{90} was found to be between 2 and 3 years (ULMAN 1972).

II-3.1 Application procedure range-finding study

As the substance is presently not registered as a commercial formulation in the European Union, it was bought as technical product (Sigma Aldrich). Since lindane was not available as a commercial formulation, it was purchased as the technical product (Sigma-Aldrich). Two stock solutions consisting of 530 mg lindane (for later concentration c_1) and 5,300 mg lindane (for later concentration c_2) were prepared, respectively, in 200 ml solubilizer (12.5 % emulgator W [cyclohexyl-isocyanate] in DMF-solution [dimethyl-formamide] plus 800 ml deionized water. The vegetation was cut the day before application to facilitate the incorporation of the test item solution into soil. 10 TME were treated with a concentration c_1 (10 mg lindane/kg dry soil, which equals an application rate of 7.5 kg of nominal active ingredient per hectare) and 10 with c_2 (100 mg/kg = 75 kg a.i./ha), respectively. 100 ml of the preparation was homogeneously sprayed onto the soil surface of the TME. The sprayer was rinsed with 200 ml of tap water; another 400 ml of tap water were applied to each TME within the next 24 h. The total amount of liquid equals approx. 10 l/m^2.

II-3.2 Application procedure - dose-response study

The lowest treatment (c_1) was at an application rate of 0.024 kg lindane/ha, corresponding to 0.032 mg lindane/kg dry-weight of 5 cm topsoil. The dose was increased by a spreading factor of about 3, resulting in c_2 = 0.075 [0.1], c_3 = 0.24 [0.32], c_4 = 0.75 [1.0], c_5 = 2.4 [3.2] kg a.i./ha [mg a.i./kg dry weight soil]. A gamma-HCH formulation of 150 g a.i./L was supplied ready-to-use by the sponsor (Bayer CropScience AG). 72 % w/w of the solvent carrier Solvesso 150 ND (ExxonMobil Chemical Belgium, Hampshire, Great Britain) was mixed

with 13 % w/w Emulgator MPS (Lanxess Deutschland GmbH, Leverkusen, Germany) and 15 % w/w lindane (JLM Marketing Inc., Tampa, FL). A spray application method was used for homogeneous application of the test item onto the soil surface of TME (hand held spray boom (Gloria 172 RTG) with a conventional hydraulic nozzle).

II-4 Data analysis

In this work, the complexity of the data ascertained required the application of multifaceted statistical methods for both univariate and multivariate datasets. Ecological and ecotoxicological methods have been combined to uncover the basic principles in the field of terrestrial model ecosystematics.

II-4.1 Data transformations

For most of the statistical univariate and multivariate tests, the data were log-transformed with y' = ln (2y+1) prior the analysis to approximate normality and homogeneity of variances requirements. The rationales of data-transformations in a Principal Response Curve analysis were described by see VAN DEN BRINK *et al.* (1995). Further analysis of influences of different transformations, criteria of significance, appropriate statistical tests and sidedness of the problem are discussed in detail in the following chapters.

II-4.2 Coefficients of variation

The Coefficient of Variation (CoV) is a standardized measure of the variability of the distribution of an endpoint (here: often the density of an organism or an organism group in soil samples). It is expressed as the %-ratio of the standard deviation **σ** to the corresponding mean **μ** and calculated by Equation II-1.

Equation II-1: Calculation of the Coefficient of Variation (CoV). KÖHLER *et al.* 2002.

$$CoV = \frac{\sigma}{\mu} \cdot 100\%$$

II-4.3 Calculation of effect thresholds

Recently, there is no guidance how to report and interpret the results of outdoor TME effect studies with natural communities (a first draft was published by LEICHER in SCHÄFFER *et al.* 2011). In order to refer to any guidance the analysis of the dataset is closely leaned against the 'Guidance document on simulated freshwater lentic field tests (outdoor microcosms and mesocosms)' (OECD 2006).

II-4.3.1 Pre-testing for distribution and variance homogeneity

Kolmogorov-Smirnov tests for normal distribution as well as Cochran's test on homoscedasticity were performed either using the software SPSS 14 (and former versions, SPSS INC. 2005), or ToxRat Pro Version 2.09 (TOXRAT SOLUTIONS GMBH 2004), or Community Analysis Package Version 4.3.06 (based on HOMMEN *et al.* 1994). After pre-testing, parametric or non-parametric tests were chosen.

II-4.3.2 Non-normal parameter distributions by Mann-Whitney U-test

The results of the bait-lamina test were analysed as total feeding activity of all soil layers and as the depth specific activity per 5 mm soil layer. Pair wise Mann-Whitney U-tests (MANN & WHITNEY 1947) between treatments and controls were performed on the numbers of open holes. The ergosterol contents of the soil were statistically compared by applying the same U-test statistics. Pair wise tests were performed with the program package SPSS 14 (SPSS INC. 2005). The non-parametric tests were applied on non-normally distributed data.

II-4.3.3 Pair-wise comparisons by Student t-Test

Differences in dry weight of the plant biomass in the range-finding study between all treatments were compared by pair wise t-tests (GOSSET 1908). Pair wise tests were performed with the program package SPSS 14 (SPSS INC. 2005).

II-4.3.4 Effect thresholds by the Williams t-Test

If prerequisites of normality and variance homoscedasticity were fulfilled, the NOEC in effect studies was calculated by means of the multiple t-tests of WILLIAMS (1971, 1972). This test is similar to the Dunnett test but has more power to detect differences between controls and treatments due to its sequential approach. The Williams test is insensitive against a non-monotonous concentration-effect relationship, since it uses the maximum-likelihood estimate of the sample means rather than the original sample mean (achieved by a moving average procedure before testing). All Williams tests were performed one-sided with $\alpha = 0.05$ (5% level of significance) on ln-transformed data (for rationale see II-4.1). Williams's tests were

performed for each sampling day separately. Transformed data matched better the prerequisites of the statistical tests that are normal distribution and homogeneity of variances. PCA sample scores and diversity indices were not transformed. For the range-finding study, the control group (N = 20) was compared to the two treatment groups (N = 10 each). For the dose-response study, 12 controls were tested against five treatment groups with 6-fold replication each. For both studies, effect thresholds of the study endpoints in the ecotoxicological experiments were determined using the multiple t-test after WILLIAMS (1971, 1972) on ln-transformed abundances for oribatids, collembolans and lumbricids. The test was also applied on the non-transformed sample scores of the PCA to derive a NOEC community and on the index scores of the diversity analysis. Effect thresholds of similarity indices were derived qualitatively as 'interpreted NOECs', because the test replication was already used to calculate the indices, so that statistical testing was not possible. 'Interpreted NOECs' can be used for reasons of precaution where statistically non-significant results appear clearly dose-dependent and ecologically relevant due to the time-course of abundances after treatment. Williams's tests were conducted using the Community analysis software (HOMMEN *et al.* 1994).

II-4.3.5 Effective Concentrations by regression analysis

EC_x-values were calculated based on ln-transformed data for the total abundance of the four taxa (collembolans, oribatids, enchytraeids, and nematodes) and in addition on higher and lower taxonomic levels as families, genera and species, if specified in the data. A Linear Maximum Likelihood Regression model (Probit analysis) was used to derive a fit for the dose-response function. EC_x-values were considered valid and further interpreted if the following criteria of the goodness-of-fit were met:

- The model fitted explained of a significant part of the variation of the data.
- The p-value as an indicator of the actual Type-I-error rate had to be smaller than 5%.
- The data were not scattered around the computed dose-response function (probability of Chi^2-criterion).
- The slope of the response function was significantly different from zero.

The results are presented in conjunction with the corresponding NOECs in order to compare the treatment level necessary for the onset of effects. Calculation on population abundances was performed using the Community analysis program (first version by HOMMEN *et al.* 1994) and on PCA sample scores for the communities using ToxRat Pro Version 2.09, TOXRAT SOLUTIONS GMBH (2004).

II-4.3.6 Prospective power analysis

Prior each ecotoxicological experiment or environmental data collection, an estimate of the expectable variation of the data obtained should be made for a proper planning of a sampling design. For the design of ecotoxicological dose-response experiments the number of and the spacing between exposure levels, the number of replicates per dose level and the number of sampling dates has to be considered to ensure the desired power of an experiment. The variation of the data could be estimated by historical control data (ISO 2004).

Equation II-2: Number of necessary replicates by means of Dunnett's multiple t-test.

$$n_0 = \left[(1+\sqrt{a}) \frac{\sigma^2}{\delta^2} (t_{\alpha,\infty,r,1-a} + z_1 - \beta)^2 \right]$$

$$n = \left[(1+\frac{1}{\sqrt{a}}) \frac{\sigma^2}{\delta^2} (t_{\alpha,\infty,r,1-a} + z_1 - \beta)^2 \right]$$

With

σ = standard deviation of the mean

z = standardized random variable

t = studentized random variable

n_0, n = number of necessary replicates of the control and treatment groups

δ = safeguarded difference (% of control)

a = number of groups (without control group).

As always with null-hypothesis testing (e.g. to find a NOEC), the limits for the acceptance of the hypothesis are arbitrarily set by the user, but mostly adjusted to common agreements. Usually, a type-I error rate of 5 % (the error of falsely rejecting the null hypothesis α) and a type-II error rate of 20 % (the probability of falsely accepting the null-hypothesis β) is considered acceptable. The power of an analysis is then defined as 1-β, i.e. the probability of correctly rejecting the null-hypothesis is usually accepted to be not more than 80 % (KÖHLER *et al.* 2002). The post-hoc power is always a function of the observed p-value and sufficiently high when significant differences have been detected (HOENIG & HEISEY 2001). Directly interdependent variables in statistical hypothesis testing are the sample size N, the mean value μ, the standard deviation SD and the α- and β-error rates. It is then possible to derive necessary samples sizes to reach a desired confidence level with a given variation of the date as a CoV. In chapter V-3.2.1 the number of replicates for a test design with a control group and five exposure levels was calculated for the multiple t-tests after Dunnett by Equation II-2. The minimum detectable differences (MDD) in chapter V for the comparison of untransformed and transformed abundance data have been calculated

Equation II-3: Calculation of the Minimum Detectable Differences (MDD) between controls and treatments in ecotoxicological tests.

$$MDD = \bar{x}_1 - \bar{x}_1 = t^* \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

With

t* = critical value of t-statistics

n = number of replicates

s^2 = variance.

based on the Williams test by means of the Community Analysis software (HOMMEN *et al.* 1994) after Equation II-3.

II-4.3.7 Principal response curve analysis

A matrix of $m_{species}$ *x* $n_{samples}$ can be resolved for its most meaningful linear combination by applying a Principal Components Analysis method (PCA). Since our experiments focused on changes of soil communities caused by low to high treatments of a toxic test substance, the analysis was constrained on that part of variation that could be explained by the factor 'nominal concentration of test item'. This can be achieved by applying a Redundancy Analysis (RDA), which is the constrained form of PCA and uses a linear response model. The Principal Response Curve method (PRC) allows depicting changes in species composition of a community due to the effect of a toxic substance over time. The relative deviation from control level is displayed on the ordinate whereas temporal changes can be tracked on the abscissa. Thus, PRC provide options for interpretation of effects and recovery after disturbance, in general. Two diagrams show on the one hand the principal community response (c_{dt}), on the other hand the relative affinity (correlation) of each species to this response (species weights b_k). The method was derived from a redundancy analysis (RDA) that constrains the results of a principal component analysis (PCA) to the treatment related variability (VAN DEN BRINK & TER BRAAK 1997, 1998, 1999). Monte-Carlo permutation-tests indicate a significant influence of the treatment regime over the whole dataset and for each sampling date. To test for significant differences between treatments and controls, a PCA was performed for each sampling date separately. This stepwise procedure of calculations yields a pictorial representation of the community's response to the treatment, the Principal Response Curve (PRC), as well as statistical threshold concentrations of the community (No Observed Effect Concentration of the community = $NOEC_{community}$). As the main result of the analysis, both the explanatory power and significance is calculated and expressed graphically. The calculation of the PRC follows a further stepwise procedure, as indicated below.

Equation II-4: Formula to calculation the Principal Response diagram of a community.

$$y_{d(i)tk} = \bar{y}_{0tk} + b_k \cdot c_{dt} + \varepsilon_{d(i)tk}$$

$y_{d(i)tk}$=Log-transformed counts of taxon k, at time t, in treatment d and in replicate i

y_{0tk}=mean abundance of taxon k in control on sampling date t

c_{dt}=Principal Response of the community in treatment d on sampling date t

b_k=weight of species k with PRC (= affinity of species k to the PRCs)

$\varepsilon_{d(i)tk}$=error term with mean zero and variance σ^2_k for replicate i of treatment d on date t for species k

Step 1: Calculation of c_{dt} and b_k values by partial RDA

The whole dataset is used, and the time as well as the treatments is introduced as dummy var-

iables (for the use of dummies see LEPŠ & ŠMILAUER (1999) in a redundancy analysis. By firstly treating time and treatment as explanatory variables that are deleted in advance, these factors are excluded of the analysis. By treating time as a covariable, the 'temporal' portion of variance is deleted from the dataset. Time-treatment interaction terms are defined thereafter. Exclusively part of the variance is used that can be explained by the treatment effect per date. Control TME are excluded as well from the analysis (by excluding an interaction term for them) to express the treatment effects as deviations from the x-axis represented by the controls as constant. Equation II-4 was used for the calculation of the principal response.

Step 2: Testing for reliability of the whole analysis

To judge the analysis' reliability to reflect noticeable portions of treatment related variance, it can be referred to several parameters. The proportion of the total variance displayed in the diagram can be deducted from the canonical sum of all eigenvalues. To test if the diagram displays a significant portion of the total variance F-type Monte-Carlo permutation tests of the whole time-series is performed. Split-plots were permuted, but whole plots were not included in the permutation procedure (2000 permutations, reduced model in CANOCO software). A whole-plot (=block) in a mixed design like a TME study is represented by one TME cylinder and consists of several split-plots. Split-plots are sub-samples that are taken out of each TME at each sampling interval. Samples within a block (=whole-plot) are dependent, levels of treatment factors are independent (=mixed design). Probabilities of type I error below 0.05 ($\alpha < 0.05$) were accepted as significant, because of the high variability in natural multivariate datasets. If all requirements are fulfilled it was proceeded with step 3. Otherwise the analysis was stopped.

Step 3: Significance of the treatment regime per sampling date by RDA

In addition, a redundancy analysis, restricted to each sampling date, followed by Monte-Carlo permutation tests, gave information if the treatments showed treatment related significant differences in community structure at this date. Therefore, $\log_{10}$-transformed nominal concentrations of the test item were used as explanatory variables.

Step 4: Calculation of $NOEC_{Community}$ by PCA and Williams-test

If there were treatment related effects at certain sampling dates with $p < 0.05$ of the Monte-Carlo-Permutations of RDA per date, a Principal Component Analysis was applied to the data of that sampling date. The resulting sample scores were used as inputs in a Williams-test (WILLIAMS 1971, 1972) in order to calculate the Community-NOEC (VAN DEN BRINK & TER BRAAK, 1998, 1999).

Step 5: Interpretation of the analysis

The species weights (b_k) and the principal response per sampling date and treatment (c_{dt}) allow to calculate the predicted response for each taxon at a given time and treatment by the term exp (b_k c_{dt}). The exponential function has to be used because the data are log-transformed (using the CANOCO default transformation y' = ln (2*y +1)) before the analysis (for the rationale see VAN DEN BRINK *et al.* 1995). Details of the permutation tests used for the PRC analysis can be found in VAN WIJNGAARDEN *et al.* 1995. One has to be aware that minor deviations from control in a time-series could be founded in a seasonal decline of populations of the untreated communities. This will be one of the main objectives of the interpretation of results. In the dose-response study, the statistical analyses for the group of collembolans by the Principal Response Curve method were conducted separately for the dataset of the first year until five months after application of the test item ('*set 5*'). This was because the sampling design for the worms was not congruent with the design for microarthropods in terms of sampling intensity (Figure II-9) Secondly, the analysis was repeated for the collembolans based on the complete dataset until one year after application ('*set 12'*). This was done to check, if the insecticide likewise affected the collembolan community in different time intervals of 5 and 12 months.

II-4.4 Classification of effects

VAN DER LINDEN *et al.* (2006) proposed a five-part classification system for effects of pesticides for soil communities based on the model of BROCK *et al.* 2006 that was deduced from

Table II-5: Classification of effects based on NOEC-values. Synopsis of two TME studies, conducted separately in the years 2005 and 2006. The study period in both tests was about one year after application of the test item. The effect classes are defined accordingly to VAN DER LINDEN *et al.* (2006).

1	**No treatment-related** effects
2	**Slight treatment-related** transient effects, usually on one or a few isolated sampling dates only
3	**Clear effects** on several consecutive sampling dates, lasting **less than 2 months** post last application of the test item in the test system
4	**Clear effects** on several consecutive sampling dates, lasting **longer than 2 months** but **full recovery within 1 year** post last application of the test item in the test system
5	**Clear long-term effects**; **full recovery not within 1 year** post last application of the test item in the test system

aquatic mesocosm studies. The proposal was also picked up by SCHÄFFER *et al.* (2011). A classification system has some major advantages for the interpretation of the complex results of a semi-field study that includes many different taxa.

It can

- give a good overview of the experimental results,
- facilitate the comparison of different experiments, and
- be used to rank transient effects and show the dose-dependency of the effects by a clear arrangement.

The definition of the specific effect classes is given by Table II-5. The classification of effects has to be done with respect to test concentrations and serves as indication of a No Observed Ecologically Adverse Effect Concentration for one of the doses tested. The No Observed Ecologically Adverse Effect Concentration (NOEAEC) has been defined as a concentration that can be classified as 'effect class 1' or 'effect class 2' (no or slight treatment related effects) and should be an integrative judgement based on all results for a particular taxon or a community endpoint like the effect thresholds derived from PRC analysis. A population or the community has been defined as resuming normal reproduction and population growth after chemical stress. Since the TME studies have been designed for persistent substances and many soil species have relatively slow reproduction rates, the time to full recovery is set to a minimum of 12 months. Due to the limited number of sampling dates in the TME studies at hand (not more than 6 samplings in the dose-response study), it was not possible to stipulate several dates without effects for assuming at least partial recovery of the soil communities. It was then decided to assume a partial recovery for the first sampling at which no statistically significant difference to controls was observed. The $NOEC_{community}$, the $NOEC_{population}$ and the time taken for complete recovery to the control level were then used to determine a No Observed Ecologically Adverse Effect Concentration (NOEAEC).

II-4.5 Ecological indicators

II-4.5.1 Similarity indices

Effect studies

The analysis of similarity indices for the ecotoxicological tests (range-finding study and dose-response study, see chapters III and IV) focused on the relative abundance of species (Stander's index) and the occurrence of rare species (Steinhaus' index). These were used to complement the PRC analysis (SMITH 1986; BOYLE *et al.* 1990; ENGELS & RATTE 1992) and calculated by the community analysis software (HOMMEN *et al.* 1994). The Stander`s index was proposed by HEIMBACH & RATTE (1997) as a suitable measure for the analysis of aquatic mesocosm data and is here adapted for the comparison of treated and control TME. It focuses on the relative abundance of each species in a community rather than on the absolute abundance value. Therefore, it is robust against the occurrence of lot of rare species, compared to e.g. the Steinhaus index. To assist the interpretation of the results the Steinhaus index is calculated and presented additionally to the Stander's index. It can deliver information on the frequency of rare species, in cases the pattern deviates clearly from the Stander's index. The Steinhaus index is calculated after Equation II-5. The Stander's (or Cosine index) algorithm of similarity is defined by Equation II-6.

Equation II-5: Calculation of Steinhaus Index of similarity.

$$S_{Steinhaus} = \frac{2 \cdot W}{\sum n_{ik} + \sum n_{jk}}$$

Where W is the sum of minor abundances (>0) of considered sample pairs; n_{ik} and n_{jk} the absolute abundance of species k in sample i or j, respectively.

Equation II-6: Calculation of Stander's Index.

$$S_{\mathrm{Stander}} = \frac{\sum p_{ij} \cdot p_{ik}}{\sqrt{\sum p_{ij}^2 \cdot \sum p_{ik}^2}}$$

Where $S_{stander}$ is Stander's index; p_{ij} , p_{ik} is the proportion of species *k* in samples *i* and *j* in relation to all species, respectively.

Classification methods

Polar ordination and classification methods required the calculation of similarity indices after Bray-Curtis (synonyms: Soerensen or Czekanowski or Dice coefficient) and Stander. The corresponding cluster analyses were calculated using the software 'SPSS' (SPSS 2005) and 'PCORD' (MCCUNE & MEFFORD 1998). To apply the binary, non-parametric Bray-Curtis index that describes the similarity of any two communities, the data were either dummy coded, which means the presence-absence of species was noted, or it was treat-

Equation II-7: Calculation of the Bray-Curtis Index

$$S_{BrayCurtis} = 100 \cdot \left(1 - \frac{\sum \left| n_{jj} - n_{ik} \right|}{\sum \left(n_{ij} + n_{ik} \right)}\right)$$

Where $S_{Bray\text{-}Curtis}$ is the Bray–Curtis index; n_{ij} and n_{ik} is the abundance of species k in samples i and j, respectively.

ed as quantitative variables. In spite of the fact that the index was developed originally for presence-absence data, it works equally well with quantitative data (ROBERTS 1986). The Bray-Curtis Index is defined by Equation II-7.

DGGE patterns

For the analysis of the DGGE band-patterns the correlation index of Pearson was used (characteristics of this index were described in BORTZ 1985). It includes information of the species abundance, i.e. on the intensity of bands in this case, respectively.

II-4.5.2 Diversity indices

Effects of lindane on the (species)-biodiversity were described using Shannon's index, considering the species richness and evenness (SHANNON 1948; BOYLE *et al.* 1990). The Shannon index depends on the species richness and on the frequency distribution of the individuals of the species and gets larger the more species were found and the higher the evenness of the community. (Equation II-9). The evenness was calculated by dividing the Shannon-index by the maximum possible value (Shannon-Index if all species are equally abundant). The influence of the number of species is neglected. The maximum evenness is one while few very dominant species result in low evenness values (Equation II-8). By plotting the Shannon index in connection with evenness and species richness, it can be deduced whether changes of the index are caused by alterations of the one or the other predictor.

Equation II-9: Calculation of the Shannon-Index of Diversity

$$H_S = -\sum p_j \cdot \ln(p_j)$$

with H_S = Shannon Index, p_j = relative abundance of species j

Equation II-8: Calculation of evenness based on Shannon-index of diversity.

$$E = \frac{H_S}{\ln(n)}$$

with E = Evenness, H_S = Shannon-Index, n = number of species

II-4.5.3 Maturity index

Maturity indices for the communities of nematodes were calculated as indicators of disturbance. The equations and classifications of BONGERS (1990) were applied.

II-4.6 Ecological gradients and classes

Multivariate ordination techniques were originally designed by plant sociologists to uncover transient patterns in the composition of vegetation (WHITTAKER 1967). A great variety of multivariate techniques of community data in ecology and ecotoxicology has been developed since then. Firstly, the polar ordination method of BRAY & CURTIS (1957) became the most popular ordination technique. Nowadays, different types of correspondence analyses dominate the scientific community, originating in the work of HILL (1973) based on weighted averages

rather than on ecological distance measures. Multivariate analyses were conducted by means of the CANOCO Software Package 4.5. The software was originally described by TER BRAAK (1988, 1990). The latest version was developed in 1998 by TER BRAAK & SMILAUER. In this thesis, various multivariate ordination techniques have been used which will be introduced in some detail in the following.

II-4.6.1 Polar Ordination

The polar ordination method is based on similarity indices as an index of the ecological distance between two objects that should reflect homologous to the ordination methods the maximum possible portion of the variance in the dataset (BRAY & CURTIS 1957). The method is the basis of more complex a robust methods, e.g. Principal Coordinate Analysis (PCoA) or non-metric multidimensional scaling (NMDS). An overview of numerous multivariate methods for the analysis of ecological data gives OTTERMANNS (2008).

II-4.6.2 Cluster analysis

Cluster analysis is based on the calculation of similarity indices or distance measures, comparable to the polar ordination method. All kinds of indices can be used, dependent on the data basis and the special purpose of the analysis. For an overview of similarity calculations, see chapter II-4.3.7 and the literature cited therein. The band patterns as the result of DGGE analysis were analysed by means of the GelCompar II software (Applied Maths, Gent, Belgium). Data were analysed as the relative intensity of a clearly discriminable band at a certain position on the electrophoresis gel. Phylotypes were identified as 'species-surrogates' on the gel and coded by theirs absolute position where the movement of the DNA fragments stopped under the given experimental conditions. In the nomenclature system, 'B60.5' means that the band stopped after 60.5 % of the reference-distance, which that is marked per definition by the least degrading component. The intensity of a band is afflicted with its relative density reflecting its dominance within the population of DNA fragments of different lengths (FROMIN *et al.* 2002). The DGGE patterns of the different treatments (c_0, c_3, c_5) were analysed for their similarity by using the correlation index of Pearson (BORTZ 1985). The unweighted pair group method with arithmetic mean (UPGMA) was applied to find clusters of most similar fungal communities (GOMES *et al.* 2003, OROS-SICHLER *et al.* 2005).

II-4.6.3 Canonical correspondence analysis

The canonical correspondence analysis is based on a weighted average method. The sample scores are computed as weighted averages of the species cores, then regressed on the environmental variables of interest, then the species scores are calculated from the regression.

Only the part of variation of the species-per-samples matrix that can be explained by a regression to the environmental variables is displayed (TER BRAAK 1986, OTTERMANNS 2004). The analysis was conducted by the CANOCO software (TER BRAAK 1988). Here, it was tested (section V-4) if differences in the climate data between the coring site in Monheim a. Rh. and the experimental field site in Aachen has profound influence on the species composition in field and corresponding TME samples. The data were square-root transformed to dampen the influence of extreme values (e.g. singletons).

II-4.6.4 Partial correspondence analysis

Ecologists are usually not interested or capable to measure all factors influencing the communities investigated, but the most decisive ones have to be hypothesized. For multivariate datasets, in order to exclude the factors of no particular interest from further analyses, the application of partial constrained ordination methods (variance decomposition) has been widely recommended (WHITTAKER 1984). The basic theory and methodology was described by TER BRAAK & PRENTICE (1988). The methodology and terminology within the 'Canoco' software is described by LEPŠ & ŠMILAUER (1999). Here, the influence of soil removal compared to the temporal variation of species data was desired to describe in chapter V-2.4. For this reason, a series of redundancy analyses (RDA) in Canoco were set up, using 'days after start of the experiment' (complies with 'time') and percentage soil of soil removal was used as explanatory variables and covariables, alternatingly. First, the variance explained by the co-variables was removed from the dataset, and the remaining information was constrained to the environmental variables. The significance of the relationships between species data and environmental data were tested by unrestricted Monte-Carlo permutation tests that were already implemented in the software. Control values only of the data obtained in the pre-study, the range-finding study and the dose-response study. In the pre-study, means of either 7 or 2 sub-samples per TME were calculated and further analysed. The parameter values were used as days after start of the experiment ('das') for the variable 'time' and as areal percentage of soil removed before the corresponding sample. Since a TME has an inner diameter of 295 mm and each of the subsample soil cores has a diameter of

Table II-6: Percentage of TME soil removed (upper 5 centimetres) by each sub-sample.

Inner diameter of TME (mm)	295
Total TME area (mm^2)	68349
Area of 1 subsample (mm^2)	1963

Number of sub-samples taken	Percent soil removed before next sampling
1	2.87
2	5.74
3	8.61
4	11.48
5	14.35
6	17.22
7	20.09

50 mm, each sample decreases the total area of a TME on the upper 5 centimetres by 2.87 % (see Table II-6).

During the dose-response study, enchytraeids have been sampled at day -1, 88 and 149 and the number of possible cores was increased by one compared to the pre-study and the range-finding study from 7 to 8. This was included in the calculations as additional soil losses. The losses due to nematode sampling were ignored in the calculation because of the negligible contribution to the total loss.

II-4.7 Geostatistical autocorrelation analysis

In order to analyse the spatial variability on the coring area, an intensive field screening was conducted (for sampling design see chapter II-1.1). Each sample location was attributed to geographical x- and y-coordinates to allow for the calculation of the distance of each sampling point to each other on the sampling grid. In a first step, an *index of patchiness* was calculated. An index indicates if the arrangement of species differs significantly from a random distribution (PERRY 1995). The *big-scale estimators* were randomly distributed over the sample area, so it was assumed that randomly distributed species would not differ from the assumptions of the model. Lloyd's index of patchiness (*liop*) was used to determine the level of aggregation. If *liop*-estimate is greater than one, this indicates that the pattern is aggregated. As *liop* increases, the degree of aggregation increases on his part. If *liop* is less than one, a random pattern can be assumed (XIAO *et al.* 1997). The following equation was used to determine *liop* for a respective set of samples:

Equation II-10: Calculation of the index of patchiness *liop* (XIAO *et al.* 1997)

$$liop = \frac{mean + \mathrm{var}\,iance / mean - 1}{mean}$$

In a second step, the *spatial autocorrelation* between samples was calculated for all species and the total abundances of the organism groups. The distance between two samples at which no autocorrelation of the abundance distribution of the species could be detected, marks an important threshold and is called the ‘range’ of the variogram. It cannot be assumed that the distribution is homogenous beyond the threshold distance. A proper coring design for TME soil cores should be adjusted to this distance. Soil cores should be possibly cored within the autocorrelation distance to get as homogenous TME as possible. The spatial autocorrelation differs for each of the species and for the total abundance of certain taxa.

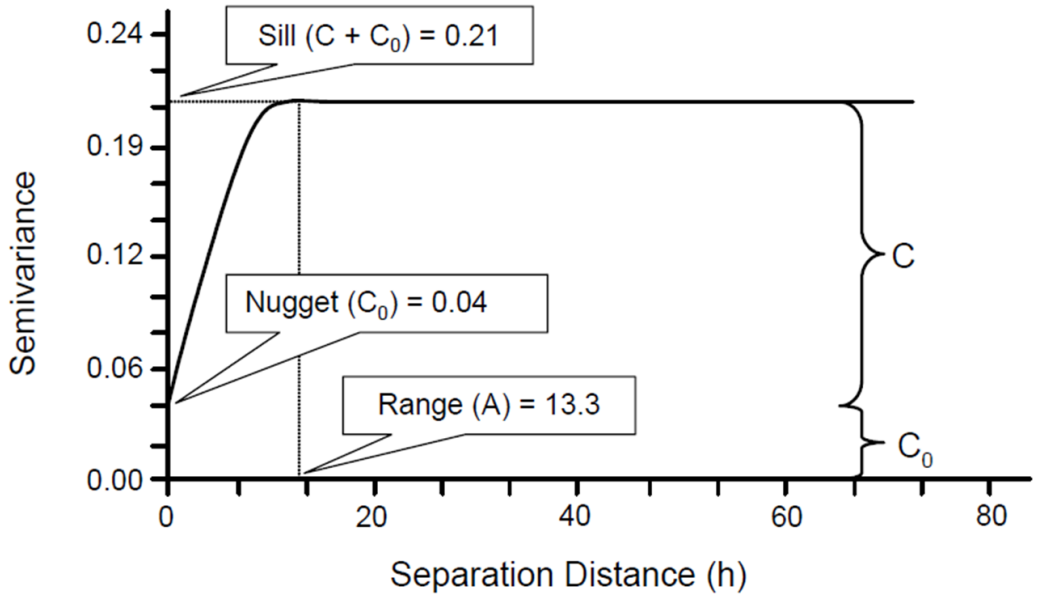


Figure II-11: Generalized variogram model and decisive points of a semivariogram curve. Graphical representation taken from GS+ user's guide (Gamma Design Software 2004).

Thus, a pragmatic expert judgement is needed to decide about the coring grid. Within the terminology of the geostatistical area, this distance is called 'range', which is the base for the conclusions of chapter V-1, based on the collembolan data as shown by Table V-3. The semivariance was calculated by the formula of ROSSI *et al.* 1992, LIEBHOLD *et al.* 1993 (Equation II-11).

Equation II-11: Calculation of semivariance.

$$\gamma(h) = \frac{1}{2n(h)} + \sum \left[x(s_i) - x(s_{i+h}) \right]^2$$

With

n(h): number of sample pairs of distance h

$x(s_i)$: abundance of first sample of a sample pair

$x(s_i+h)$: abundance of second sample of sample pair.

The relation between semivariance and sample distance can be represented graphically as a semivariogram and modelled to find the best-fit function and its parameters. The model gives evidence of the nugget (y-intercept=C_0), the sill ($C+C_0$) and the range (A_0). The nugget is the variance due to sampling errors or due to the fact that the range is below the scale under investigation (mainly dependent on the minimum distance between samples due to the maximum possible spatial resolution of the methodology). The sill (model asymptote) marks the distance above which no autocorrelation occurs. The range marks the separation distance over which no dependence occurs (i.e. structural variance is displayed in the range) (also sometimes distinguished between effective range A and model's range A_0). In the case at hand, it indicates the maximum distance at which a 'homogenous set of soil cores' could be expected. The last step of the geo-statistical analysis was to *interpolate* between the sampling points. This procedure could facilitate the interpretation of results because the sampling in the current study

was not done by using a regular pattern but a random design. Thus, the distances between samples were unequal and thus it was difficult to judge on the large-scaled gradients occurring on the coring area. This analysis was closely combined with the index of patchiness analysis (as shown by Table V-3). The result was a map of the area sampled (on different grids: *bsc*, *ss1*, *ss2*), by using *kriging* algorithms. Lloyd's index of patchiness was calculated using Equation II-10. Comparative cokriging and semivariance analyses as well as visualisation mapping were done using a variety of free software and shareware as SGems, SADA, Variowin, SAM, Explostat; however, the final analyses were conducted by means of the GS+ 9.0 software (GAMMA DESIGN SOFTWARE 2004).

III

Effects of lindane to soil communities: range-finding, design and method development

This chapter documents the technical design and the results of an ecotoxicological experiment using open and intact soil cores as Terrestrial Model Ecosystems (TME), which contain a diverse grassland community of soil organisms. By installing the TME outdoors, soil organisms were expected to reflect natural dynamics and interactions. The aim of the study was to investigate whether outdoor TME could detect changes of the community structure of oribatids and collembolans after treatment with high dosages of the model compound γ-HCH (lindane). Lindane has a DT_{90} in common European agricultural soils of between 40 and 70 days (HERBST & VAN ESCH 1991) and high potential toxicity to soil invertebrates based on standard toxicity testing (e.g. LOCK *et al.* 2002). Due to its bio-accumulative potential and possible long-distance transport it is classified as a persistent organic pollutant (POP); Lindane has been banned in the European Union and many other countries worldwide for the use in agricultural pest control (EUROPEAN COMMISSION 2000a).

III-1 Results

III-1.1 Environmental parameters

One of the most important factors affecting the density and occurrence of soil organisms is the soil moisture. Hence, some effort was given to its measurement. Figure III-1 shows major fluctuations of soil humidity during the summer months. Shortly after heavy rain events, the water content tended to decline rapidly due to strong evaporation. This effect was more clearly visible in the upper soil layer (approx. 5 cm depth from surface), whereas the moisture of deeper layers of about 30 cm distance to the soil surface were buffered much better. The impact on the performance is discussed below.

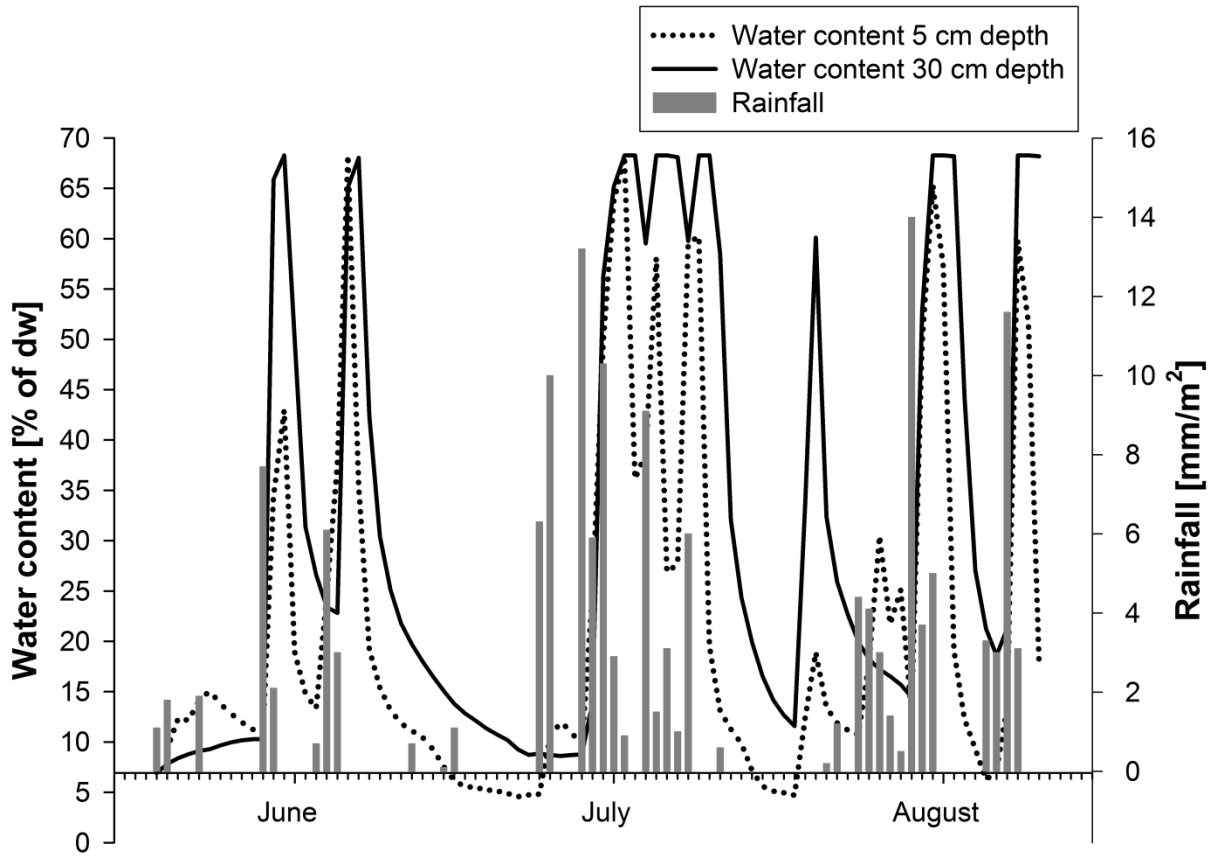


Figure III-1: Water content as calculated from measured matrix potential and rainfall (available on the Internet: www.wetteronline.de [2005]). A maximum water content of 68 % was assumed to be valid for this soil type.

III-1.2 Effects on collembolans

In sum, 29 collembolan species belonging to 12 families were found (Table III-1). Isotomids

were the most abundant family, at 69 % of the total abundance as mean of all treatments and dates, followed by entomobryids (21 % of the total).

The untreated community was dominated by two isotomid species, *Lepidocyrtus cyaneus* and *Desoria trispinata* at 26 and 24 % of all individuals per year on average, followed by *Lepidocyrtus lanuginosus* (14 %), *Parisotoma notabilis* (8 %), *Isotoma viridis* (6 %) and *Isotomurus palustris* (6 %). The species mentioned above live in the humus- or litter layer without exception.

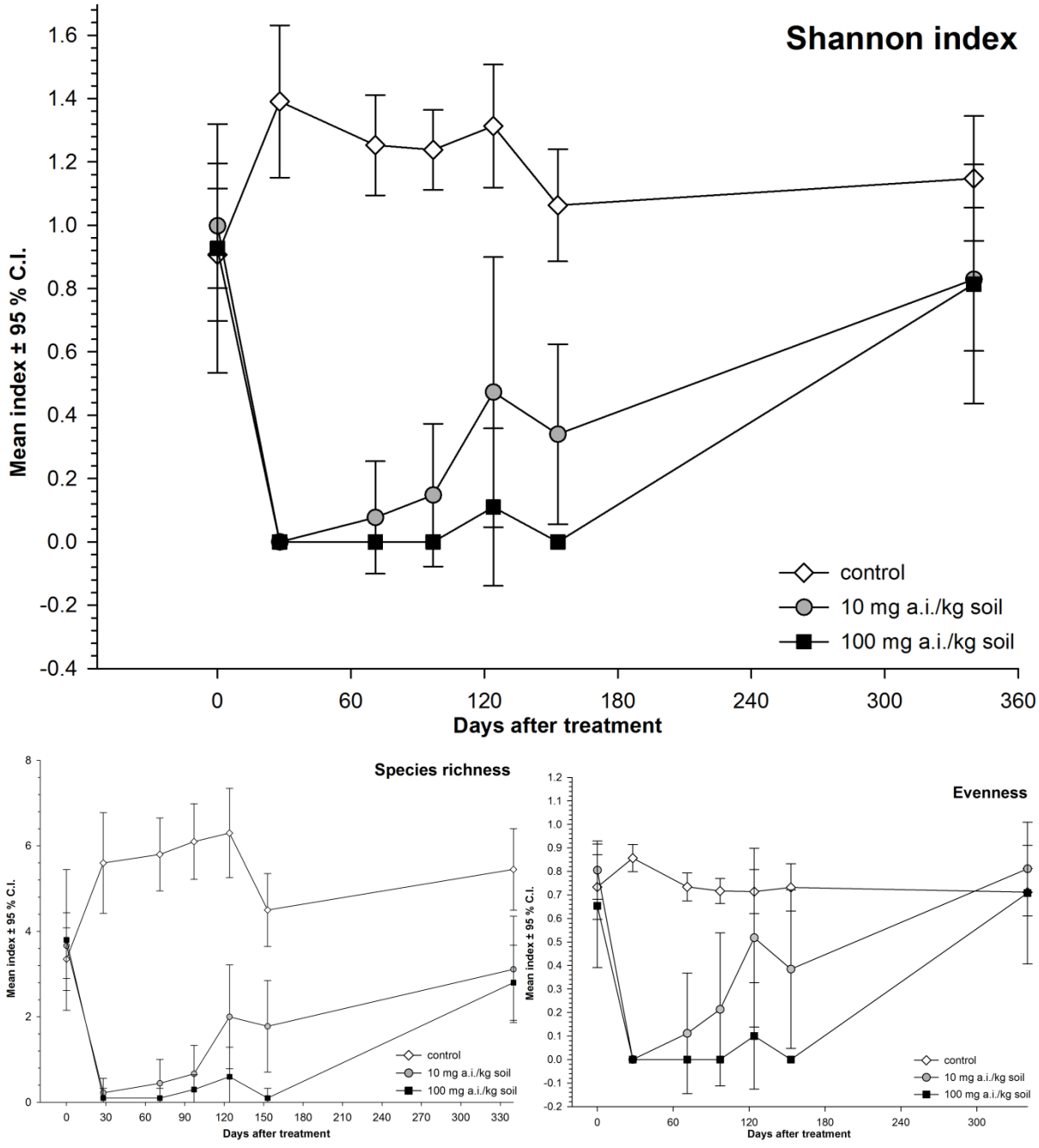


Figure III-2: Diversity after Shannon for collembolans. Additionally, evenness and species richness are given, in order to facilitate interpretation of results.

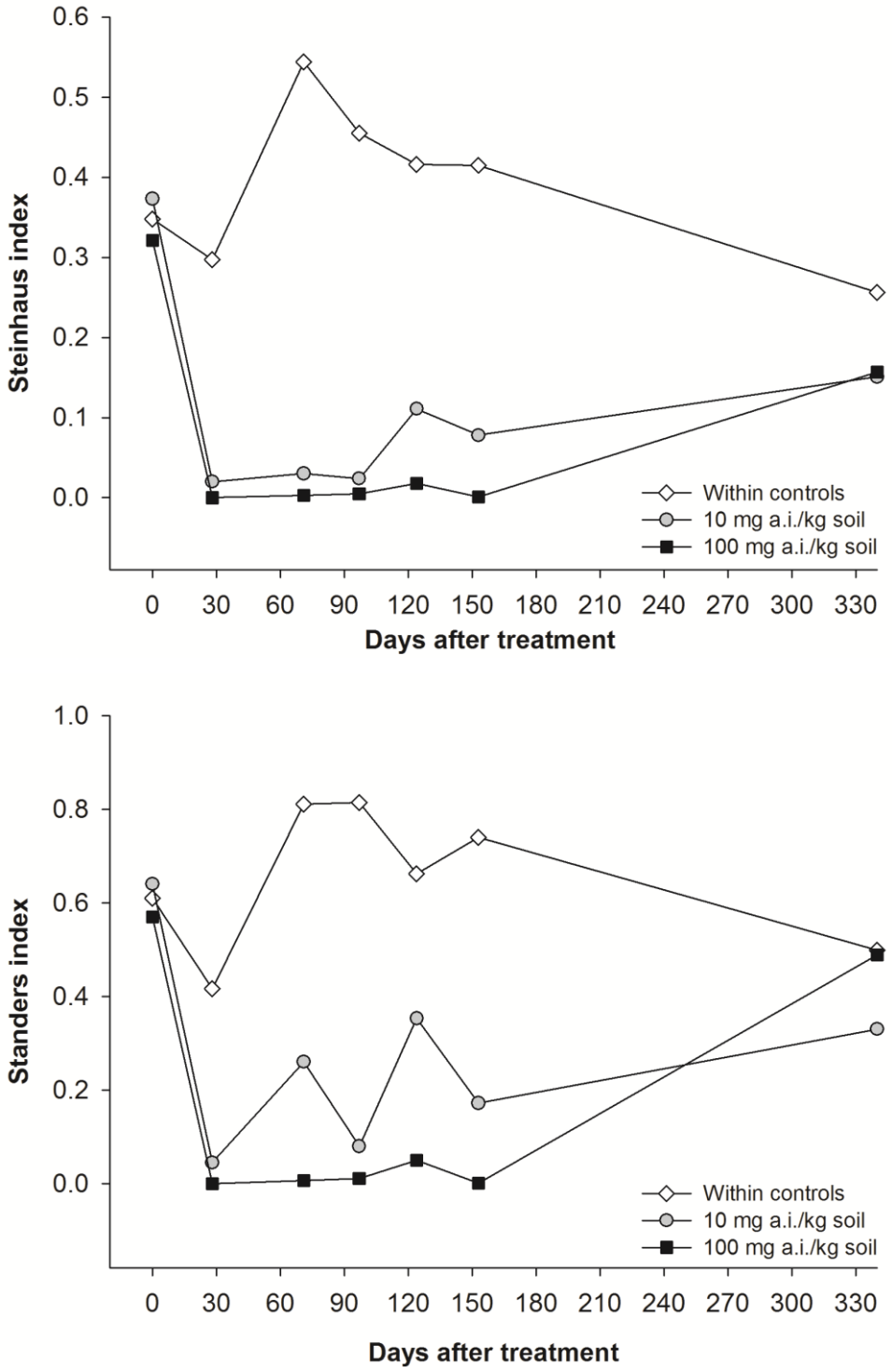


Figure III-3: Similarity within controls and between controls and treatments.

All juvenile specimen and ambiguously identified individuals have not been included in the community analysis and are thus not shown by Figure III-5. In 2005, the total abundance and the collembolan populations showed a relative maximum of the mean of 44 individuals per sub-sample in April 2006 (Figure III-4).

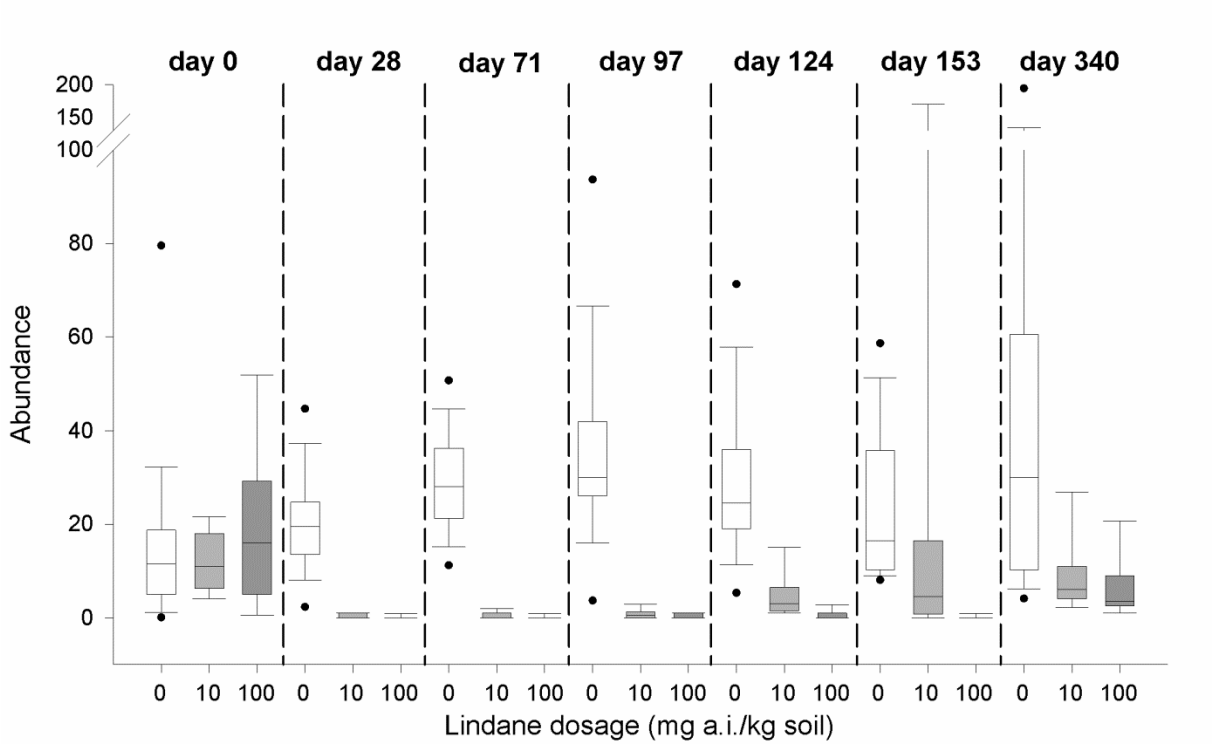


Figure III-4: Boxplots of total abundance of collembolans. Open bars control group (N = 20), light grey 10 mg a.i./kg soil, dark grey 100 mg a.i./kg soil. Filled circles $5^{th}/95^{th}$ %percentile, if not present no or too little data points beyond $10^{th}/90^{th}$ % percentiles

The smallest mean abundance of 14 individuals per sub-sample was registered in May 2005. Amongst the organisms groups captured by the TME experiments, the community of collembolans and the rare findings of Gamasid mites (results not shown) were particularly affected by the treatment. The total abundance of collembolans was significantly lowered by both lindane concentrations c_1 and c_2 during the complete study period (Figure III-4 Williams test; two-sided $\alpha < 0.05$; $N_{controls} = 20$; $N_{treatments} = 10$). The abundance of the dominant species was also a sensitive endpoint to reflect the effects of the treatments. *D. trispinata* showed initial effects of both treatments, followed by a recovery of the mean abundance in c_1. Large variation was observed for the control population of *D. trispinata* at day 340, which may have masked the statistical difference from the untreated control TME, leading to an erroneous interpretation of recovery.

In general, the treatments caused an irreversible decrease in abundance during the whole experimental period (i.e. NOEC < 10 mg a.i./kg soil in Table III-1).The Shannon diversity and evenness of collembolans were reduced significantly at all dates except the last sampling at day 340 (Table III-1). However, a mean species number of three was observed in the treated TME at day 340, which was compared to the controls (> 5) clearly lowered. Similar to the other endpoints, the PRC showed great homogeneity at day 0, contrasted by pronounced deviations from control level after treatment (Figure III-6). The treatment regime significantly altered the community structure of collembolans until day 340 (Table III-1).

Table III-1: Species found in the TME-range-finding study and the NOEC values (mg lindane/kg dry soil) of all end-points for collembolans, oribatids, lumbricids, plant biomass and feeding activity. Williams multiple t-Test, α<0.05)

	Lindane NOEC (mg a.i./kg soil)					
	Days after treatment					
	28	71	97	124	153	340
Endpoints (collembolans)						
PRC	<10	<10	<10	<10	<10	<10
Diversity Shannon	<10	<10	<10	<10	<10	100
Diversity Evenness	<10	<10	<10	10	<10	100
Diversity taxa richness	<10	<10	<10	<10	<10	<10
Similarity (Steinhaus' index)	<10	<10	<10	<10	<10	100
Similarity (Standers' index)	<10	<10	<10	<10	<10	100
Total abundance	<10	<10	<10	<10	<10	<10
Lepidocyrtus cyaneus (Tullberg, 1871)	<10	<10	<10	<10	<10	<10
Desoria trispinata (MacGillivray, 1896)	<10	<10	10	<10	10	100
Lepidocyrtus lanuginosus (Gmelin, 1788)	<10	<10	<10	<10	100	<10
Parisotoma notabilis (Schäffer, 1896)	<10	<10	<10	<10	<10	100
Isotoma viridis (Bourlet, 1839)	<10	<10	<10	<10	<10	100
Isotomurus palustris (Müller, 1776)	<10	<10	<10	<10	100	100
Lepidocyrtus lignorum (Fabricius, 1775)	<10	<10	<10	<10	<10	100
Isotoma anglicana (Lubbock, 1873)	100	<10	<10	<10	<10	na
Sphaeridia pumilis (Krausbauer, 1898)	<10	100	<10	100	100	100
Desoria tigrina (Nicolet, 1841)	<10	<10	<10	<10	<10	<10
Sminthurinus aureus (Lubbock, 1862)	<10	<10	100	100	100	100
Brachystomella parvula (Schäffer, 1896)	<10	<10	<10	<10	<10	<10
Orchesella spec. (Templeton, 1835)	100	100	100	10	100	na
Entomobrya spec. (Rondani, 1861)	-	-	-	-	-	-
Heteromurus nitidus (Templeton, 1835)	-	-	-	-	-	-
Pseudosinella alba (Packard, 1873)	-	-	-	-	-	-
Tomocerus minor (Lubbock, 1862)	-	-	-	-	-	-
Tomocerus vulgaris (Tullberg, 1871)	-	-	-	-	-	-
Ceratophysella spec. (Börner, 1932)	-	-	-	-	-	-
Xenylla spec. (Tullberg, 1869)	-	-	-	-	-	-
Desoria neglecta (Schäffer, 1900)	-	-	-	-	-	-
Folsomia spec. (Willem, 1902)	-	-	-	-	-	-
Frisea mirabilis/truncata agg. (Tullberg, 1871)	-	-	-	-	-	-
Neanura muscorum (Templeton, 1835)	-	-	-	-	-	-
Stenaphorurella quadrispina/denisi agg. (Börner, 1901)	-	-	-	-	-	-
Mesaphorura krausbaueri (Börner, 1901)	-	-	-	-	-	-
Deuterosminthurus repandus (Bourlet, 1842)	-	-	-	-	-	-
Dicyrtomina spec. (Lubbock, 1862)	-	-	-	-	-	-
Sminthurus viridis (Linnaeus, 1758)	-	-	-	-	-	-
Endpoints (oribatids)						
PRC	<10	<10	<10	10	10	10
Diversity Shannon	<10	<10	<10	<10	<10	<10
Diversity Evenness	<10	<10	<10	<10	100	<10
Diversity taxa richness	<10	<10	<10	<10	<10	<10
Similarity (Steinhaus' index)	<10	<10	<10	<10	10	10
Similarity (Standers' index)	<10	<10	<10	<10	10	10
Total abundance	<10	<10	100	10	10	10
Scheloribates laevigatus (C.L. Koch, 1836)	<10	<10	100	100	10	10
Achipteria coleoptrata (Linne, 1758)	<10	<10	<10	<10	<10	<10
Galumna obvia (Berlese, 1915)	100	<10	<10	<10	<10	<10
Eupelops occultus (C.L. Koch, 1835)	<10	<10	10	<10	<10	<10
Platynothrus peltifer (C.L. Koch, 1839)	<10	<10	100	100	<10	100
Minunthozetes semirufus (C.L. Koch, 1841)	100	<10	100	100	100	100
Liebstadia similis (Michael, 1888)	100	100	100	100	100	<10
Ctenobelba pectinigera (Berlese, 1908)	-	-	-	-	-	-
Nothrus spec. (C.L. Koch, 1835)	-	-	-	-	-	-
Belba spec. (von Heyden, 1826)	-	-	-	-	-	-

Table III-1 continued

	Lindane NOEC (mg a.i./kg soil)					
	Days after treatment					
	28	71	97	124	153	340
Gustavia fusifer (C.L. Koch, 1841)	-	-	-	-	-	-
Gustavia microcephala (Nicolet 1855)	-	-	-	-	-	-
Oppiella nova (Oudemans, 1902)	-	-	-	-	-	-
Ramusella clavipectinata (Michael, 1885)	-	-	-	-	-	-
Oribatula tibialis (Berlese, 1916)	-	-	-	-	-	-
Tectocepheus velatus agg. (Michael 1880)	-	-	-	-	-	-
Endpoints (lumbricids)						
Diversity Shannon	ns	ns	ns	ns	ns	10
Diversity Evenness	ns	ns	ns	ns	ns	10
Diversity taxa richness	ns	ns	ns	ns	ns	100
Similarity (Steinhaus´ Index)	ns	ns	ns	ns	ns	100
Similarity (Standers´ Index)	ns	ns	ns	ns	ns	100
Total abundance	ns	ns	ns	ns	ns	100
Allolobophora spec. juv.	ns	ns	ns	ns	ns	10
Lumbricus castaneus (Savigny, 1826)	ns	ns	ns	ns	ns	<10
Lumbricus terrestris (Linnaeus, 1758)	ns	ns	ns	ns	ns	<10
Allolobophora caliginosa (Savigny, 1826)	-	-	-	-	-	-
Allolobophora cupulifera (Tétry, 1943)	-	-	-	-	-	-
Allolobophora icterica (Savigny, 1826)	-	-	-	-	-	-
Allolobophora rosea (Savigny, 1826)	-	-	-	-	-	-
Dendrodrilus rubidus (Savigny, 1826)	-	-	-	-	-	-
Endpoints (plant biomass)						
Dry weight	n.s	ns	<10	<10	<10	ns
Endpoints (feeding activity)						
Total	100	ns	ns	ns	ns	ns

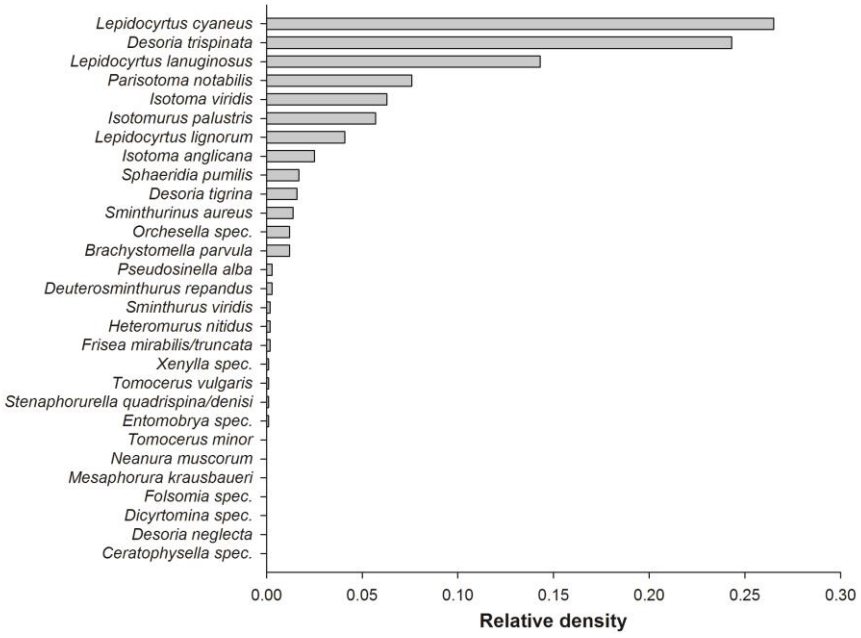


Figure III-5: Dominance spectrum of all sampling dates (control samples only) for collembolans.

PRC statistics Significance of 1st canonical axis (Monte Carlo permutation test)		% of total variance captured by		% of treatment variance displayed in 1st PRC
Collembolans				
Eigenvalue	0.213	Time	18.9	
F-ratio	92.255	Difference between replicates	52.7	**75.1**
p-value	0.0005	Treatment	28.4	
Oribatids				
Eigenvalue	0.145	Time	7.7	
F-ratio	48.346	Difference between replicates	72.6	**73.8**
p-value	0.0005	Treatment	19.7	

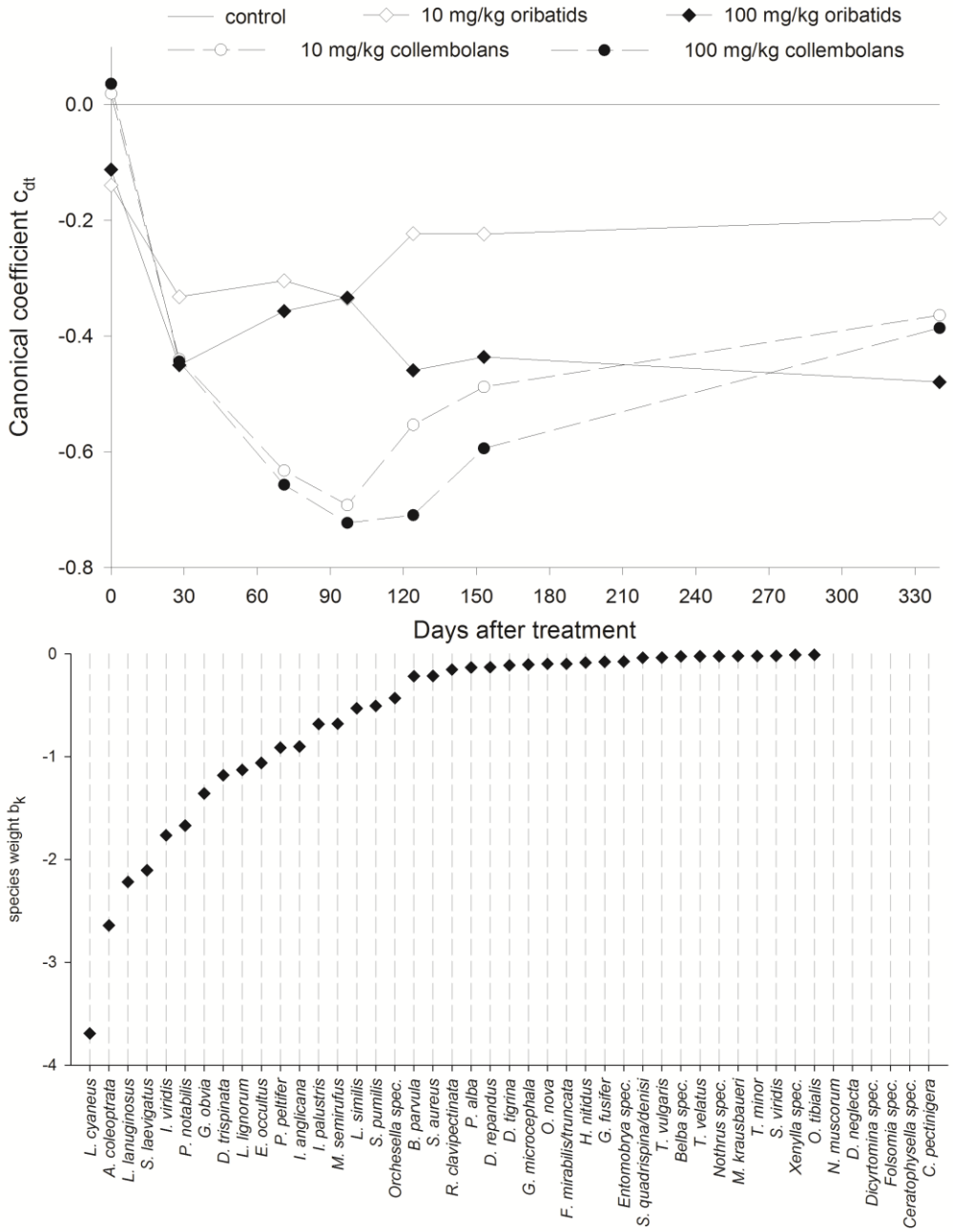


Figure III-6: PRCs of collembolans and oribatids in the TME range-finding study 2005. Middle: c_{dt} –values representing mean canonical coefficients of the first axis of the RDA analysis of treatment related effects. Bottom: b_k values representing the relative accordance of the response of single populations of collembolans and oribatid mites with the principal response.

The Principal response curve analysis of the collembolan community in TME showed clear and

long-lasting effects of the model compound lindane on the structure of community until one year after application. At all dates, treatments were significantly different from controls. Most species (besides three very rare ones) follow the basic pattern of the PRC and the most dominant species *L. cyaneus, L. lanuginosus, P. notabilis* (compare Figure III-5) showed the strongest affinity to the result of PRC analysis. There were only slight differences between the c_1 and c_2. Due to the more pronounced convergence of the high treatment to control level, both treatment groups were approaching the same level at the end of the sampling period

The similarity between the TME replicate samples per treatment was generally low between 30 and 40 % prior the application of test item, for both the Steinhaus and the Standers index. It remained relatively constant in the control TME in the first study year. The convergence of the treated TME towards the control level after one year of exposure is not necessarily due to recovery, but probably partly owing to decreasing similarity in controls.

III-1.2.1 Effect classes collembolans

The Table III-2 summarizes and clearly shows the magnitude and longevity of effects on collembolans due to the two application rates c_1 and c_2. The definition of the effect classes is given in chapter II-4.3.7. The decrease of the population density lasted at most for five month after treatment. The majority of the collembolan populations recover one year post application. However, most importantly, the structure of the whole community was still affected at the end of the study period as indicated by the PRC analysis. The method of the classification of effects, similar to aquatic mesocosm studies is considered highly useful to facilitate the interpretation of the overall results of a study and to determine the no observed adverse effect concentration for the environmental risk assessment of the particular plant protection product.

Table III-2: Effect classification of collembolans in the TME range-finding test. Results for 10 and 100 mg a.i./kg soil. See chapter II-4.4 for classification criteria.

Endpoints Effects of γ-HCH	Range-finding 10	 100
Principle Response	5	5
Shannon index	4	4
Evenness	4	4
Taxa richness	5	5
Total abundance	5	5
Brachystomella parvula		
Entomobrya spec.		
Desoria trispinata	4	4
Isotoma anglicana	5	5
Isotoma viridis	4	4
Isotomurus palustris	4	4
Lepidocyrtus cyaneus	5	5
Lepidocyrtus lanuginosus	5	5
Lepidocyrtus lignorum	4	4
Mesaphorura macrochaeta		
Orchesella spec.	1	2
Parisotoma notabilis	4	4
Sminthurinus aureus	3	3
Sphaeridia pumilis	2-3	2-3
Lowest Community-NOEC	<	
Lowest population-NOEC	<	
NOEAEC	?	

III-1.2.2 Response patterns of collembolan populations

In the following, the effects of the model compound lindane on the most important populations

of collembolans are presented by plotting their mean abundances against time, and theirs response patterns have been classified using criteria as the specific sensitivity or eye-catching seasonal components of the population density. Specifically, the following criteria were applied:

- First, the community response in the PRC analysis has been classified. Species that were clearly following or opposing the principal pattern are shown in an own graph.
- Second, if at least one NOEC for the specific population at any sampling date occurred, the species was considered relatively susceptible.
- Third, if the population has been abundant and dominant compared to the whole community.
- For reasons of distinctness, in the next section differences between control and treated TME were considered statistically significant if the probability of type-I error was smaller than 10 %.

Pattern I - Initially sensitive, outbreaks

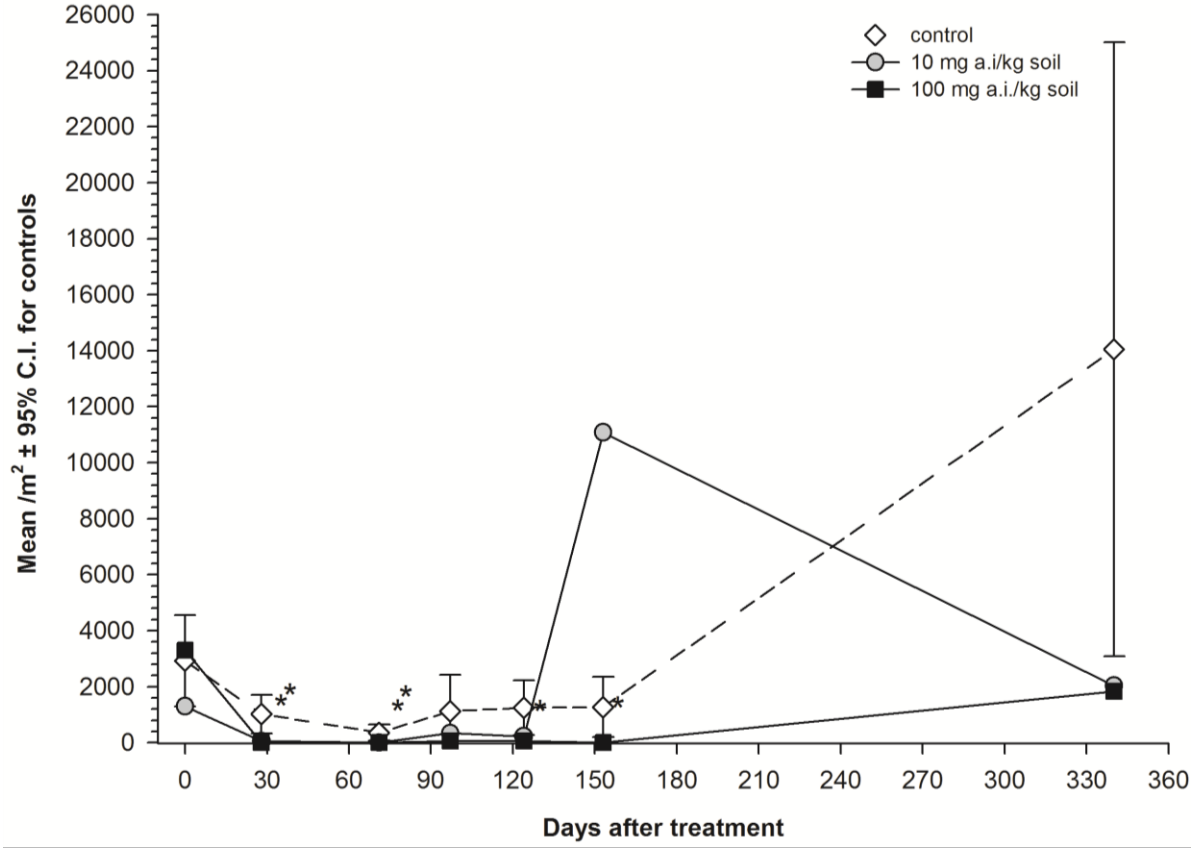


Figure III-7: Mean abundance (± 95% C.I. for controls) of *Desoria trispinata* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α=0.1; $N_{controls}$ = 20, N_{low} = 9, N_{high} = 10).

Desoria trispinata was the most abundant collembolan species in this study, and the second most dominant one over all sampling dates. It tended to stochastic outbreaks, as observed in one TME of c_1 5 months after treatment and in the control TME one year after treatment. The

species is described as probably introduced and only occurring in soils with unnaturally high organic contents (flower pots) promoting mass occurrences (FJELLBERG 2007). The species was also found in field samples of certain sampling dates. Coincidently, these characteristics resulted in a high amount of variability of the population abundance of each of the treatments.

There were significant differences between the treatments c_1 and c_2 and controls 28 days and 71 days after treatment. The TME with low application rates recovered 97 days after treatment, whereas the effects of the high treatment group lasted for at least another 2 months. One year after application, no significant differences in both the low and the high treatment groups were registered.

Figure III-7 shows that this was mainly due to extreme high variance in control TME. The mean values of the control are still 7-times higher than in the treatments. It can be stated that the adverse effects on this species lasted for nearly one year after application. It is noticeable that high peaks of *D. trispinata* occurred later in the course of the study. At the beginning of the experimental period, *Isotoma anglicana* occurred in low abundances. The maximum of the population density was reached 71 days after application in mid-June. That was slightly earlier than for the sister species *Isotoma viridis*, indicating weak competitive exclusion between the

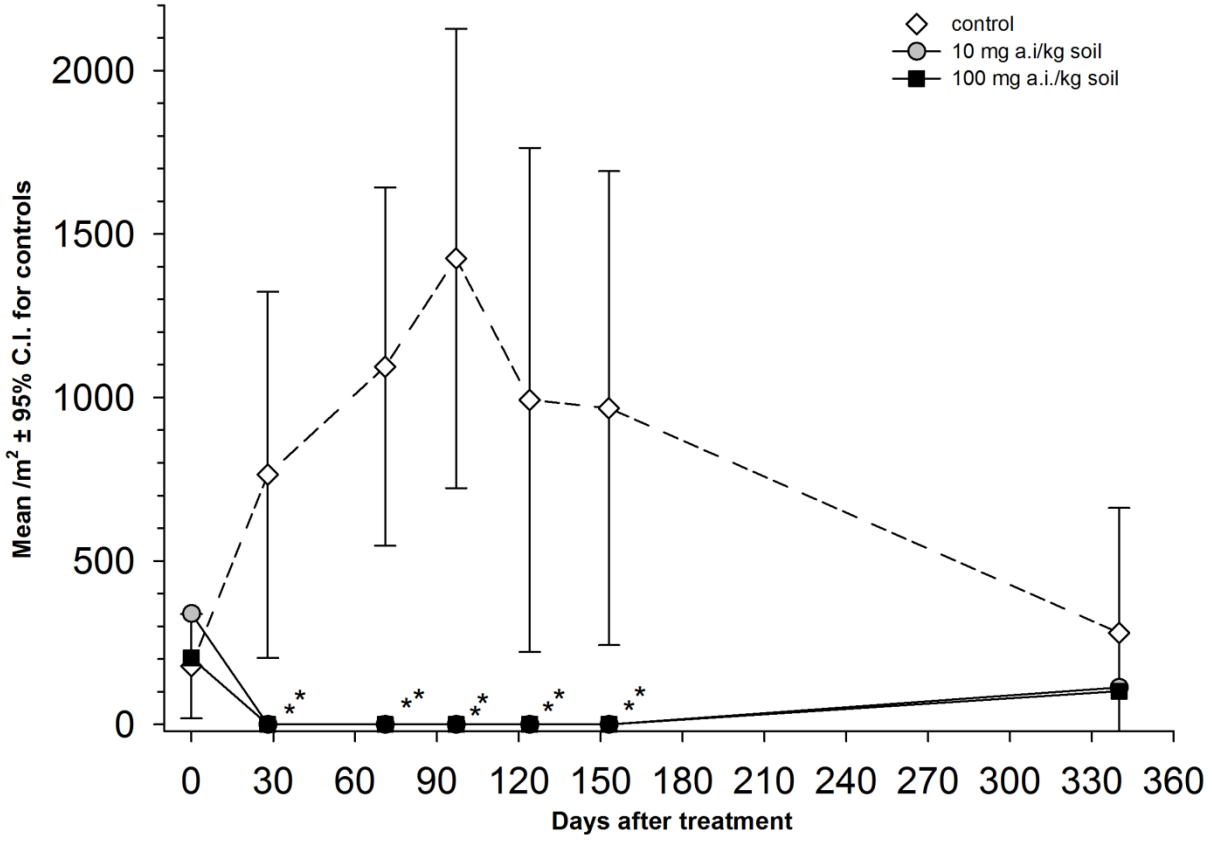


Figure III-8: Mean abundance per square metre of *Isotoma viridis* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α<0.1)

two species. At this point, the difference in abundance of both c_1 and c_2 was statistically significant. The population density decreased clearly at the end of the vegetation period, but the statistical testing did not indicate effects of the treatments at the sixth sampling date in October. The population dynamics of *Isotoma viridis* was similar to that of *I. anglicana*; it reached climax somewhat later (end of July 2005) than the species formerly described (see above). It was very sensitive towards the substance applied, showing significant differences over the whole first growing season. At the last sampling date, there was a trend to convergence, in this case not only due to decreasing control abundance. In both treatments, abundance increased from nearly zero (5 months after treatment) to more than 100 individuals per square meter one year after treatment. At the same time, the control abundance dropped from the maximum density of 1400 individuals / m² to the level of the treatments (Figure III-8).

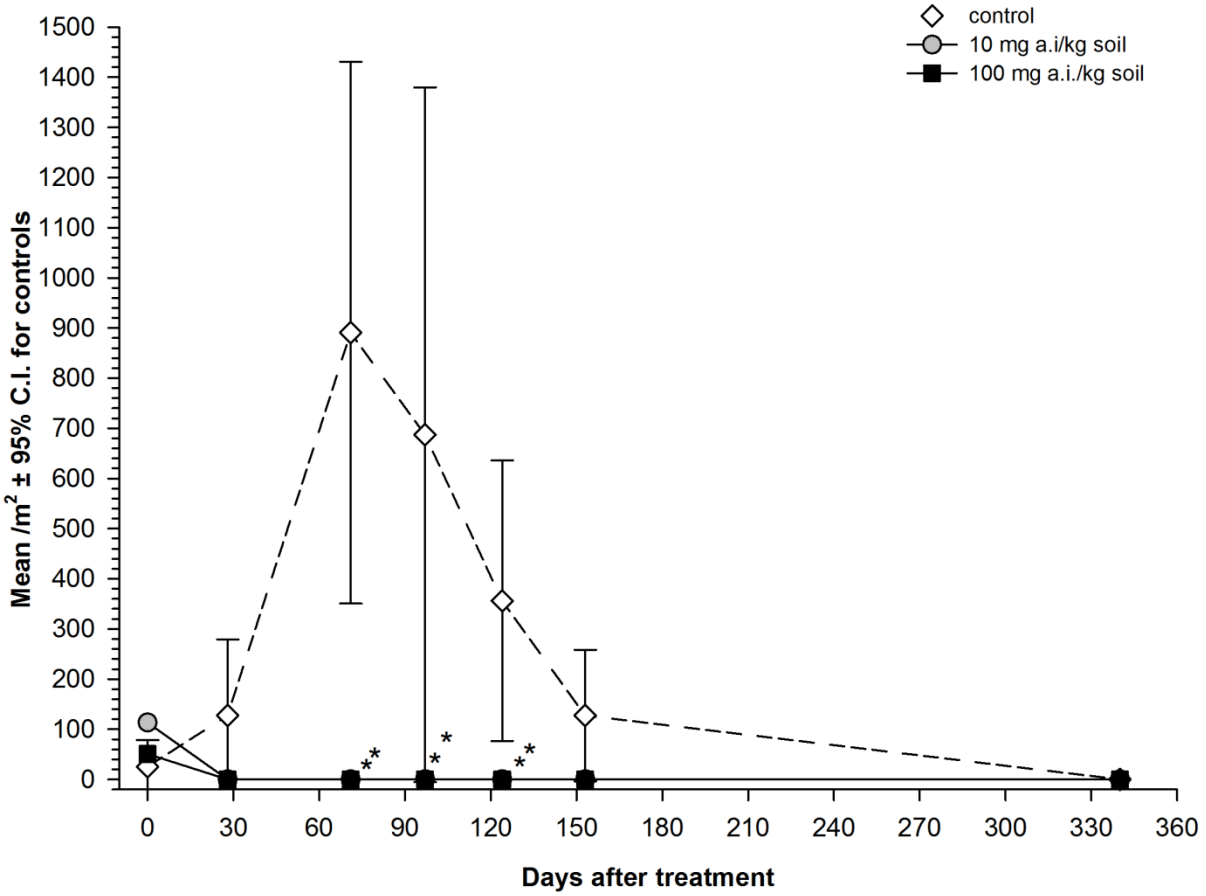


Figure III-9: Mean abundance per square metre of *Isotoma anglicana* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α<0.1)

Pattern II - Recovery at the end of the experiment

Compared to *I. viridis* and *I. anglicana* the isotomid species *Isotomurus palustris* showed a very different response pattern. Nowadays *I. palustris* is considered to be a species group that lumps the species *I. palustris*, *Isotomurus maculatus* and other species of the genus I*sotomurus* formerly suspected as colour varieties of *I. palustris* (CARAPELLI *et al.* 2001). In this work, the

distinction between the different species was not made.

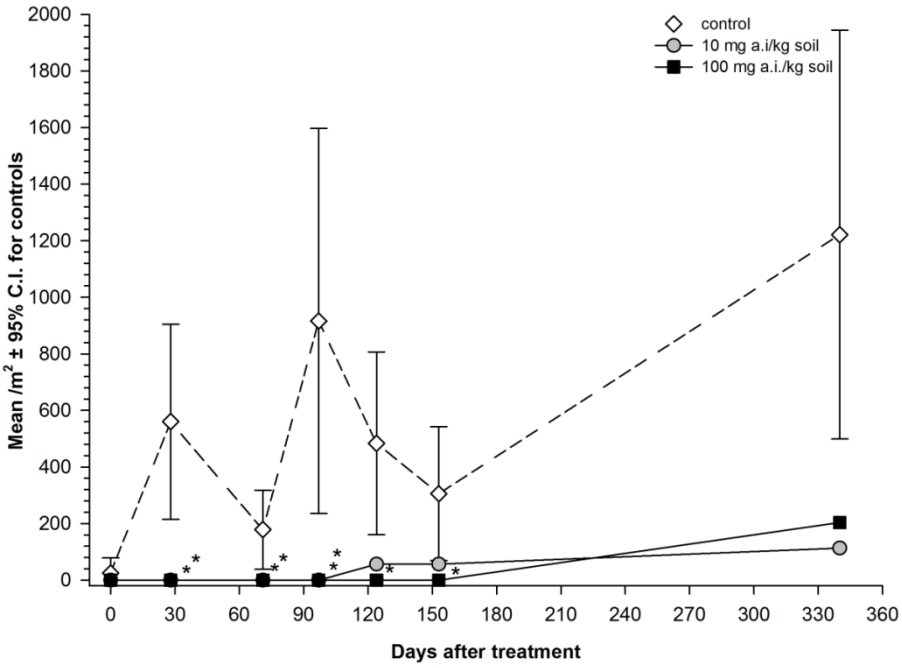


Figure III-10: Mean abundance of *Lepidocyrtus lignorum* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α<0.1)

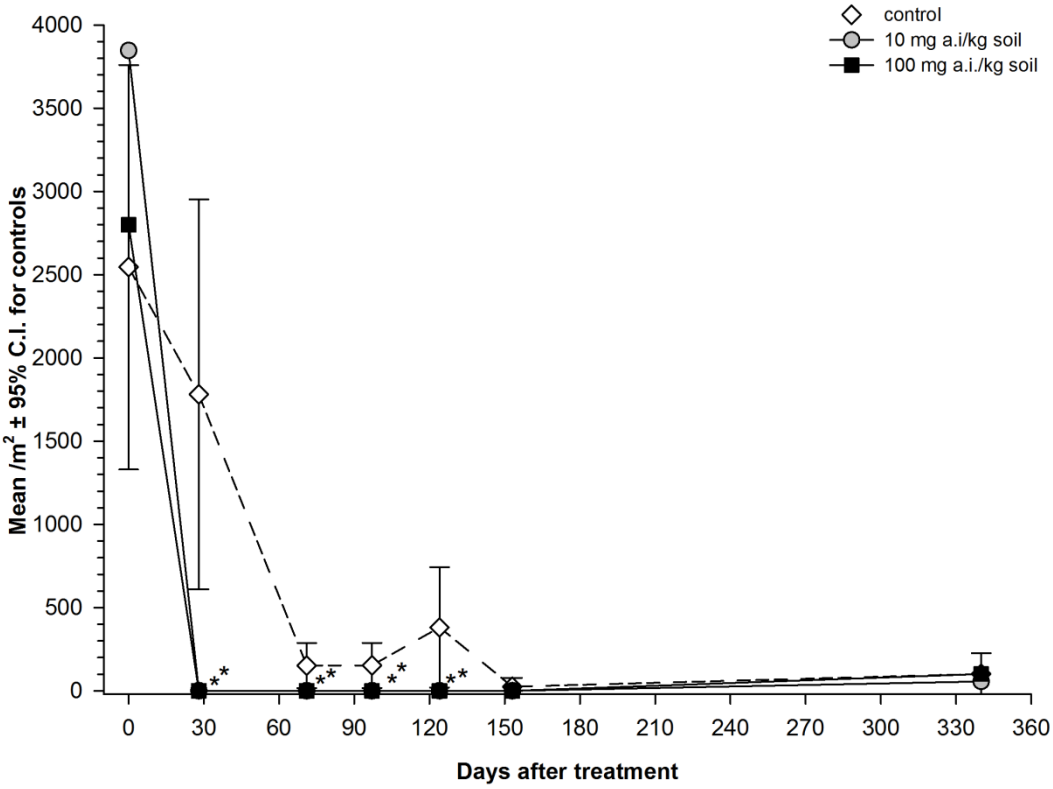


Figure III-11: Mean abundance per square meter of *Isotomurus palustris* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α<0.1)

The population maximum was reached in spring, before application of the test item. The species has adapted to moist conditions (HOPKIN 2006). It showed a clear and long-lasting reduction of abundance due to the application of the test item. Though it seemed to recover five months after treatment, based on the mere lack of statistical significance, this interpretation has to be judged in the light of a regression of control populations.

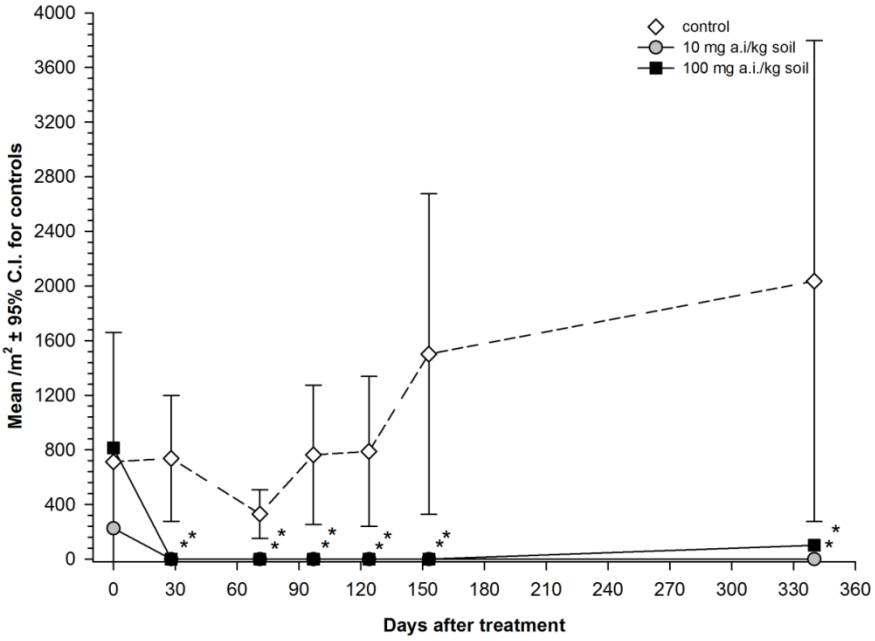


Figure III-12: Mean abundance *Lepidocyrtus lanuginosus* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α<0.1)

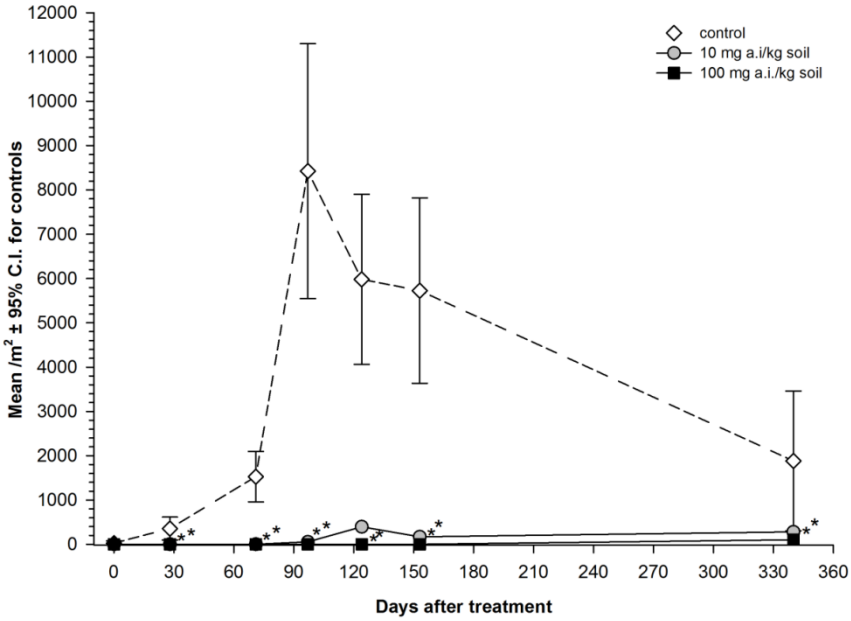


Figure III-13: Mean abundance per square meter of *Lepidocyrtus cyaneus* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α<0.1)

Three abundant species of the genus *Lepidocyrtus* found in this study: *L. cyaneus*, *L. lanuginosus* and *L. lignorum*. They showed clear seasonal differences of population dynamics. The early species *L. cyaneus* was the most susceptible towards the model compound, whereas the further two species did not show significant differences between c_1 and control TME.

Pattern III - Constant occurrence, high susceptibility

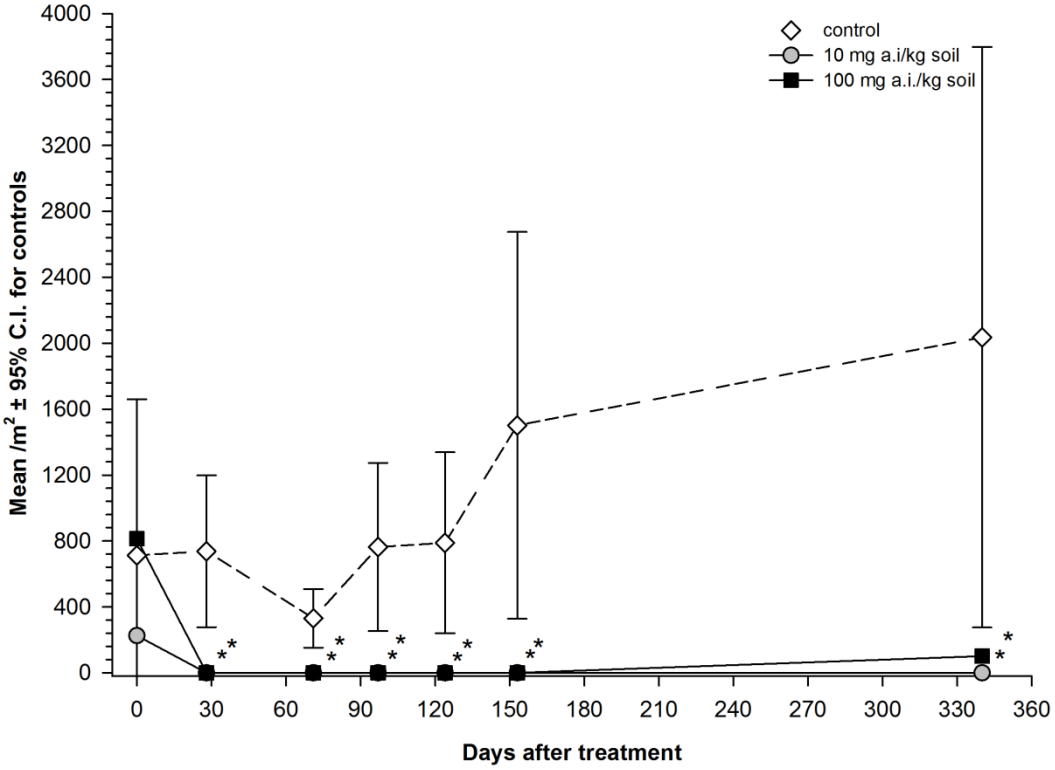


Figure III-14: Mean abundance per square metre of *Parisotoma notabilis* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α<0.1)

Parisotoma notabilis was one between the few species that allowed statistical analysis and occurred in steady numbers at each of the seven sampling dates. It was very susceptible not showing the vaguest trend to convergence one year after application.

III-1.3 Effects on oribatids

In the present study, 16 different oribatid species occurred. Hence, oribatids were slightly less species diverse than the collembolans. The most dominant species within the group of oribatids was *Scheloribates laevigatus* (34 % of the total abundance from day 0 to day 340), followed by *Achipteria coleoptrata* (29 %), *Galumna obvia* (9.7 %), *Eupelops occultus* (8.1 %), *Platynothrus peltifer* (6.0 %) and *Minunthozetes semirufus* (5.7 %). The dominance spectrum is documented by Figure III-15. Those species were found consistently within the samples, and all were identified as typical for temperate grassland. Low mite densities are likewise typical for grasslands as low diversity (TOSCHKI 2008).

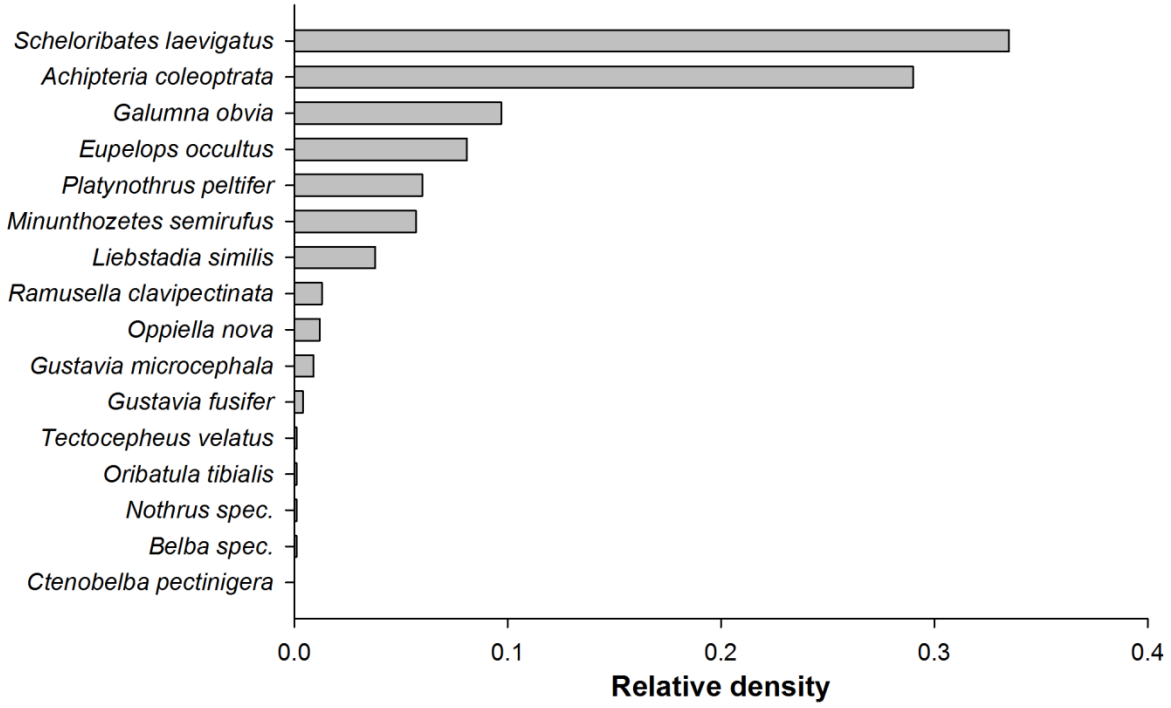


Figure III-15: Dominance spectrum over all sampling dates (control samples only) for Oribatid mites.

The total number of oribatids of the untreated TME increased until day 124 (Figure III-17). The maximum average number in controls was about 13 individuals per sub-sample. Initially, the total abundance of oribatids was significantly reduced by the low and high lindane concentrations c_1 and c_2 (day 28, day 71). From day 124 to the end of the study period, no significant difference between the control level and c_1 was detectable. At the elevated concentration, c_2 oribatids did not recover from the treatment within the study period (Table III-1 gives an overview over the NOECs obtained).

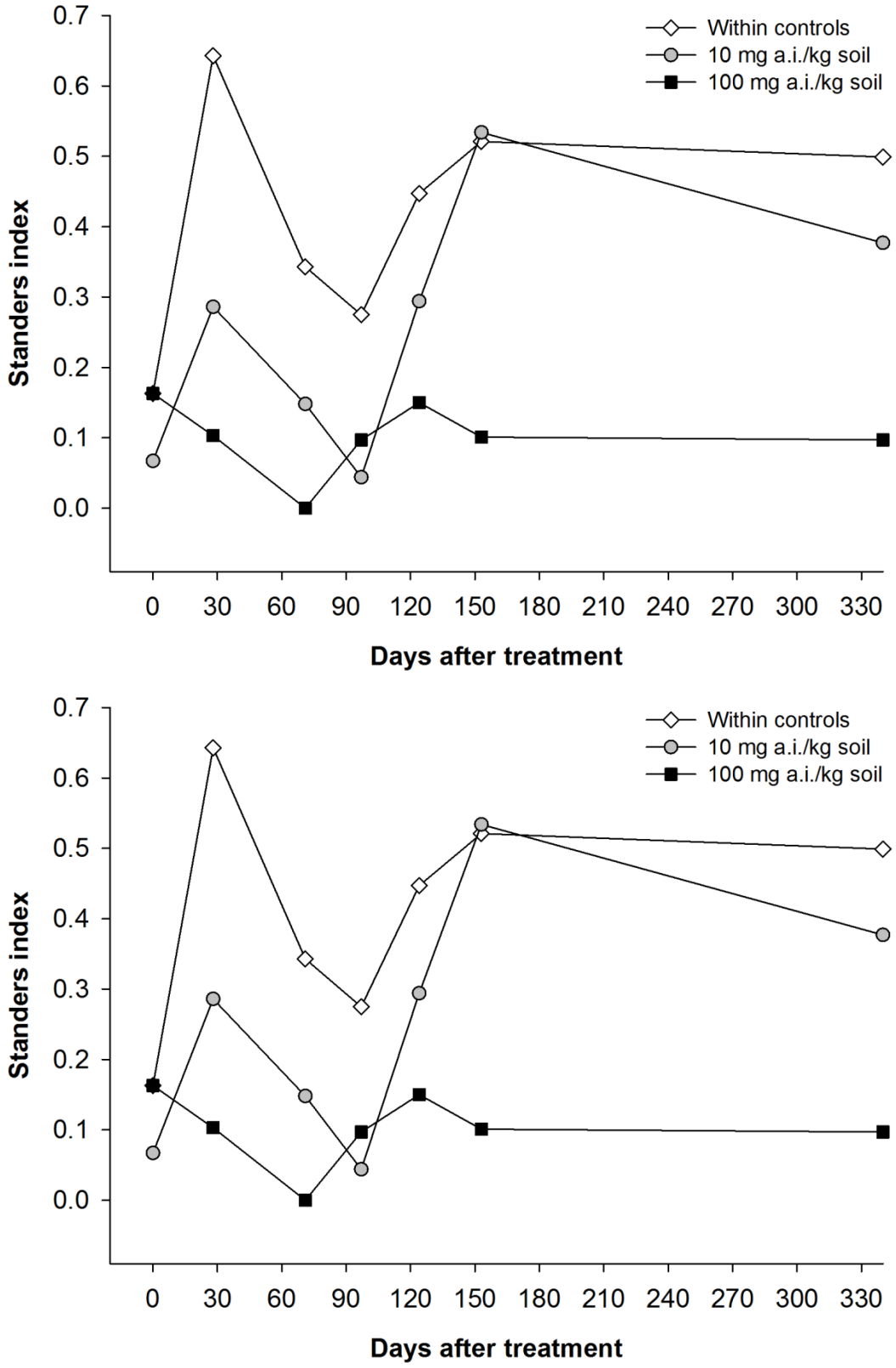


Figure III-16: Similarity indices of Oribatids.

The majority of individuals that occurred at c_1 after treatment were identified as *S. laevigatus*. This species was highly overrepresented in treated TME compared to controls (approx. 70 % relative density vs. 35 %). Steady and abundant taxa were defined as contributing at least 2 %

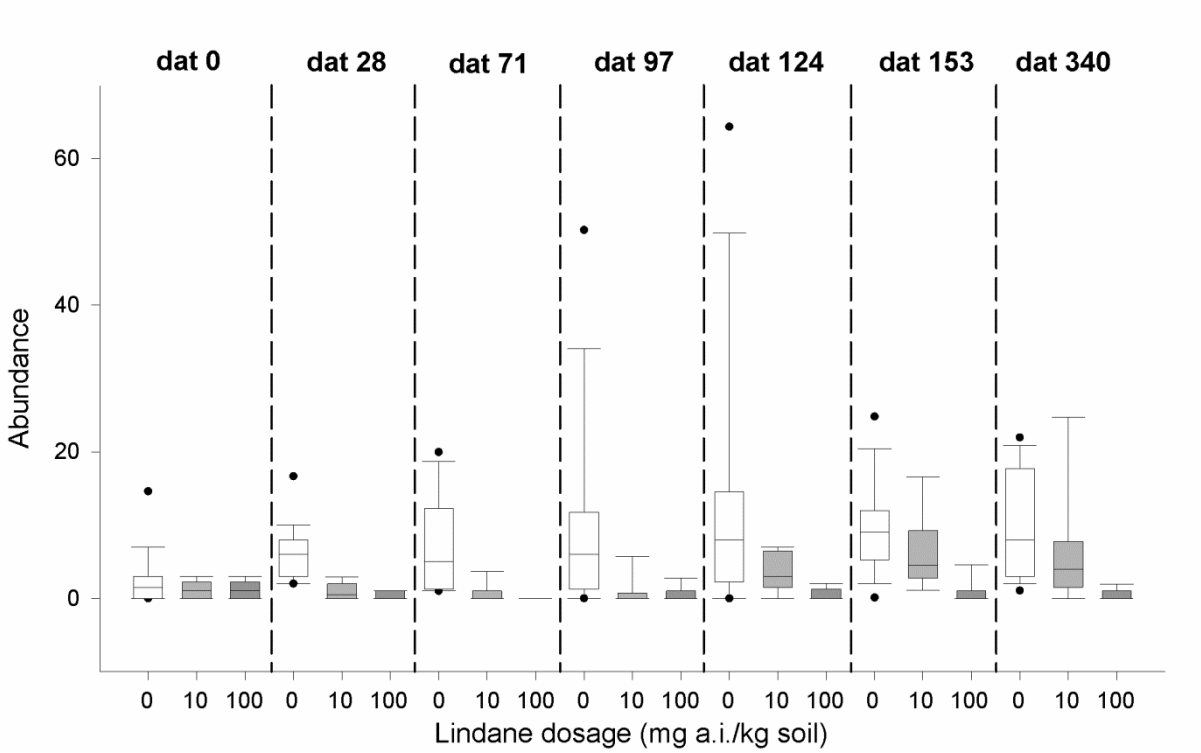


Figure III-17: Boxplots of total abundance of oribatids. Open bars control group (N = 20), light grey 10 mg a.i./kg soil, dark grey 100 mg a.i./kg soil. Filled circles $5^{th}/95^{th}$ %percentile, if not present no or too little data points beyond $10^{th}/90^{th}$ % percentiles.

of the whole community, including eight taxa. Those taxa were also focused in the following analyses of the overall response of the community of oribatid mites, as obtained by the analysis of the total abundance, the PRC and the particular populations.

The community structure of oribatids was strongly influenced from day 28 to day 340 by c_2, as indicated by the PRC analysis. In contrast, at the lower concentration c_1 initial effects were observed but afterwards recovered at day 124. However, not confirmed by the statistical tests, the community of oribatids did not unambiguously recover to the control level as shown by the PRC analysis (Figure III-6). There was no tendency to converge, even at the last sampling date. Juveniles followed the overall response; they could belong to each of the species found and thus reflecting an 'average' response of the community. The community of oribatids appeared to be very stable over time, indicated by a very low percentage of explained variance by sampling dates, i.e. the factor time in PRC analysis explained 7.7 % of the total variability of the dataset, after constraining to the treatment. All endpoints except for the abundance of *G. obvia*, *Liebstadia similis* and *M. semirufus* were initially affected by both treatments. At the end of the experimental period, the indices of similarity (Figure III-16) between treatments and controls, the PRC and the total abundance had recovered. A number of endpoints, i.e. the diversity and abundance of *A. coleoptrata* and *G. obvia* did not recover until the last sampling date (Table III-1).

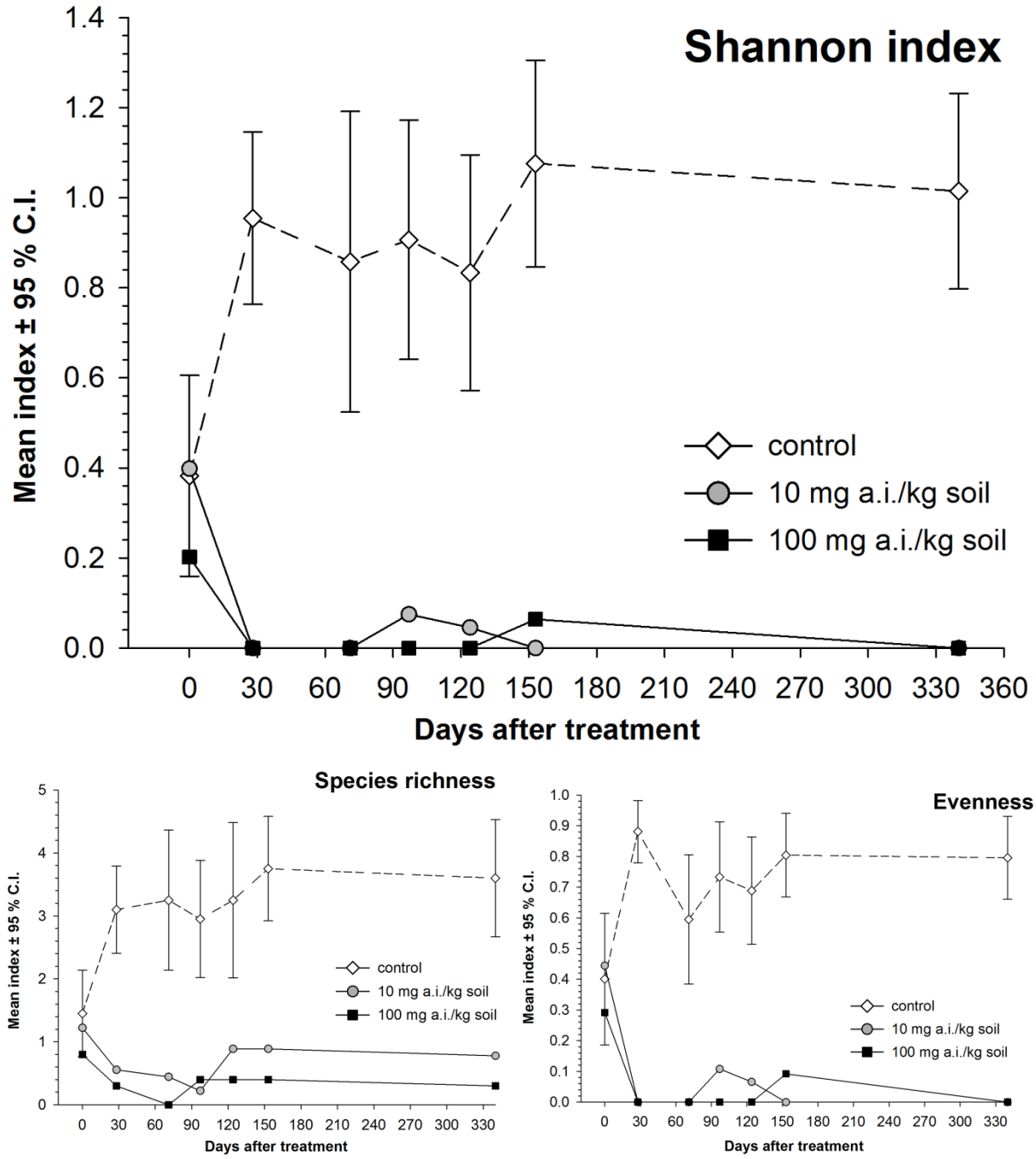


Figure III-18: Diversity indices of oribatids.

III-1.3.1 Effect classes oribatids

As Table III-3 shows, most of the endpoints, as well for the communities measures and for the single populations were classified as 'effect class 5' (definition of effect classification chapter II-4.3.7), i.e. no recovery of the initial effects was seen until the end of the experimental period. Most of the abundant species showed no recovery. The total abundance was assigned to be effect class 3 because at the sampling date day 97 no statistical difference between the control and c_1 was detected. For this reason, according to the definition of the effect classes, the recovery was apparent by statistical reasons rather than ecological. Because the lower treatment caused consistently class-five effects regarding most endpoints, a no observed ecologically adverse effect concentration could not deduced.

Table III-3: Effect classification of oribatids in the TME range-finding test. Results for 10 and 100 mg a.i./kg soil. See chapter II-4.4 for classification criteria.

Endpoints Effects of γ-HCH	Range-finding 10	 100
Principle Response	4	5
Diversity Shannon	5	5
Diversity Evenness	5	5
Diversity Species richness	5	5
Similarity (Steinhaus´ Index)	4	5
Similarity (Standers´ Index)	4	5
Total abundance	3	5
Achipteria coleoptrata	5	5
Eupelops occultus	5	5
Galumna obvia	5	5
Liebstadia similis	5	5
Minunthozetes semirufus	2	2
Platynothrus peltifer	3-4	3-4
Scheloribates laevigatus	3	5
Lowest-community NOEC	<	
Lowest population NOEC	<	
NOEAEC	<	

III-1.3.2 Response patterns of oribatid taxa

For reasons of clarity and keeping the focus, some populations were excluded from detailed descriptions. The depicted were chosen by the following criteria: First, the response in the PRC analysis was analysed. A species is shown if following or opposing clearly the principal pattern. Second, a species is shown in the following if at least one NOEC for the specific population at any sampling date was computed. Third, a population is shown if it was abundant and dominant compared to the whole community. Single individuals were excluded from the analyses anyway.

Pattern I: Low sensitivity, partial recovery

The term 'low sensitivity' refers to concentrations of lindane that were applied in this study. It is not meant a general ranking of the species. The term 'partial recovery' refers to effects on the populations that were not recovered until the end of the study in one of the two treatments c_1 or c_2. *Scheloribates laevigatus* (Figure III-19) was the only species that was shortly affected by the low treatment and that fully recovered four months after application (effect class 3 for c_1, Table III-3). It was one of the few species occurring in the samples taken immediately after treatment. Significant effects of the treatment c_2 lasted until the last sampling date.

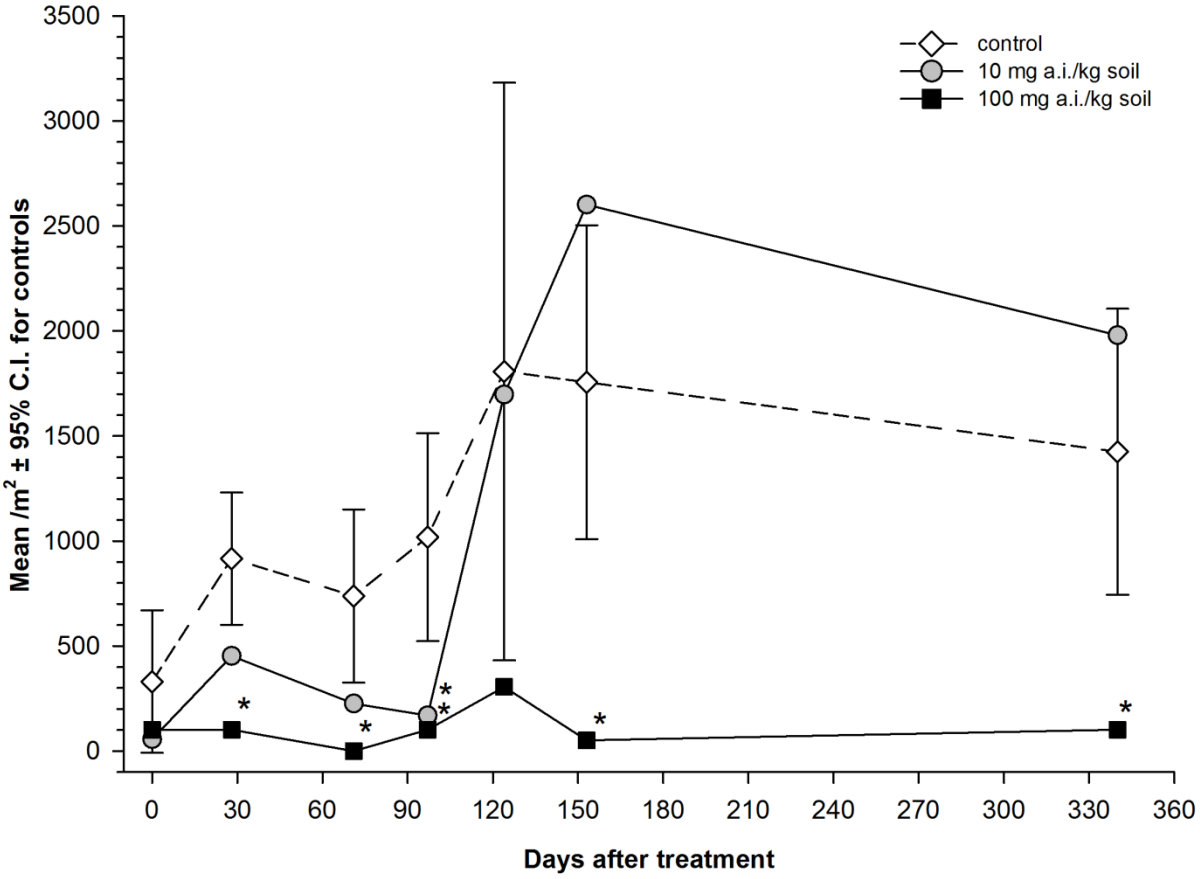


Figure III-19: Mean abundance per square meter of *Scheloribates laevigatus* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α=0.1).

Pattern II: Strong and significant effects, constant abundance in controls

Achipteria coleoptrata (Figure III-20) was one of the most abundant species in TME, strongly affected by both c_1 and c_2. The species showed seasonal maxima of abundance four months after application, i.e. in mid-September 2005, but no recovery was observed until the end of the study.

Pattern III: Low control abundance, delayed answer

The oribatid species *Galumna obvia* (Figure III-21) was found in very low densities over the complete sampling period, and its abundance was continuously increasing in control TME. A possible immigration from outside the TME could not be excluded in the reported experiment. Subsequent studies showed that the density of microarthropods in the gravel layer of the experimental facility is rather high. However, the TME-wall seems to act sufficiently as a barrier against immigration and emigration (FRÖMGEN 2008, STRAUCH 2009). The treated TME did not follow this general trend, which was seen as indicating an effect on reproduction of this species. Populations of *Galumna obvia* did not recover within the study period; the TME populations extinct.

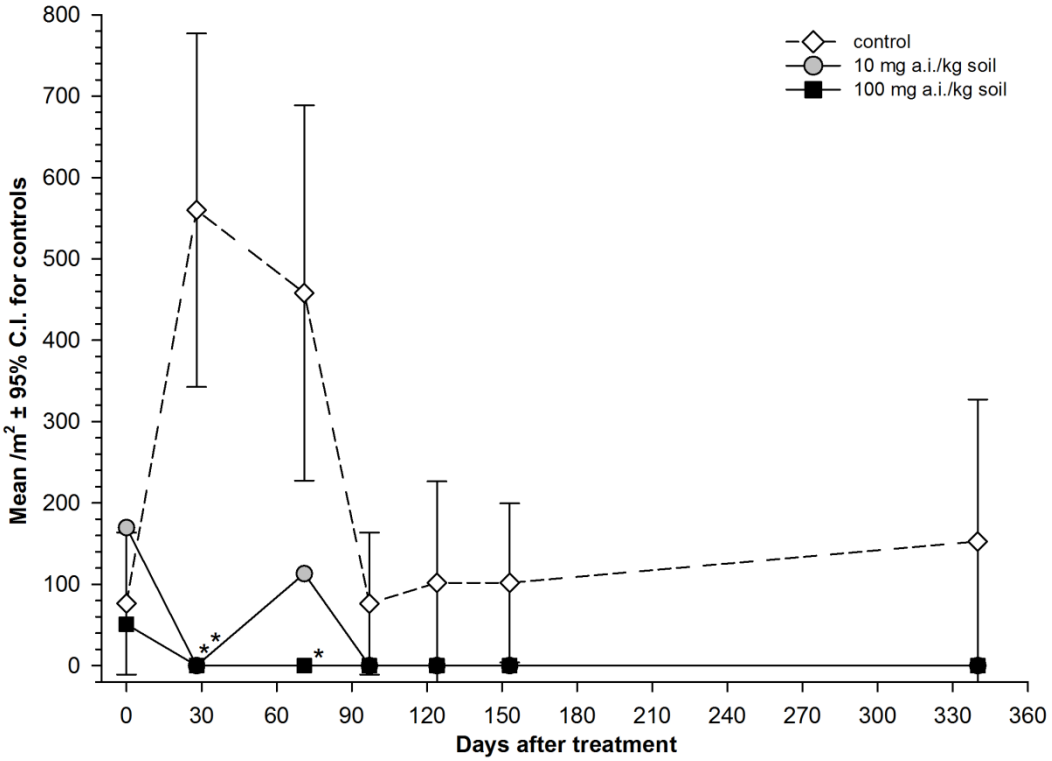


Figure III-20: Mean abundance per square meter of *Achipteria coleoptrata* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α=0.1)

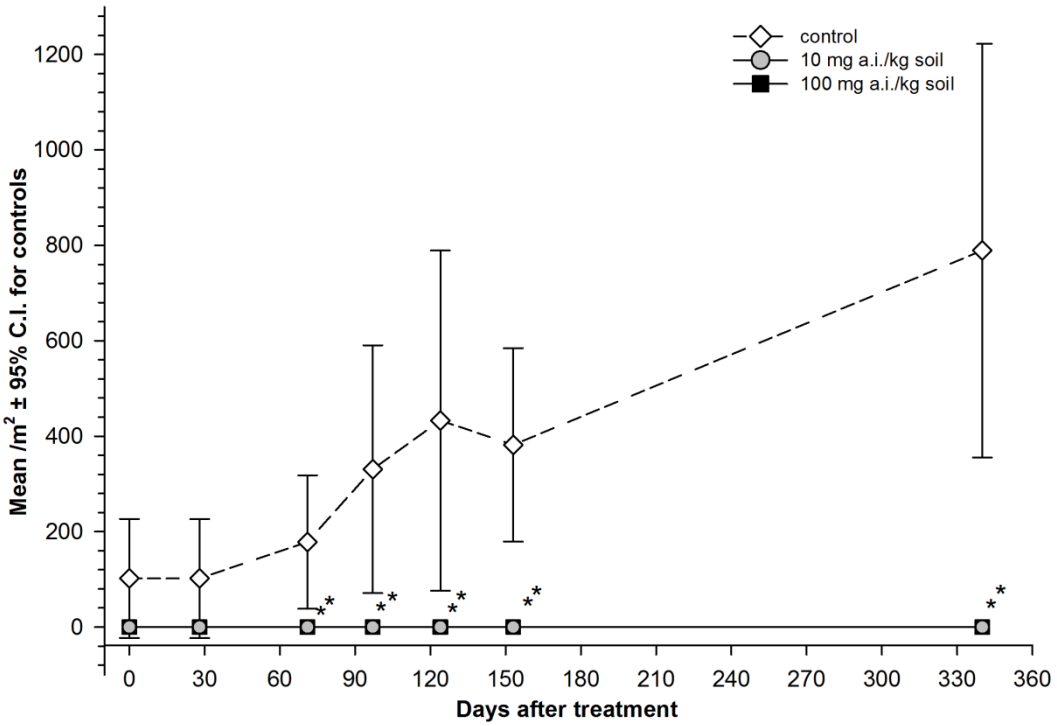


Figure III-21: Mean abundance per square meter of *Galumna obvia* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α=0.1)

Pattern IV: No statistically significant answer, but clear qualitative response

Liebstadia similis (Figure III-22) showed no statistically significant differences of treatments to control, however, in the light of qualitative inspection there was a clear decrease of abundance from the day of application to the end of the study period. Considering the median other than the arithmetic mean abundance was considered, this conclusion did not endure.

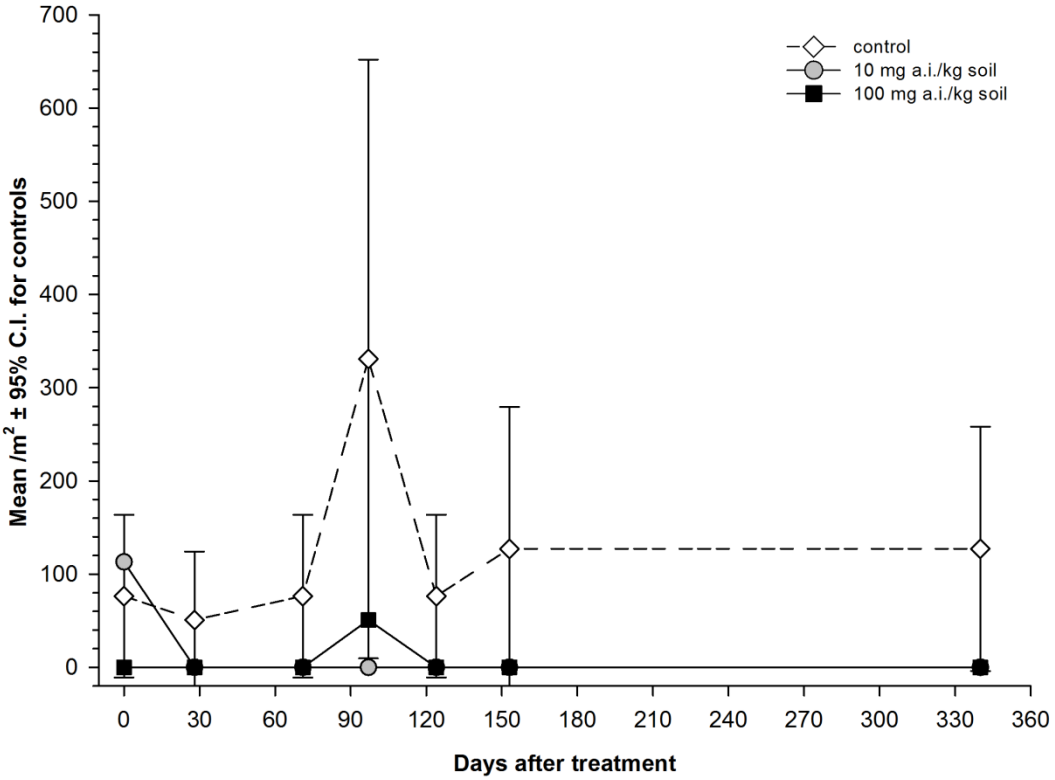


Figure III-22: Mean abundance per square meter of *Liebstadia similis* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α=0.1).

Pattern V: slow growth, integrating factors (taxonomic resolution)

For the juvenile oribatids, the population growth was not fast enough to outrange the low abundance of the treatment TME. Generally, the abundance of juveniles is the sum of all individuals, which were not determinable to species level because of insufficient characteristics of their taxonomic traits. Effectively, no statistical differences were detectable immediately after the application of the test item. The control variability was high, but the obvious differences between controls and treatments after application were significant from day 71 onward. At the last sampling date, no significant differences were obtained because of clearly decreasing control abundance, which was hampering the statistical analysis.

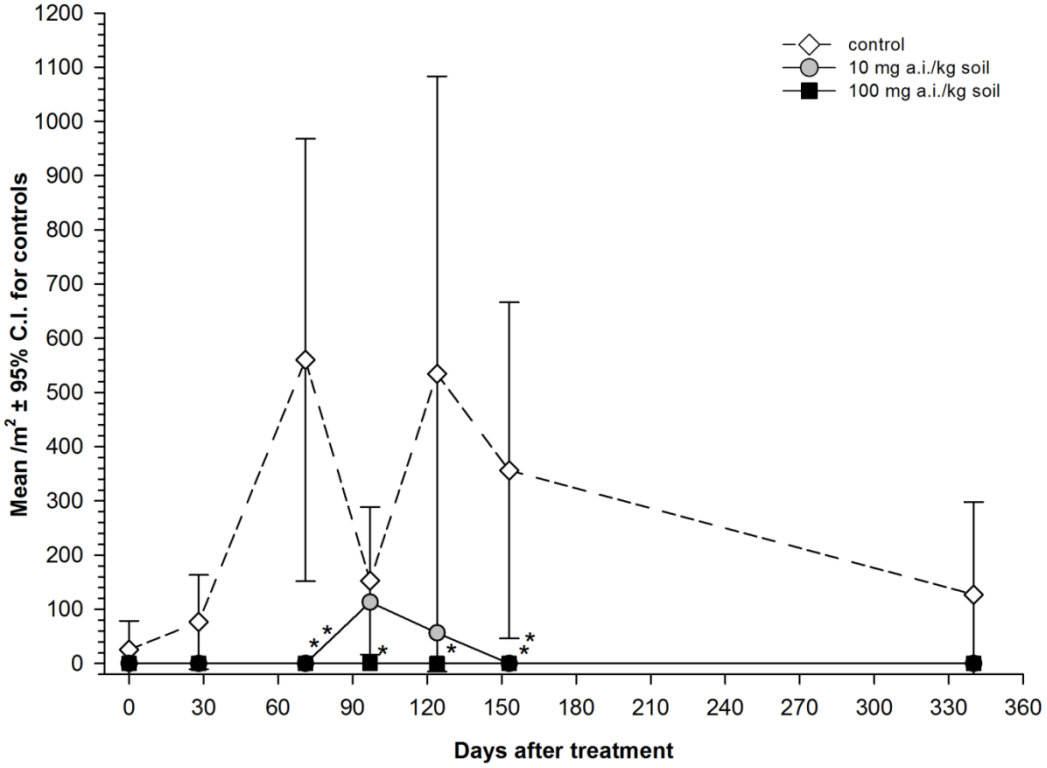


Figure III-23: Mean abundance of *Oribatid juveniles* in the TME range-finding study. Asterisks indicate significant differences of treatments compared to control (Williams multiple t-Test; one-sided smaller; α=0.1).

III-1.4 Effects on lumbricids

Seven members of the family Lumbricidae were found (Table III-1). Immediately after treatment, 140 individuals came to the soil surface and died within the next 2 days. After termination of the experiment, 559 lumbricids were extracted out of 40 TME. The differences in mean numbers between untreated and treated TME were small and not significant (results not shown). There was no clear effect of the test compound on total earthworm density 1 year after application. The number of juveniles of the genus *Allolobophora* was lowered in the higher lindane concentration c_2, indicating a measurable effect on the reproduction of endogenic lumbricids. The diversity (Shannon indices, evenness) at the lower lindane concentration $c_1$was slightly lowered; c_2 caused a significant decrease of the Shannon index and evenness compared to control (Table III-1).

III-1.5 Effects on feeding activity

The intensity of the feeding activity was correlated with the vertical distance of the exposed holes to the soil surface. It was reduced by 50 % in a 7-8 cm depth below soil surface compared to the top soil layers (0.5-1.5 cm). Neither c_1 nor c_2 significantly affected the total feeding ac-

tivity over all soil layers weeks after application (Figure III-25, Figure III-24). The consumption rate upon treatment at the high concentration c_2 was even slightly higher compared to control and c_1 (for the significance of the results refer to Table III-1).

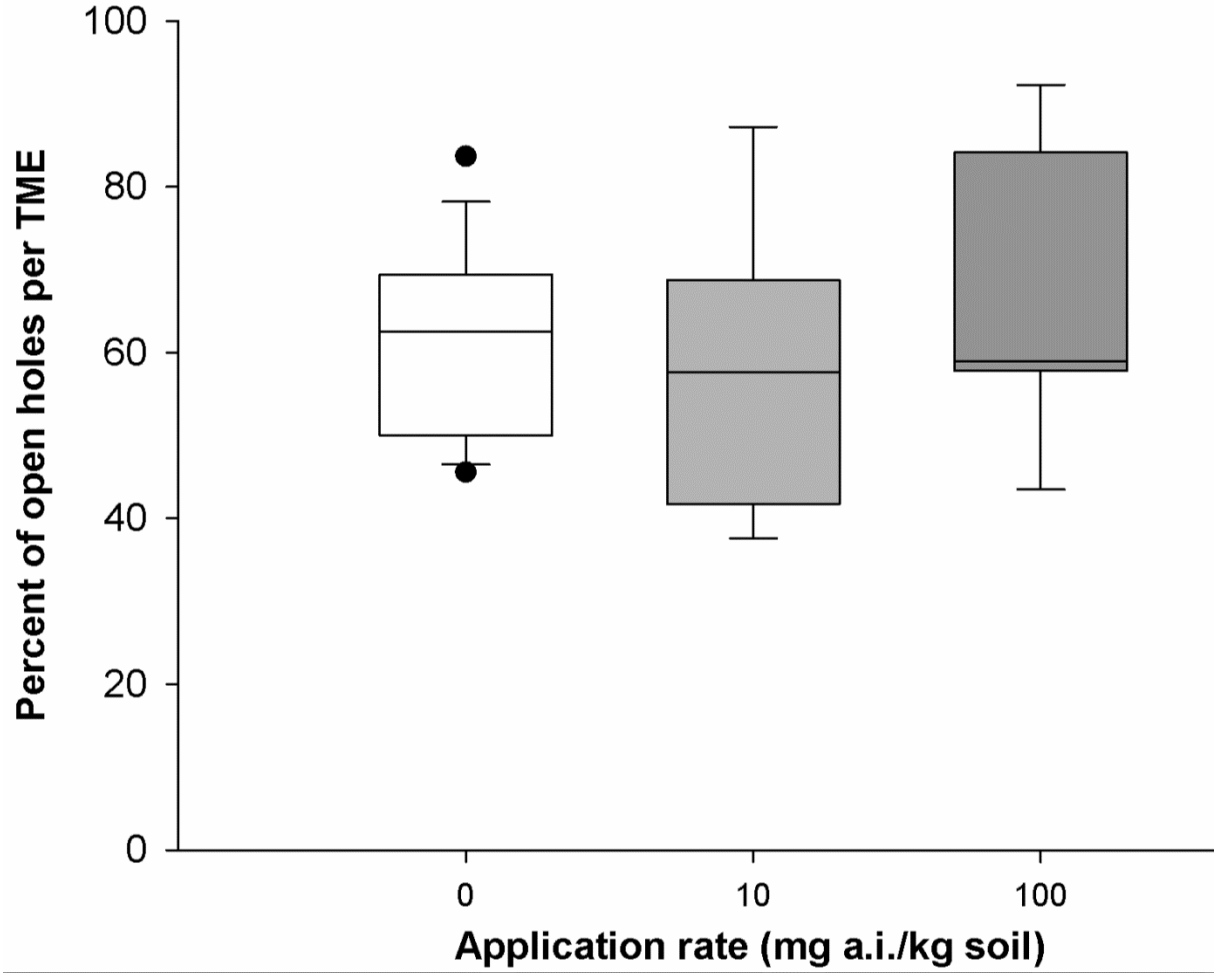


Figure III-24: Boxplots of total feeding activity in TME. Percentage of open holes of 7 bait-lamina sticks with each 16 holes in 0.5 cm vertical distance; maximum number of open holes = 112). Exposure period: 14 days after treatment with model compound lindane. N = 20 for control and 10 for treated TME. Filled circles 5^{th}/95^{th} percentile, if not present, no data points beyond 10^{th}/90^{th} percentiles.

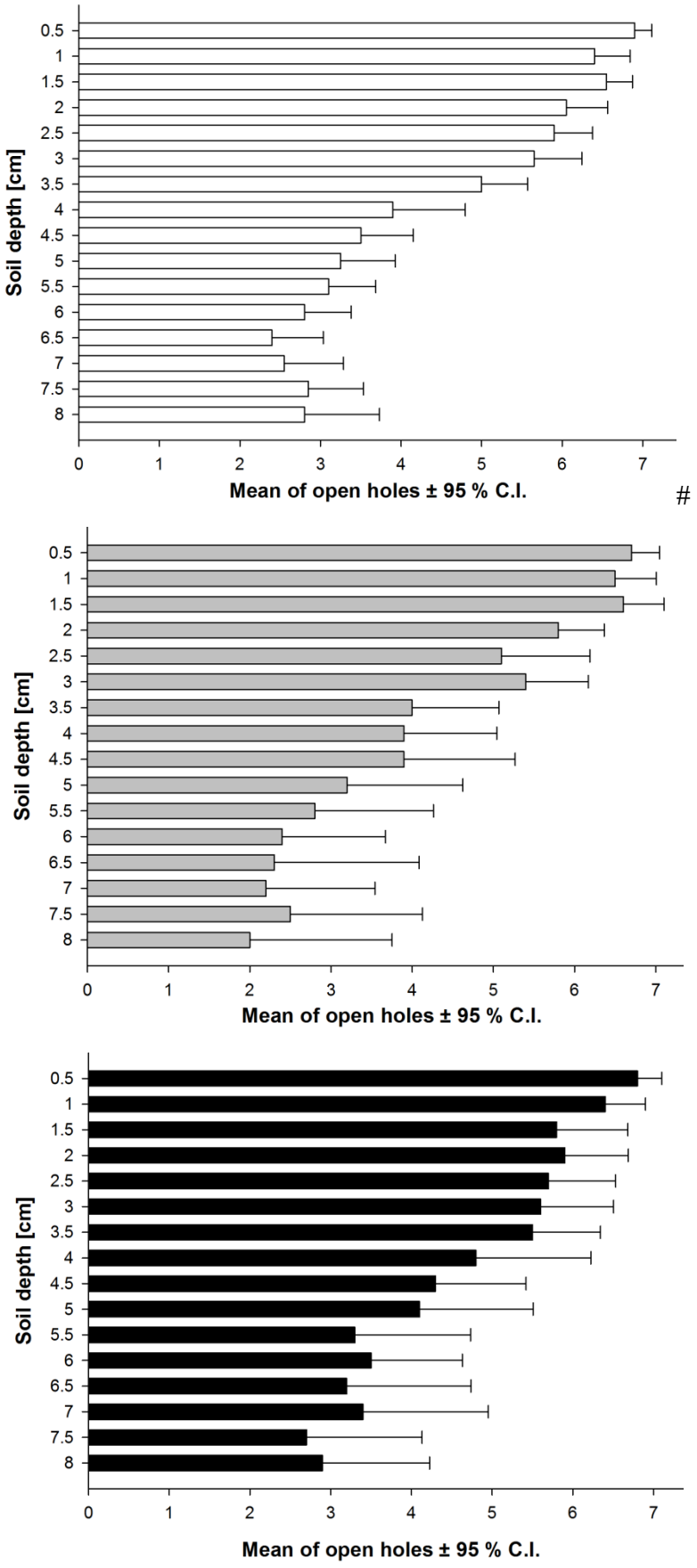


Figure III-25: Mean values of feeding activity per soil layer. Open bars: controls; light grey shaded bars: c_1; dark bars: c_2. N = 20 and 10 for controls and treatments, respectively. A period of 14 days after application was recorded.

III-1.6 Effects on plants

In the current setting, effects on plants could be analysed twofold as effects on the total plant biomass by measuring the fresh and dry weight of the cut grass layer of each TME, or as effects on the community structure of TME vegetation. The weight gain of biomass after application of the lindane formulation at the lower concentration c_1 was significantly higher at all sampling days compared to control TME (Figure III-26). The mean yield was threefold that of the control level at day 97, still twice as much as the control at day 124 and yet significantly different at day 153. The difference in biomass at the elevated concentration c_2 compared to the untreated controls was less pronounced but significantly altered until day 124. At day 153 the difference was too small to be detected by statistical means.

Two weed species were covering dominantly the TME surfaces of all replicates and all treatments: *Bromus hordeaceus* and *Holcus lanatus* (Table III-4, Figure III-27). Regular accessory species were *Taraxacum officinale* and *Arrhenaterum elatior*. Differences between the treatments were seen regarding the dominance of the species. In control-TME *B. hordeaceus* covered on average 70 % of the TME area, whereas it was less abundant in treated-TME, covering

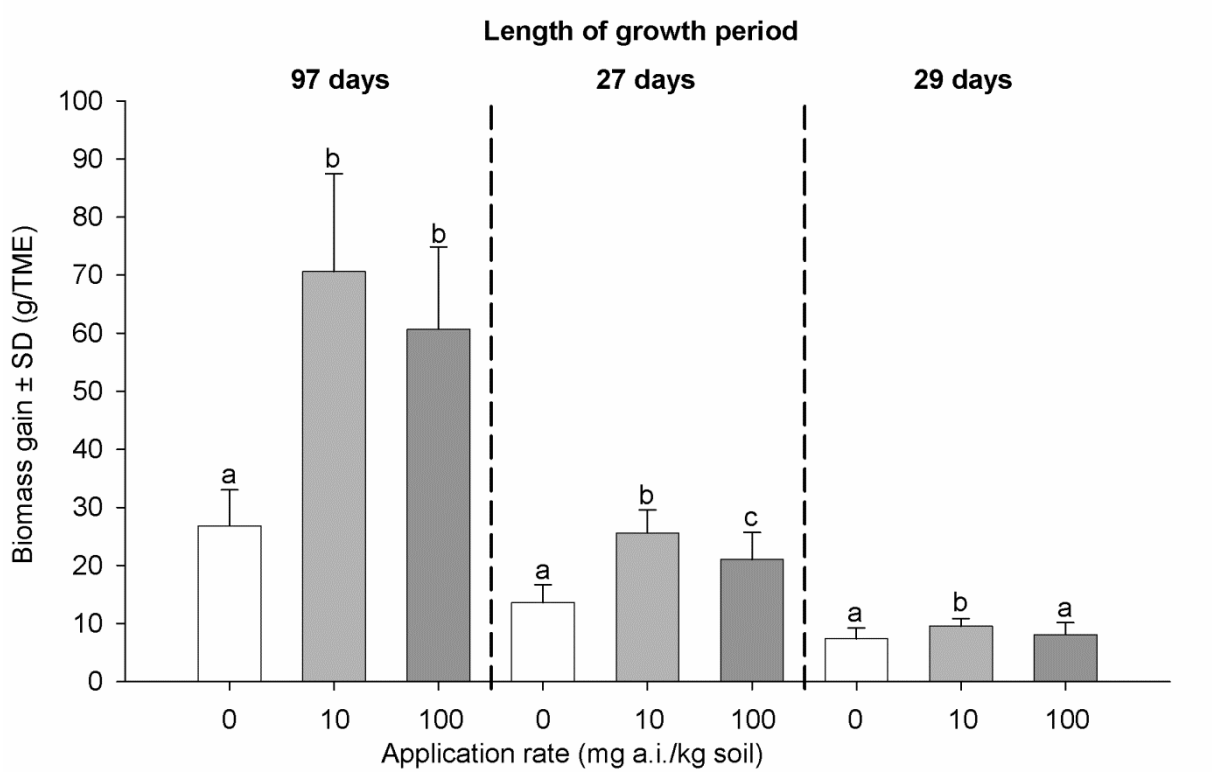


Figure III-26: Biomass gain (dry weight) of TME vegetation ± SD (g/TME) between two sampling dates (length of the growth period in days is indicated above). Open bars: control group (N = 20), light grey bars: 10 mg a.i./kg soil, dark grey bars 100 mg a.i./kg soil. Treatment groups of one sampling date were not significantly different in a Student's t test with $\alpha < 0.05$ if sharing the same letter (a), (b) or (c).

50 and 35 % in c_1 and c_2, respectively. *H. lanatus* showed an opposite pattern, being more dominant in the treatments. To which extent the shift in community composition could be ascribed to a fertilising effect was subjected to the discussion below.

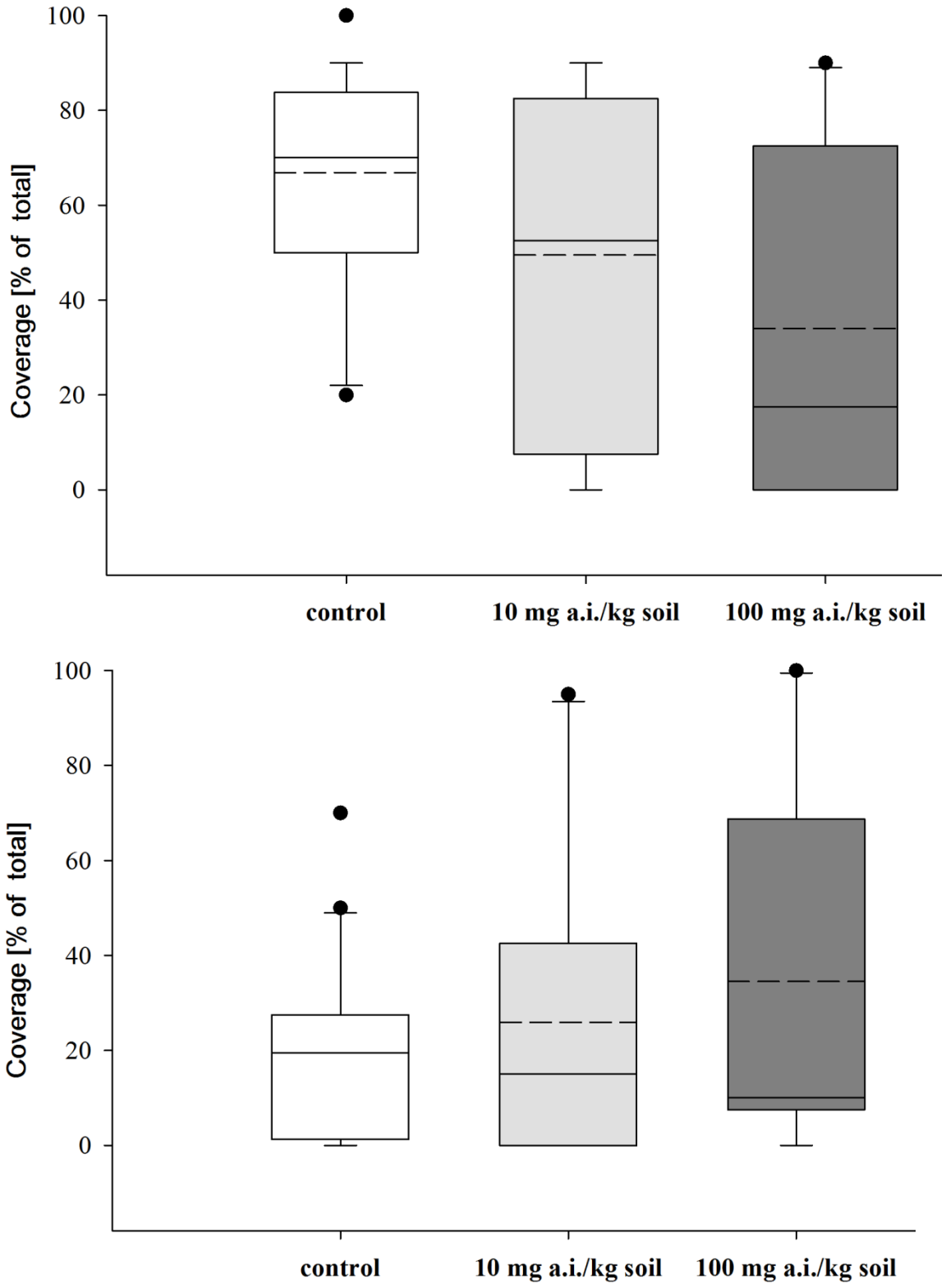


Figure III-27: Relative cover of the second most dominant plant species *Holcus lanatus* (below) and the most dominant species *Bromus hordeaceus* (above) in treated and control TME.

III-2 Discussion

Most species of microarthropods found in the studied soil are common in temperate grasslands; many of them usually occur in a wide range of open-land habitats (WEIGMANN & KRATZ 1981; TOSCHKI 2008). Treatments at concentrations c_1 = 7.5 kg a.i./ha and c_2 = 75 kg a.i./ha caused significant and long-lasting effects on the communities of microarthropods as expected since the applied rates were 5-100-fold higher than the normal agricultural practice previously lindane was banned. Usually 0.75-1.5 kg a.i./ha lindane was applied for soil treatment (FAO/WHO 1968). Laboratory LC_{50} data for the most susceptible collembolan *Onychiurus armatus* was reported to be approximately 0.05 mg a.i./kg soil (FRAMPTON *et al.* 2006) and between 0.14 and 0.66 mg a.i./kg soil for *F. candida* (LOCK *et al.* 2002), respectively. The densities of populations of microarthropod species in TME-sub-samples varied strongly over time and between TME. After logarithmic transformation of the raw data, the coefficients of variation were usually between 10 and 40 % (compare chapter V-1). By applying robust and common statistical test procedures (Dunnett's t-test or Williams multiple t-test), average deviations of 20 % compared to control levels are detectable. The detection levels show large variations on their part. The analyses of the limits of detection will be discussed extensively in chapter V-3. It is considered essential to provide information about the variability of a test system in order to be in a position to judge the power of evidence that can be deduced from the systems.

III-2.1 Collembolans

The community of collembolans was significantly affected by the treatment as indicated by both PRC and diversity analyses. In particular, the PRC analysis indicated that the community was not fully recovered until the end of the study period, i.e. 340 days after application. The effects of the low and the high lindane concentrations were statistically detectable, although the large variation between the TME-replicates mitigated against statistical significance. Similar results were observed for the total abundance of collembolans. Based on available data, we cannot distinguish sensitive from tolerant endpoints because the number of test concentrations was too small to demonstrate a concentration-response relationship and effects on all levels of investigation were strong (i.e. on abundances and on community parameters). The experiment was originally designed as a range-finding study with two application rates, so those limitations were expectable. Dose-response experiments can be used to establish distinct effect classes based on lumped parameters such as the total abundance of a taxon or its community composition. Effects on single populations reflect heterogeneous response patterns to the treatment,

even without applying a dose–response design; thus it is possible to assign categories of differing sensitivity (‘effect classes’) to certain species of collembolans. There were species that showed initial effects and recovered at the end of the study period. *S. aureus*, S. pumilis, *I. viridis*, *D. trispinata*, *P. notabilis* and *I. palustris* all returned to control level again. Others remained less abundant after that time (*L. cyaneus*, *L. lanuginosus*). For all of the species, effect thresholds have been calculated and NOECs deduced (Table III-1). Due to very high concentrations of lindane in the topsoil, most species were affected by the elevated concentration c_2. Most of the species belonged to the family Isotomidae, which are known to be epedaphic or hemiedaphic life forms and thus highly exposed to the toxic compound near the soil surface. The compound is expected to affect those organisms directly, as they were unable to avoid exposure actively. Members of edaphic families were found occasionally; lacking steadiness and abundance, the group refused proper statistical analysis. One of the dominant species, *D. trispinata*, is not a typical resident of Central Europe’s grassland. Most likely, this species was introduced from Japan (where it is the typical dominant in grasslands) and established non-permanent populations as a very fast colonizing compost species (TANAKA 1970). Its apparent recovery is solely due to one extreme outlier sub-sample that led to a greatly elevated average number. It was assumed that the recovery of most of the collembolan species are recruited from autochthonous resources, because the construction of the TME constrained immigration and the species pool was rather constant over time and similar to that of the coring area. It was clearly shown that significant but transient toxic effects occurred. Particularly, this was the case for sub-dominant species (e.g. *P. notabilis*, *I. viridis*); however, the most dominant species did not recover during the experimental time under the experimental conditions. This includes *D. trispinata* that ‘simulated’ recovery through very high outlier abundances.

III-2.2 Oribatids

The records of oribatids showed, similar to the collembolan results, that lindane treatments significantly altered the community composition and the diversity indices over the complete experimental period. Contrasting the effects on collembolans, the community structure (Figure III-6) and the total abundance (Figure III-17) recovered 4 months after treatment in c_1, but not the diversity. It is up to expert judgment to decide which of the endpoints is most relevant to maintain proper ecosystem functions in the long-term. Once a species is extinct in the TME, the PRC analysis will not consider it as important. In this case, the statistical methodology could be relatively insensitive at detecting small differences in community composition. On the population level, one species of oribatids (*S. laevigatus*) showed a remarkable pattern of recovery after

a pronounced decrease in numbers immediately after treatment. The relative density was doubled compared to the control. This may be the consequence of one of the following scenarios. A fast mode of reproduction that is not affected by the treatment renders *S. laevigatus* superior over the other species. However, there is no evidence in the literature that *S. laevigatus* could be a pronounced r-strategist. Fast repopulation from outside the TME may have occurred, although the construction of the TME and the presumed migration range of soil organisms should have made immigration very improbable, as already mentioned for the collembolans above. Thus, the interpretation of these findings cannot be deduced from the data and has to be subject to further experimentation. Oribatid mites are thought to be more sensitive towards pesticides but have been investigated to a lesser extent than collembolans (LEBRUN & VAN STRAALEN 1995). Our results, however, do not indicate that oribatids of single populations recover less frequently or less completely from the treatments compared to collembolans. Differences of the potential recovery could depend either on the toxicological sensitivity towards the test compound, or on a limited capability of reproduction (CIANCIOLO & NORTON 2006). It is hypothesized that parthenogenetic species reproduce faster than sexually reproducing species and thus are superior in the recovery process. This, however, was not the case. In particular, the dominant species *S. laevigatus*, *M. semirufus* and *G. obvia* are those that reproduce sexually, and feed mainly on fungi (SIEPEL 1996). These species revealed high sensitivity and did not recover at the high lindane concentration c_2 until the end of the study period. Only one parthenogenetic species (*P. peltifer*) was steadily found in very low numbers. The proportion of 10 % of parthenogenesis in the group of oribatids is unusually high among all arthropods, but the coring area exhibits a proportion far below average for the group of oribatids. After the application date, the herbivorous *P. peltifer* was extinct in both treatment groups. There is no evidence from our results that differences of feeding or reproduction modes may be responsible for shifts in sensitivity.

III-2.3 Lumbricids

Earthworms were sampled once after the last sampling date for microarthropods, not in a continuous time-series. The rationale behind this limitation was that the standard sampling procedures for earthworms recommended the use of the formalin extraction method or hand sorting of all animals (ISO 2003a). Both methods would destroy the integrity of the test systems and would render it impossible to sub-sample the TME after extraction of lumbricids. Since no time-series data were available for lumbricids, no PRC analysis was performed. With *Lumbricus terrestris* and *L. castaneus*, two out of six species were significantly affected by the lindane

treatments one year after application (Table III-1). These species reproduce relatively slow and are likely to be found as facultative epigaeics at least temporarily in the upper soil layers (GISI *et al.* 1997). They were exposed to lindane over a long period, and effects on reproduction have most probably caused the deviations from control level rather than direct toxicity. Juveniles of the genus *Allolobophora* were also affected by the higher concentration c_2, possibly pointing to a reproductive inhibition of this group which appeared beside the insensitivity of adults. Contrasting the limitations of the test design concerning extraction methodology, the group was more viable and reproductive than expected in TME. Clearly effects of the insecticide lindane were detected even one year after the application. Due to the limitations discussed above (small-scale test systems, variability, destructive sampling reducing number of sampling events etc.) and the fact that tiered test systems for the assessment of risks posed to earthworms are established and implemented in the regulation of plant protection products, it is not recommended and required to consider earthworm testing in TME approaches.

III-2.4 Feeding activity

The feeding activity by means of bait-lamina sticks was assessed by using standardized procedures and uniform equipment (VON TÖRNE 1990) unless results were afflicted with a high degree of temporal variation (LARINK & KRATZ 1994). Feeding activity was not affected by the treatments at day 14, even at the higher application rate of 100 mg a.i./kg soil. The relation between bait-lamina consumption and abundance of earthworms, enchytraeids or soil microarthropods was well described in the literature (FÖRSTER *et al.* 2004; FILZEK *et al.* 2004; RÖMBKE *et al.* 2006). The non-parametric Spearman's rank correlation index between the parameters 'total control abundance of mesofauna at day 0' and 'total control feeding activity at day 14' was determined. Our analysis did not indicate that the mesofauna was responsible for the bait-lamina consumption (Figure III-28). On the other hand, an effect of the treatments on the consumption rates was expected because many populations of soil macro- and mesofauna recorded in this study were affected by the treatments initially or on a larger time scale. However, this expectation was not backed up by our dataset (significance of Spearman correlation >0.05). If the microarthropods were affected but feeding activity was not, other groups of organisms e.g. microorganisms, fungi, earthworms, enchytraeids whose densities were not recorded simultaneously to the bait-lamina test must have been responsible for the bait-lamina consumption. Because the earthworms were not recorded coincidentally, it was not possible to correlate the results of the feeding rates to earthworm densities.

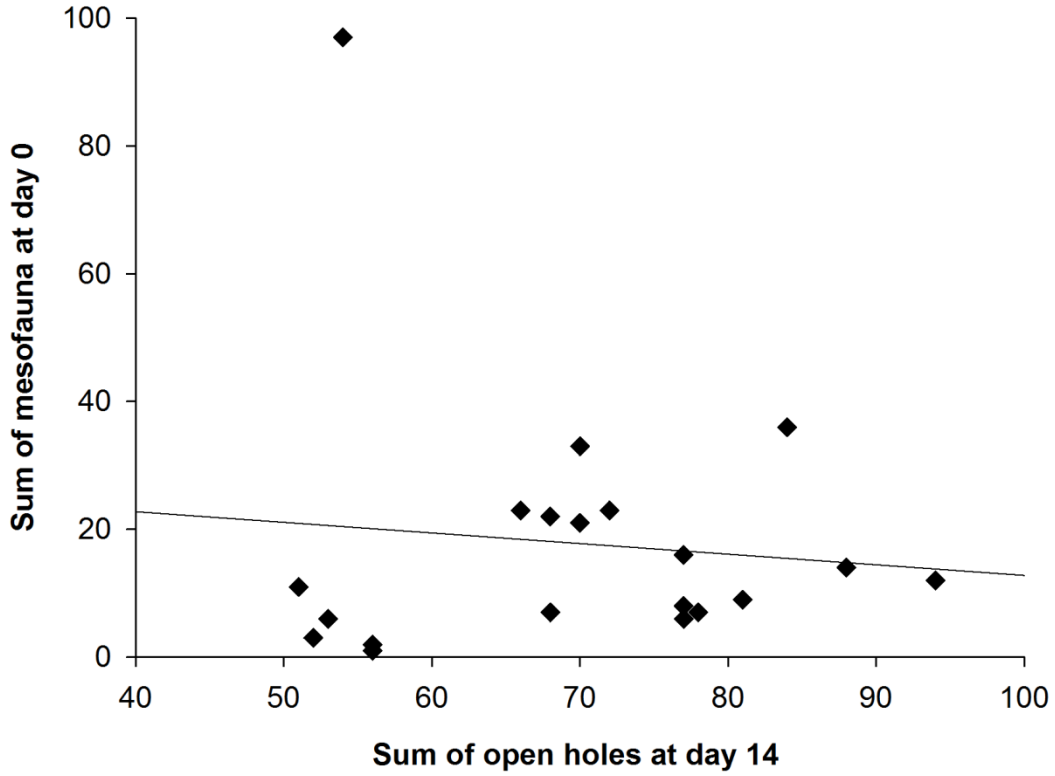


Figure III-28: Sum of mesofauna abundance and feeding activity in the dose-response study. Correlation visualized by the linear trend line. Spearman correlation analysis could not find significant relationships between any pair of variables ($p < 0.05$).

We further concluded that those groups were not affected by the treatment within the (unknown) detection limit of the bait-lamina test. Thus, we speculate that most of the organic matter consumption is attributed to the activity of microorganisms or, alternatively, that the effect could merely not be detected, even though lindane as a toxic model compound was applied at rates that were higher by orders of magnitude compared to standard agricultural practice. Furthermore, it would be possible that e.g. earthworms, which were most probably affected by the treatment from the very start of the experiment, used alternative food sources facilitated by the enhanced biomass production in the treatment TME (Figure III-26).

In-depth analyses of the data and the use of correction factors as for biomass or soil organic matter that delete excess variation from the data would possibly reveal effects of the treatment. However, those data were not gathered in our project. It is proposed to plan future TME experiments more consistently to enable further statistical corrections of interrelated endpoints. In conclusion of the present study, the interpretation of the bait-lamina results remains rather speculative and inconsistent to the preliminary expectations.

III-2.5 Plants

The treated TME showed significantly increased growth rates of plant biomass compared to controls, indicating a fertilizing effect of the formulation chosen (Figure III-26). The biomass stimulation was more clearly seen for the application rate c_1. It can be assumed that opposed effects of toxicity and fertilization led to the minor increase of growth rates under c_2. High-rates of lindane are known to inhibit plant growth. LICHTENSTEIN *et al.* (1962) showed amongst others that 30 ppm of lindane decreased the growth of different corn and pea species by 51 %. It was observed during the experiments that plant leafs and shoots were yellowing within hours after direct overspray, furthermore the soil structure was less compact than in the untreated controls for the whole study period. A conclusion of these findings is that the general promotion of the plant biomass was very likely due to the solvent carrier DMF that was included in the formulation; DMF can be partly metabolized into bioavailable nitrogen as the metabolite formamide (GESCHER 1993) or directly as ammonium (VEERANAGOUDA *et al.* 2006; JONES *et al.* 1966). The amount of nitrogen added by lindane application equals 490 kg/ha, which is a multiple of the normal agricultural practice (up to 230 kg/ha, usually exceeding 170 kg/ha). Due to the low toxicity of DMF (EC_{50} /LC_{50} aquatic organisms much greater than 100 mg DMF/l, BMU 2001) this compound is not considered to influence the outcome of the experiments with regard to the strong toxic effects of lindane. The promoting effect is likely assigned to fertilization through nitrogen supply rather than to the promotion of the microbial community. Nitrogen enrichment altered neither the communities of soil microbes nor the biomass of soil fungi but led to slight declines in microbial biomass (TRESEDER 2008).

Table III-4: Plant taxa occurring on the TME surface 79 days after the date of application on 28 July 2005.

Treatment	control															10 mg a.i./kg soil										100 mg a.i./kg soil									
TME No.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	1	3	4	7	8	9	11	12	13	14	2	5	6	10	15	16	17	18	19	20
Bromus hordeaceus	69	79	70	80	90	85	20	80	80	20	70	50	49	70	100	40	60	85	50	90	80	90	40	50	10		80		90	55	70	30			80		5	65		90
Holcus lanatus		10	20	20	10	10	39	19	20	70	20	50		30		40	20		5		20		20	4	80	95		30		10			50	10	10	100	95	10	60	10
Taraxacum officinale	25	10	10			5	39	1		10	10		50				10	5	20			10		45	10			15	10	30	10	10	30					15		
Arrhenatherum elatius																20			5	10			40					50				60	20	45	10			10	40	
Dactylis glomeratus																															20									
Poa trivialis							1																			5								45						
Ranunculus repens	5																10	5									20			1										
Festuca pratensis cf.													1						20									5												
Lolium perenne																																								
Cirsium arvense																									1					4										
Agropyron spec. cf.							1																																	
Trifolium spec.	1	1																			25	20	30	20	20	25	15	25	20	20	25	35	20	30	30	20	20	30	35	20
mean growth height [cm]	15	15	25	20	20	15	15	25	20	25	15	15	20	25	20	30	30	15	25	30	398	240	261	328	284	379	297	324	476	325	330	302	271	217	199	294	257	242	225	165
fresh weight [g]	150	191	247	168	166	90	180	161	137	179	124	131	154	157	140	139	195	127	160	136																				

III-2.6 Methodological conclusions

Suitability as higher-tier option in environmental risk assessment (ERA) the TME-method was found to be suitable for testing the impact of as a model compound on soil arthropod communities. TME can be maintained outdoors for a period of up to 1 year as shown by this study. Thus, TME can be used for long-term testing of persistent substances. High application rates of lindane, i.e. approximately 10-100-fold concentrations compared to recommended field rates) caused strong and long-lasting effects on microarthropods. Our TME approach was based on the use of grassland soil with a more diverse and more sensitive community of microarthropods compared to arable soils. However, further research is needed to establish differences in sensitivity of such organisms in in-crop- and off-crop-scenarios.

III-2.6.1 Outdoors versus indoors

The TME were intentionally set up outdoors. It was presumed that natural communities and their specific diversity would remain constant for an extended period of time under variable and natural environmental conditions i.e. a natural water regime and fluctuations of temperature. On the contrary, it is known that under constant conditions, specialized 'best-fit species' may become dominant (BEGON *et al.* 1998). Indoor systems have been shown to be suitable for short- or mid-term experiments, but hold the risk of fast population changes after a few months; a corresponding example is given by KRIEG *et al.* (2007), general considerations can be found in DAEHLER & STRONG (1996).

III-2.6.2 Sequential sampling

The sub-sampling within a TME results in a loss of soil surface of less than 20 % which does not substantially influence the integrity of the whole system (as expected by the results of SCHNEIDER *et al.* 2007). Our own additional studies suggested that the reduced surface area does not influence the soil communities of micro-arthropods (to be published). It was considered to be essential to follow-up a TME over the whole experimental period as a coherent experimental unit.

III-2.6.3 Numbers and extraction issues

The numbers of microarthropods extracted from all soil samples were rather low, which can be partially explained by the methodology. As mentioned by KOOLHAAS *et al.* (2004), the extraction in a 3-week period can influence the numbers of animals because of egg hatching. Hence, the thermal extraction procedure was restricted to 2 weeks accepting the consequences of lower numbers of animals compared to longer extraction times, particularly of juveniles. In addition, the 'upside-down' orientation of soil cores within the extraction canister may favour the extrac-

tion of epigeic rather than endogenic species.

III-2.7 Outlook

The results of this range finding experiment were taken as a rationale to further develop the outdoor TME methodology. We provide evidence for the general suitability of the TME method to reflect community and taxon level effects of the persistent and toxic model compound lindane. A relatively high number of untreated TME were used to get robust estimations of the systematic natural variability. We will continue to design reliable dose-response tests as the next step towards a sound experimental tool for higher-tier risk assessment. It is assumed that dose-response studies would enhance the statistical power of the test, yet exemplified for aquatic mesocosm tests as guided by OECD (2006). Based on the results of this study, the methodology seems to be suitable to be used in the regulatory context of the assessment of pesticides. Nevertheless, protection goals, data requirements and the conceptual framework have to be defined in the near future.

IV

Effects of lindane to soil communities: dose-response relationship

The second long-term TME experiment was designed to develop a soil community higher-tier semi-field system that is suitable to detect effects of persistent compounds on the community structure of soil organisms and to achieve a long-term stability of the test systems in order to demonstrate recovery potential within one year. By using the insecticidal model compound lindane, we studied its effects at application rates that were used according to good agricultural practice before its ban in many countries worldwide. It was purposed to test for the applicability of a dose-response design that should deliver statistical robust endpoints, i.e. NOEC and EC_x-values of populations and communities of soil organisms. Those are generally considered more informative and thus favourable over NOEC approaches for risk assessment purposes (LASKOWSKI 1995, EUROPEAN COMMISSION 2002b), since a NOEC highly depends on the chosen treatment levels (CRANE & NEWMAN 2000), and oppositely an EC_x calculation extrapolates to concentrations that were not actually tested. Risk assessment procedures should set the *x* appropriately to a corresponding NOEC in order to define a negligible level of effect (ISNARD *et al.* 2001). JAGER (2011) published very recently an opposite opinion in 'Environmental Science & Technology'. Briefly summarized, he criticized that the EC_x is far from being an independent measure of intrinsic toxicity of a substance but has many biases similar to the historical NOEC-approach. The EC_x-values depend on the arbitrarily chosen exposure time, the specific endpoint, on a non-varying exposure and other factors. The expectation that effects close to the usual application rates of lindane would be less pronounced as compared to previous studies where manifold rates were used was confirmed by the present study. On the spatial scale of a TME, the most important groups of organisms belong to the size class of mesofauna such as collembolans, oribatid mites, nematodes and enchytraeids. They were therefore considered essential for a holistic assessment of ecotoxicological effects in (model-) ecosystems. Together with the soil fungi, they represent from primary consumers to secondary predators an almost complete set of the trophic guilds present in temperate soils.

IV-1 Results

Five taxa groups, namely collembolans, oribatid mites, enchytraeids, nematodes, and fungi were tested in same system indeed, the results were analysed in separate sections of the thesis. A direct comparison is due to the large range of the abundances and susceptibilities towards the test substance lindane not possible without applying strongly distorting transformations. Therefore, the analysis of the study results was done without an integrative analysis.

IV-1.1 Effects on collembolans

The lowest control abundance was recorded in June, one month after application at day 26, with a mean number of 10 individuals per sample. The highest number of collembolans was found in May, one year after application at day 351 with a mean of 25 individuals per sample

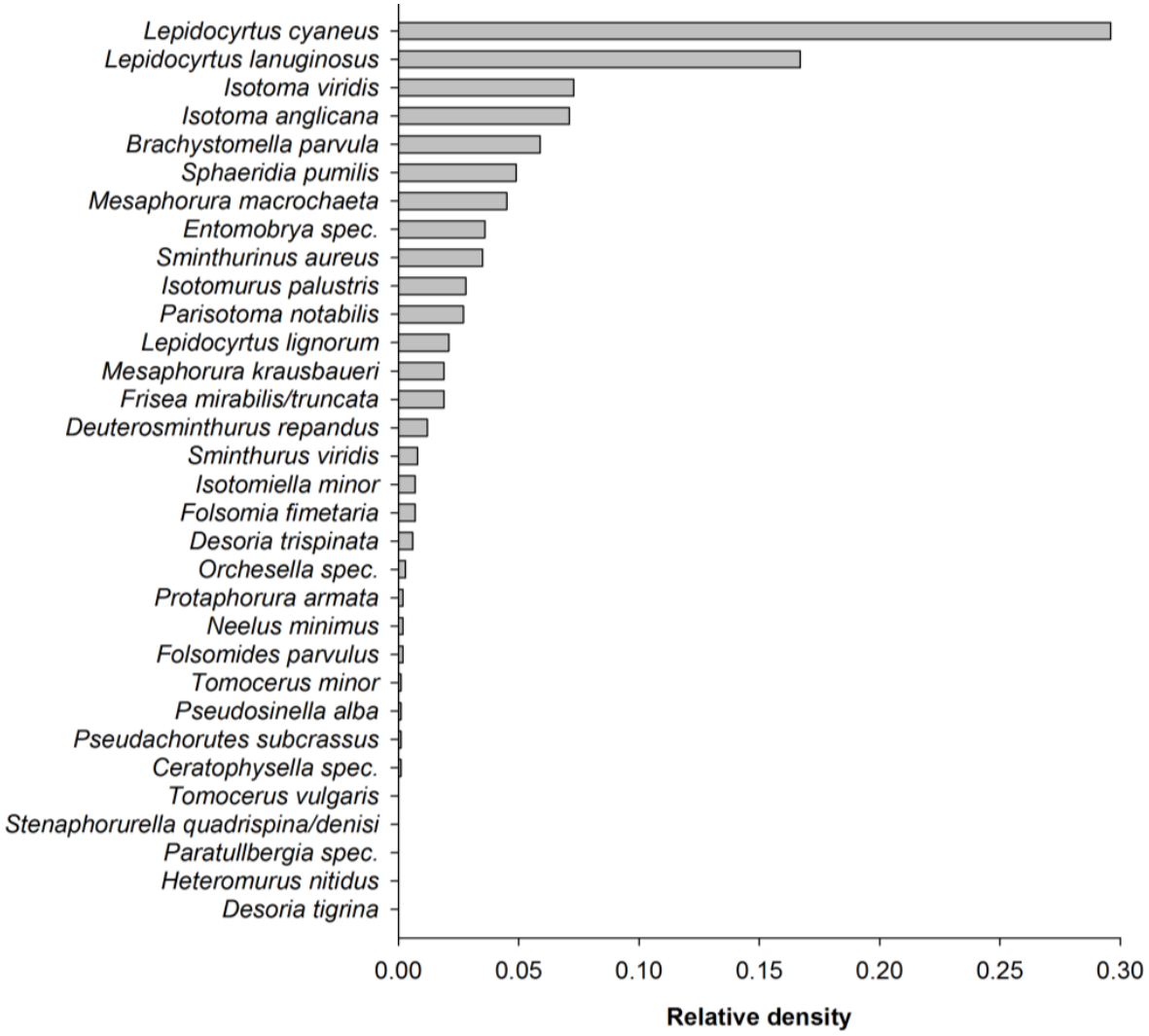


Figure IV-1: Dominance spectrum of collembolans. Figure is based on mean control values of all dates.

(corresponding to 12700 individuals per square-meter, Figure IV-5). During the pre-treatment period, there were minor differences in mean abundance of collembolans. However, the foreseen highest treatment group was significantly lowered compared to untreated TME-samples. In total 32 collembolan species were found. By far the most common species was *Lepidocyrtus cyaneus*, providing about 30 % of all individuals in control samples over all sampling dates. The species is epi- and hemi-edaphic and characterized by a relative high mobility. Further 17 % of the total control abundance was provided by *Lepidocyrtus lanuginosus*. All in all the five most dominant species (additional to the two aforementioned: *Isotoma viridis* 7.3 %, *Isotoma anglicana* 7.1% and *Brachystomella parvula* 5.9 %) built two thirds of the total collembolan abundance (refer to Figure IV-5). The responses of single collembolans species to the application of lindane in the dose-response study can be seen in the appendix.

The application of lindane resulted in a significant decrease of the total collembolan abundance at day 26. This reduction was significant between controls and the two highest treatments c_4 and c_5 (NOEC = 0.32 mg a.i./kg dry weight); further initially significant effects were also observed for two of the dominant species. *L. lanuginosus* and *I. anglicana* showed NOECs of 0.32 mg a.i./kg dry weight soil at day 26. Some of the springtail populations showed significant deviations from the control level only at the highest concentration c_5, e.g. *Sminthurinus aureus* ($NOEC_{day\ 26}$ = 1.0 mg as/kg dry soil). Other species responded to lower dosages. *I. viridis* had a $NOEC_{day\ 26}$ of 0.1 mg a.i./kg dry weight soil. Only for the latter two species, effects had been evident until the next sampling date at day 88. Although the Principal Response Curves of the collembolan dataset (*set 5* and *set 12* joined) in Figure IV-2 showed high variation at the pre-treatment date (day -1), a significant effect on the collembolan community could be observed at day 26 and day 88 (Figure IV-2 and NOEC = 0.32 mg a.i./kg dw at day 26 in Table IV-1). Of all variation within the collembolan dataset, 8.1 % and 7.8 % could be attributed to the treatment regime. Finally, 24.1 and 29.7% of the variance explained by treatment was captured by the 1st canonical axis of the PRC and is shown in the diagram. However, the treatment regime significantly influenced the community composition of collembolans for set 5 but not for set 12 as indicated by the Monte-Carlo permutation tests on the total variance (over the complete study period). The treatment effects at single sampling dates were significant at days 26 and 88 (2000 Monte-Carlo-Permutations over the samples of one sampling date), identical for both datasets because set 12 was merely complemented by the data of the last sampling at day 351. The data until day 149 was the same for set 5 and set 12. The response of the collembolan community was not strictly dose-related. Deviations from the control community were significant at day 26 for c_4 and c_5 (NOEC = 0.32

mg a.i./kg dw soil; Williams test on PCA sample-scores, two-sided, $\alpha < 0.05$, Table IV-1). However, three months after application, the two highest treatments still showed the largest negative deviation to the control level, qualitatively indicating a long-lasting effect of the treatment. At the end of the test-period of one year, all communities recovered near to the control level. Species were regarded as being particularly susceptible to lindane treatment, which have high species weights (b_k-values as indication of the correlation of the species to the overall response, Figure IV-2). Species weights about zero indicate very low correlation and an indifferent response to the treatment. In particular, species belonging to the genus *Isotoma* and *Lepidocyrtus* were highly connected to the overall response pattern. Coincidently, species with very low abundances did not provide much variation (i.e. 'information') and appeared with b_k-values near zero. Those findings were consistent regardless of the complete dataset (*set 12*) being used or the analysis being restricted to *set 5* (b_k-plot of Figure IV-2). The diversity index of Shannon and the evenness as an alternative community endpoint did not respond to the treatments, but the species richness was significantly lowered in the two highest treatments at day 26 immediately after application of the test item (Figure IV-4). As Table IV-1 summarizes, most effects of lindane on collembolans appeared for a short term only and the endpoints recovered three months after exposure. Admittedly, the NOEC for the aggregated species *Stenaphorurella quadrispina/denisi* and *Parisotoma notabilis* was at c_4, but this was due to a single find in the former and due to promotion of abundance (mean abundance of four individuals/sample in c_5 compared to two individuals in the control) in the latter case.

During the pre-treatment period, the similarity within controls and between controls and treatments was low (30-40 % for the Steinhaus index, 40-60 % for Stander´s index). There were no fundamental changes within the controls and compared to most of the treatment groups immediately after application of the test substance. C_4 responded clearly to lindane, the c_5-group showed the steepest decline in similarity from day -1 to day 26. This was fact for both indices, which showed slight differences in the order of treatment groups due to the different methods of calculation since the Stander-index focused on relative densities, whereas the Steinhaus-index focuses on the absolute abundance of species. The highest treatment caused rather a shift in relative densities of the species than of the absolute abundance. No statistical threshold concentrations were calculated for this endpoint, but it was concluded that the three highest concentrations of lindane caused a slight community shift, resulting in a lowered similarity compared to the control group.

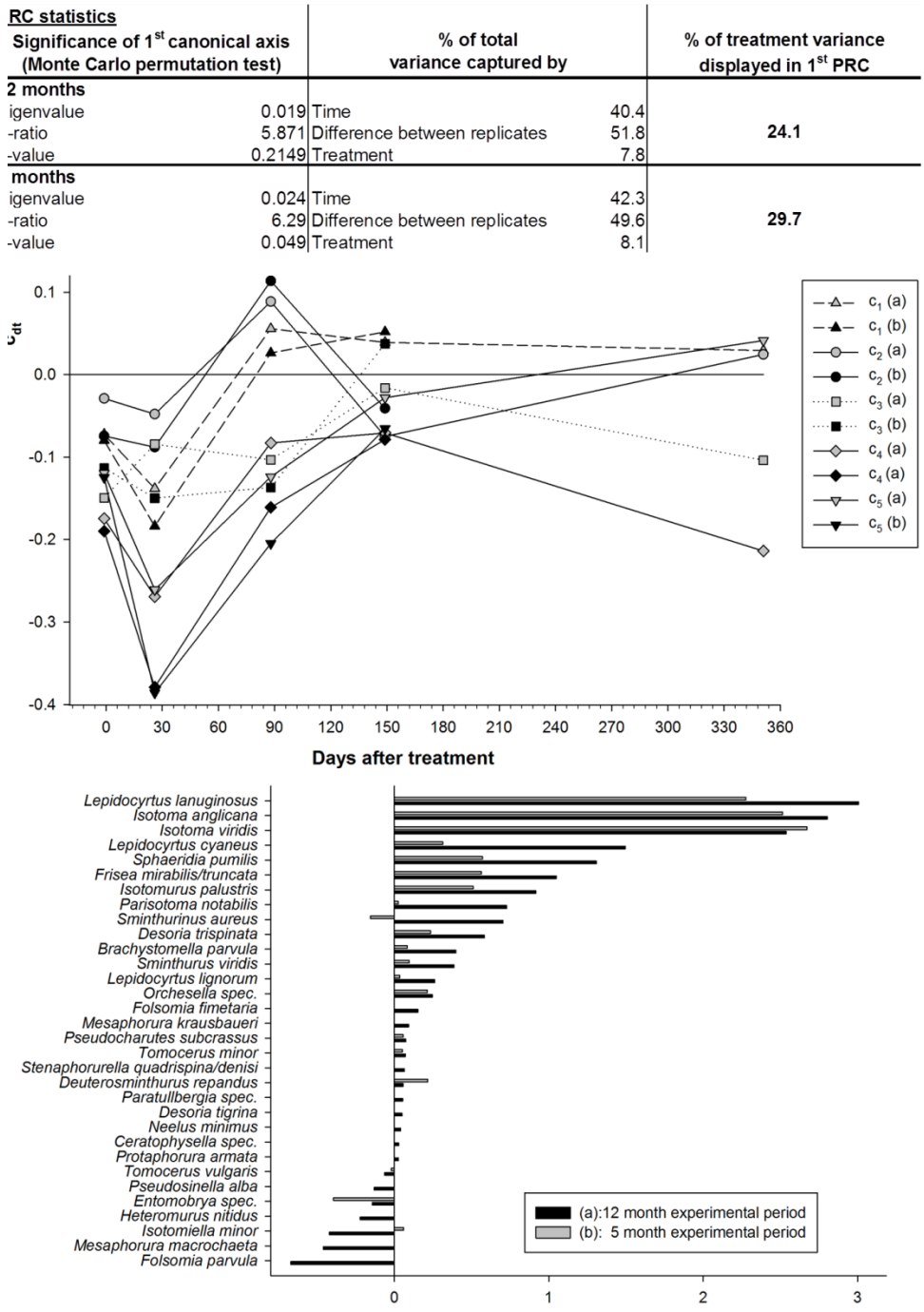
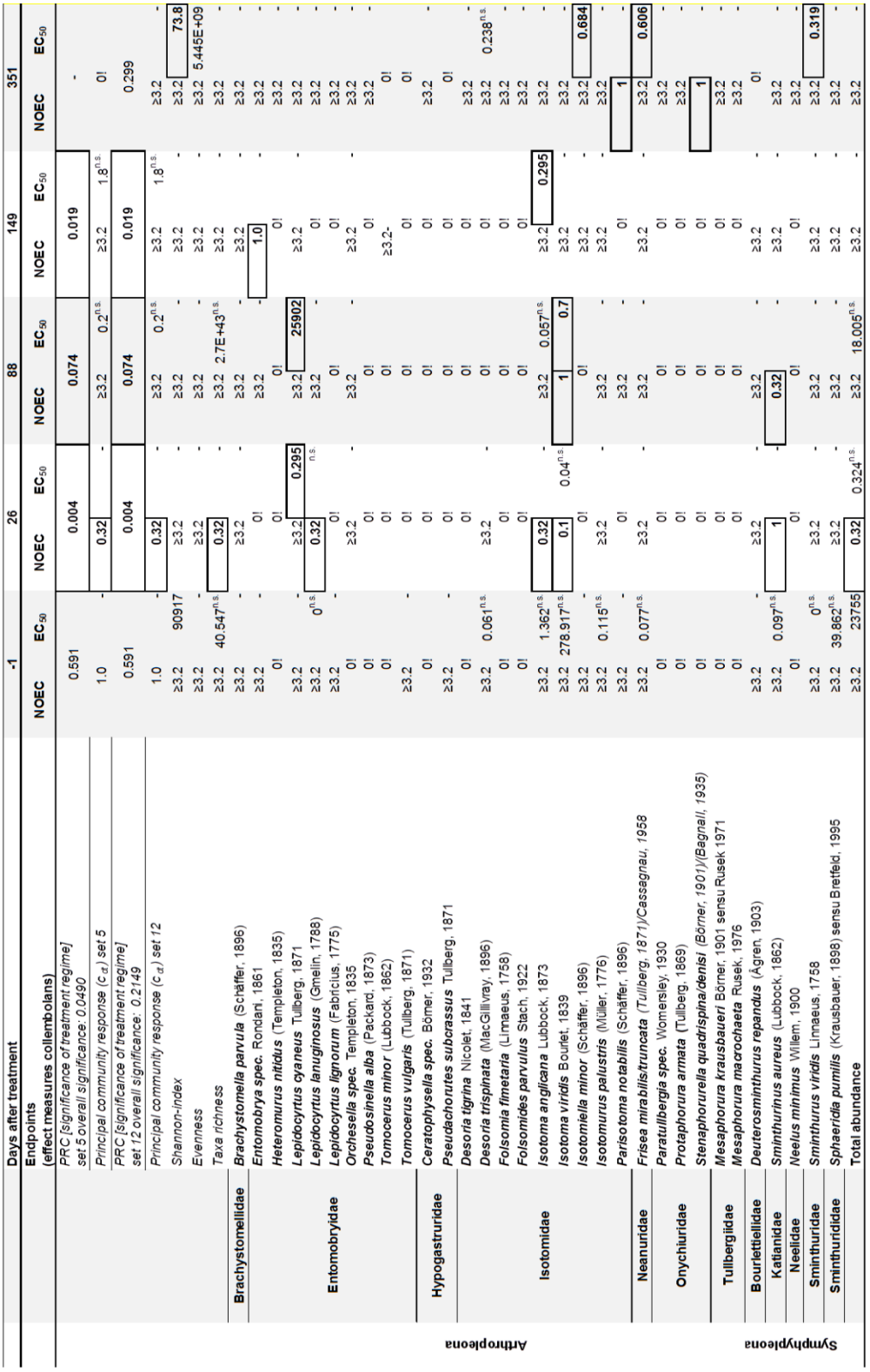


Figure IV-2: Principal Response Curve of collembolans as obtained in the TME dose-response test 5 and 12 months after exposure of lindane in concentrations of c_1 = 0.032, c_2 = 0.100, c_3 = 0.320, c_4 = 1.000, c_5 = 3.200 mg a.i./kg dw soil. The control is set to zero and is represented by the abscissa. The first canonical axis of a Redundancy Analysis (RDA) with time as covariable and lindane treatments as explanatory variables is shown. The ordinate of the PRC diagram shows the canonical coefficients (c_{dt}-values). The significance of the overall treatment regime is given in the table provided with the figure; significance of the treatment regime per sampling date is indicated by Table IV-1 (α<0.05 accepted significant). Significant differences of treatments in comparison to control by applying the Williams test (two-sided, α <0.05) are shown in Table 1. Species with high (decrease of mean abundance in case of treatment related decrease of c_{dt} values) and low (mirror-inverted response) b_k-values are supposed to follow the response pattern in a similar manner as shown by the diagram.

Table IV-1: Species lists, effects on the abundance and on the communities endpoints for the groups of collembolans, if sampled at the given date and applicable to analyse. 0!: no variance at date, species did not occur ; ≥3.2: no effects including the highest treatment; -: statistics not applicable because no dose-response relation given, slope of the function <0.001 ; n.s.: no significant effect concentration, but calculation possible, slope not <0.001; hatched cells: not sampled.



		Days after treatment	-1		26		88		149		351	
		Endpoints (effect measures collembolans)	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}
		PRC [significance of treatment regime] set 5 overall significance: 0.0490	0.591		0.004		0.074		0.019		-	
		Principal community response (c_{at}) set 5	1.0	-	0.32	-	≥3.2	$0.2^{n.s.}$	≥3.2	$1.8^{n.s.}$	0!	
		PRC [significance of treatment regime] set 12 overall significance: 0.2149	0.591		0.004		0.074		0.019		0.299	
		Principal community response (c_{at}) set 12	1.0	-	0.32	-	≥3.2	$0.2^{n.s.}$	≥3.2	$1.8^{n.s.}$	≥3.2	-
		Shannon-Index	≥3.2	90917	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	73.8
		Evenness	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	5.445E+09
		Taxa richness	≥3.2	$40.547^{n.s.}$	0.32	-	≥3.2	$2.7E+43^{n.s.}$	≥3.2	-	≥3.2	-
Arthropleona	**Brachystomellidae**	***Brachystomella parvula*** (Schäffer, 1896)	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	-
	Entomobryidae	***Entomobrya* spec.** Rondani, 1861	≥3.2	-	0!		≥3.2	-	1.0	-	≥3.2	-
		Heteromurus nitidus (Templeton, 1835)	0!		0!		0!		0!		≥3.2	-
		Lepidocyrtus cyaneus Tullberg, 1871	≥3.2	-	≥3.2	0.295	≥3.2	25902	≥3.2	-	≥3.2	-
		Lepidocyrtus lanuginosus (Gmelin, 1788)	≥3.2	$0^{n.s.}$	0.32	n.s.	≥3.2	-	0!		≥3.2	-
		Lepidocyrtus lignorum (Fabricius, 1775)	≥3.2	-	0!		0!		0!		≥3.2	-
		***Orchesella* spec.** Templeton, 1835	0!		≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	-
		Pseudosinella alba (Packard, 1873)	0!		0!		0!		0!		≥3.2	-
		Tomocerus minor (Lubbock, 1862)	0!		0!		0!		≥3.2-		0!	
		Tomocerus vulgaris (Tullberg, 1871)	≥3.2	-	0!		0!		0!		0!	
	Hypogastruridae	***Ceratophysella* spec.** Börner, 1932	0!		0!		0!		0!		≥3.2	-
		Pseudachorutes subcrassus Tullberg, 1871	≥3.2	-	0!		0!		0!		0!	
	Isotomidae	***Desoria tigrina*** Nicolet, 1841	0!		0!		0!		0!		≥3.2	-
		Desoria trispinata (MacGillivray, 1896)	≥3.2	$0.061^{n.s.}$	≥3.2	-	0!		0!		≥3.2	$0.238^{n.s.}$
		Folsomia fimetaria (Linnaeus, 1758)	0!		0!		0!		0!		≥3.2	-
		Folsomides parvulus Stach, 1922	0!		0!		0!		0!		≥3.2	-
		Isotoma anglicana Lubbock, 1873	≥3.2	$1.362^{n.s.}$	0.32	-	≥3.2	$0.057^{n.s.}$	≥3.2	0.295	≥3.2	-
		Isotoma viridis Bourlet, 1839	≥3.2	$278.917^{n.s.}$	0.1	$0.04^{n.s.}$	1	0.7	≥3.2	-	≥3.2	-
		Isotomiella minor (Schäffer, 1896)	≥3.2	-	0!		0!		≥3.2	-	≥3.2	0.684
		Isotomurus palustris (Müller, 1776)	≥3.2	$0.115^{n.s.}$	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	-
		Parisotoma notabilis (Schäffer, 1896)	≥3.2	-	0!		≥3.2	-	0!		1	-
Symphypleona	**Neanuridae**	***Frisea mirabilis/truncata*** *(Tullberg, 1871)/Cassagnau, 1958*	≥3.2	$0.077^{n.s.}$	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	0.606
	Onychiuridae	***Paratullbergia* spec.** Womersley, 1930	0!		0!		0!		0!		≥3.2	-
		Protaphorura armata (Tullberg, 1869)	0!		0!		0!		0!		≥3.2	-
		Stenaphorurella quadrispina/denisi *(Börner, 1901)/(Bagnall, 1935)*	0!		0!		0!		0!		1	-
	Tullbergiidae	***Mesaphorura krausbaueri*** Börner, 1901 sensu Rusek 1971	0!		0!		0!		0!		≥3.2	-
		Mesaphorura macrochaeta Rusek, 1976	0!		0!		0!		0!		≥3.2	-
	Bourletiellidae	***Deuterosminthurus repandus*** (Ågren, 1903)	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	-	0!	
	Katianidae	***Sminthurinus aureus*** (Lubbock, 1862)	≥3.2	$0.097^{n.s.}$	1	-	0.32	-	≥3.2	-	≥3.2	-
	Neelidae	***Neelus minimus*** Willem, 1900	0!		0!		0!		0!		≥3.2	-
	Sminthuridae	***Sminthurus viridis*** Linnaeus, 1758	≥3.2	$0^{n.s.}$	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	0.319
	Sminthurididae	***Sphaeridia pumilis*** (Krausbauer, 1898) sensu Bretfeld, 1995	≥3.2	$39.862^{n.s.}$	≥3.2	-	≥3.2	-	≥3.2	-	≥3.2	-
		Total abundance	≥3.2	23755	0.32	$0.324^{n.s.}$	≥3.2	$18.005^{n.s.}$	≥3.2	-	≥3.2	-

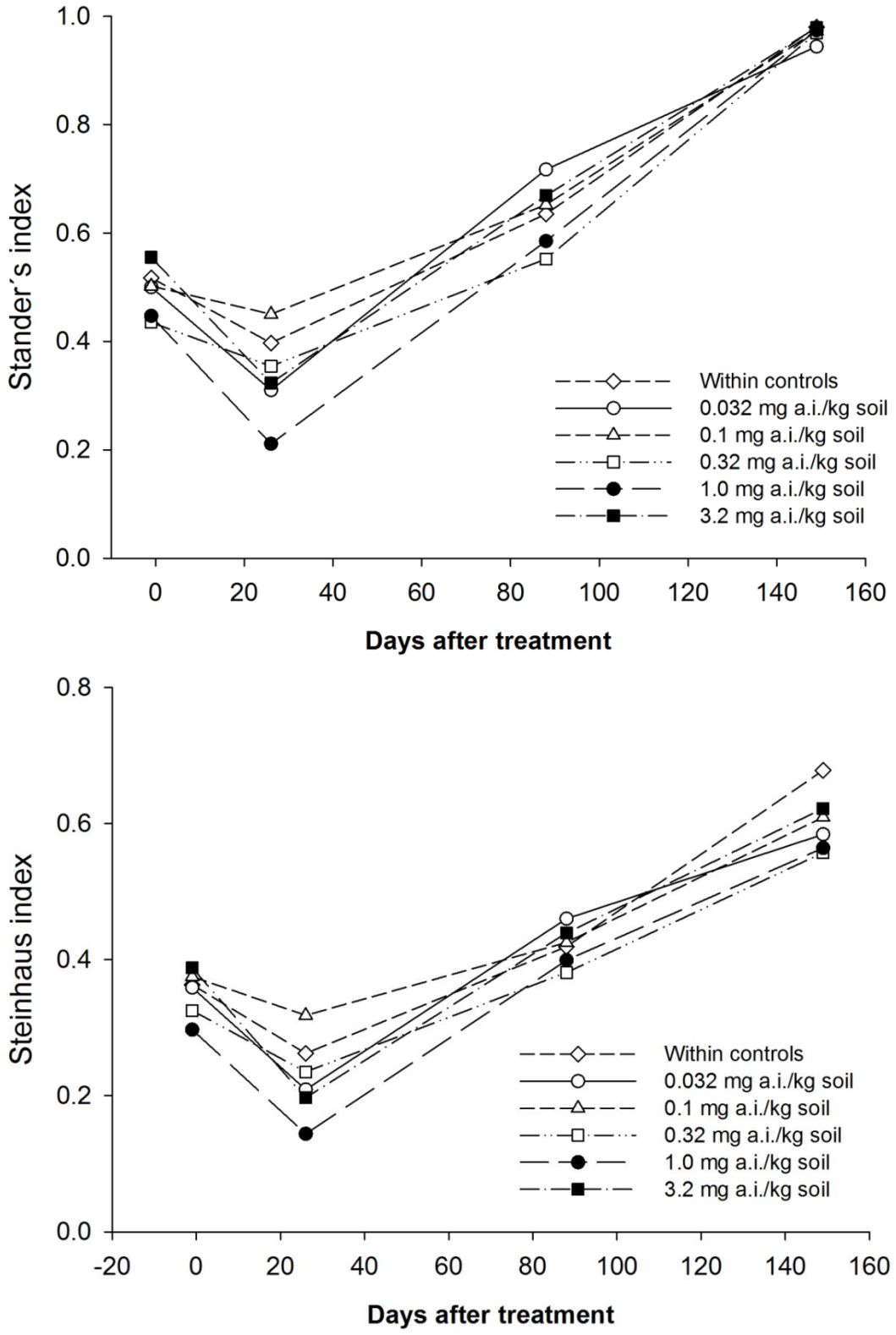


Figure IV-3: Similarity indices of Collembolans in the dose-response study with model compound lindane. 12 and replicates for control and treatment groups, respectively.

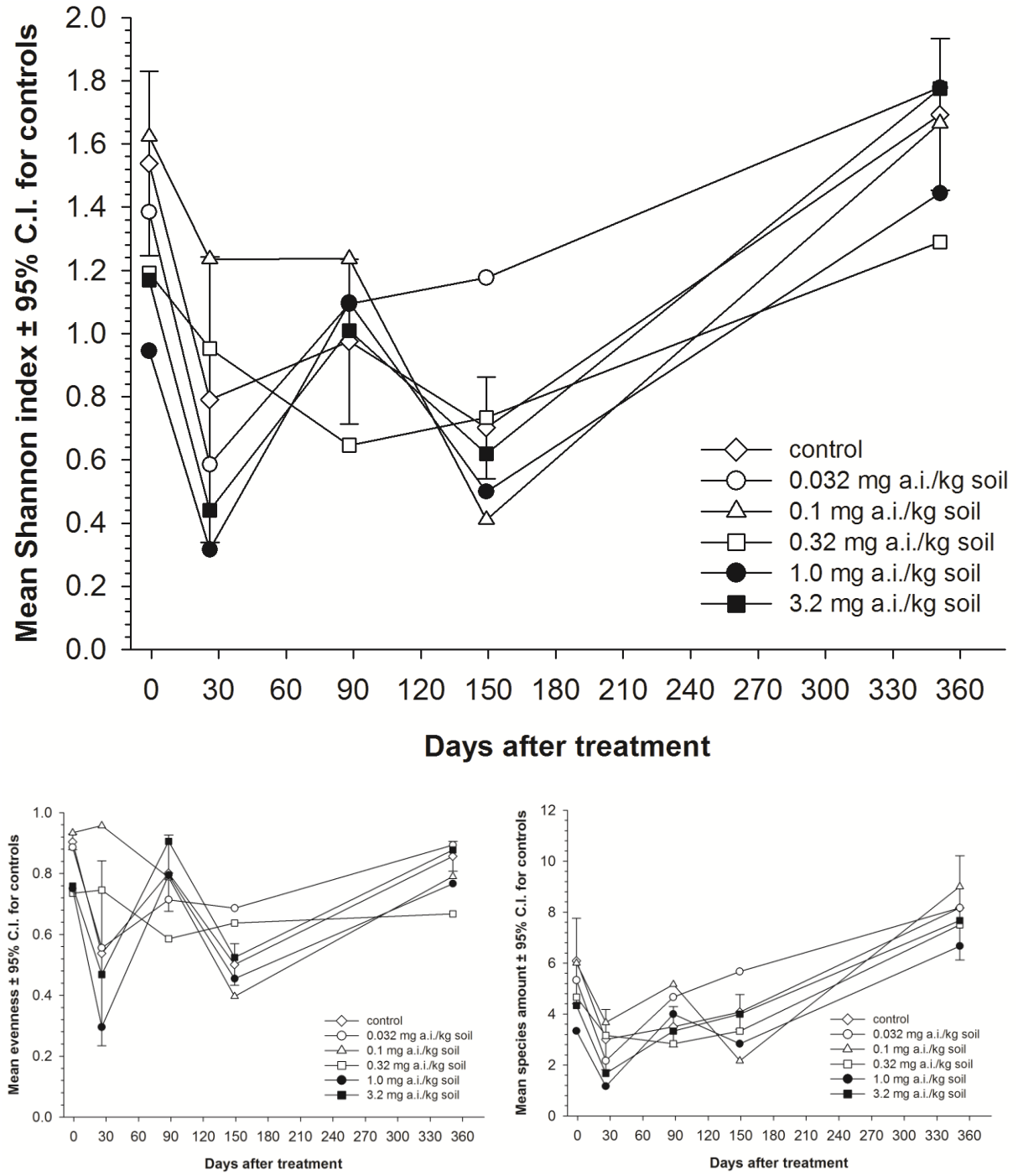


Figure IV-4: Diversity of Collembolan community: Shannon´s H', Evenness and species richness. FRor the significance of treatment differences to control by William's multiple t-Test (two-sided; $\alpha<0.05$) compare Table IV-1.

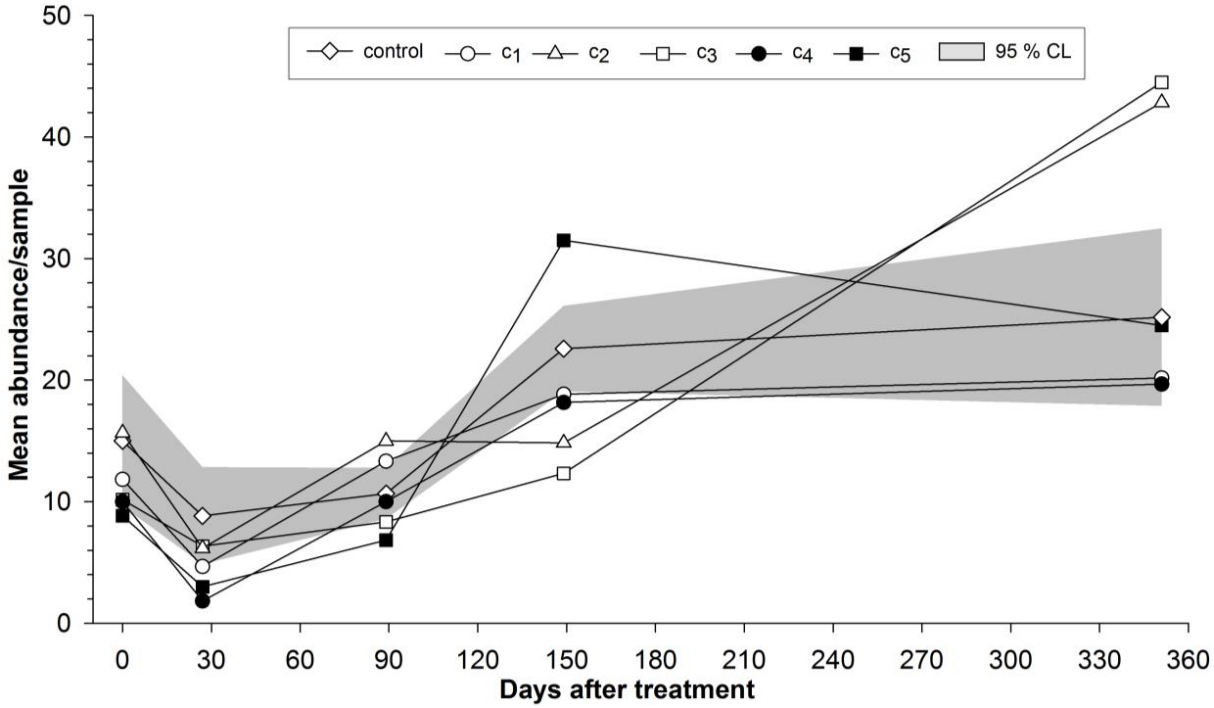


Figure IV-5: Arithmetic mean (± 95 % confidence interval for the controls) of collembolan abundance ($N_{control}$ = 12 $N_{treatment}$ = 6 samples per TME and date) as obtained in the dose-response study.

IV-1.2 Effects on oribatid mites

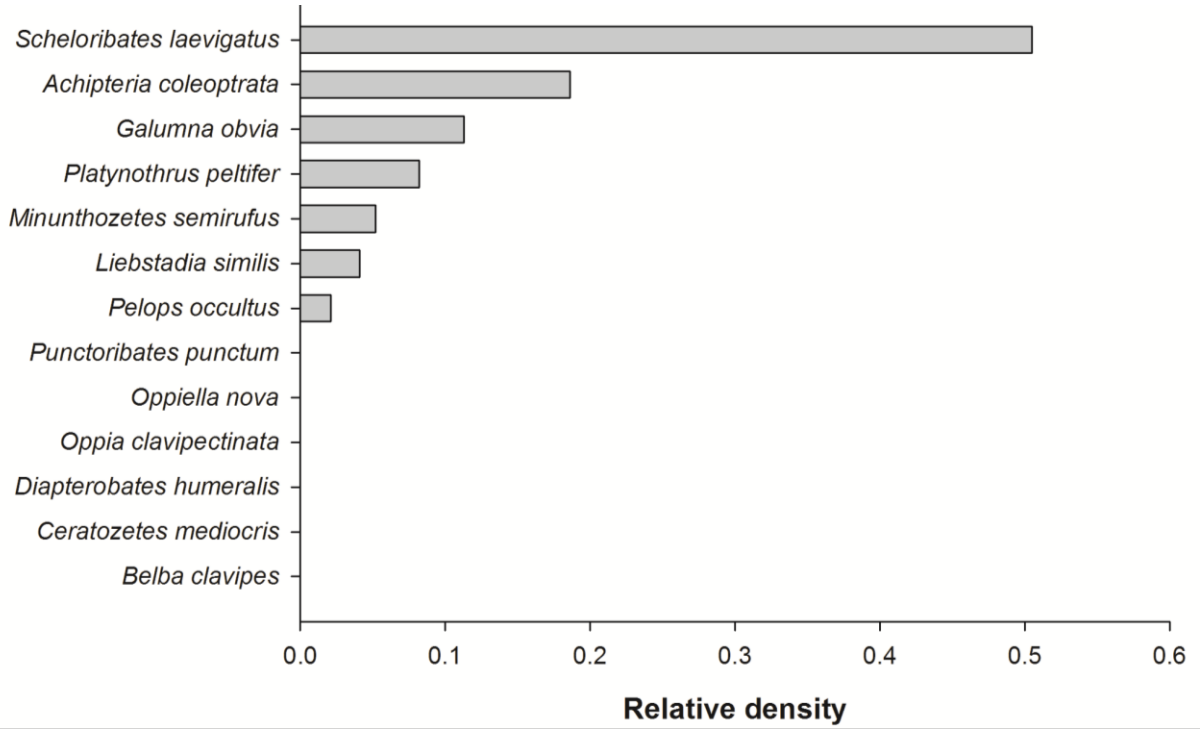


Figure IV-6: Dominance spectrum of oribatids. The diagram is based on mean control values of all dates. Species without bars did not occur in the control samples but in the treatments at any sampling date.

Very low abundances of oribatids were recorded (compare Figure IV-10). Control and treatment group means per sample were between one and four individuals per sample corresponding to densities of 500-2000 individuals per square meter; none of the samples contained more than ten different species.

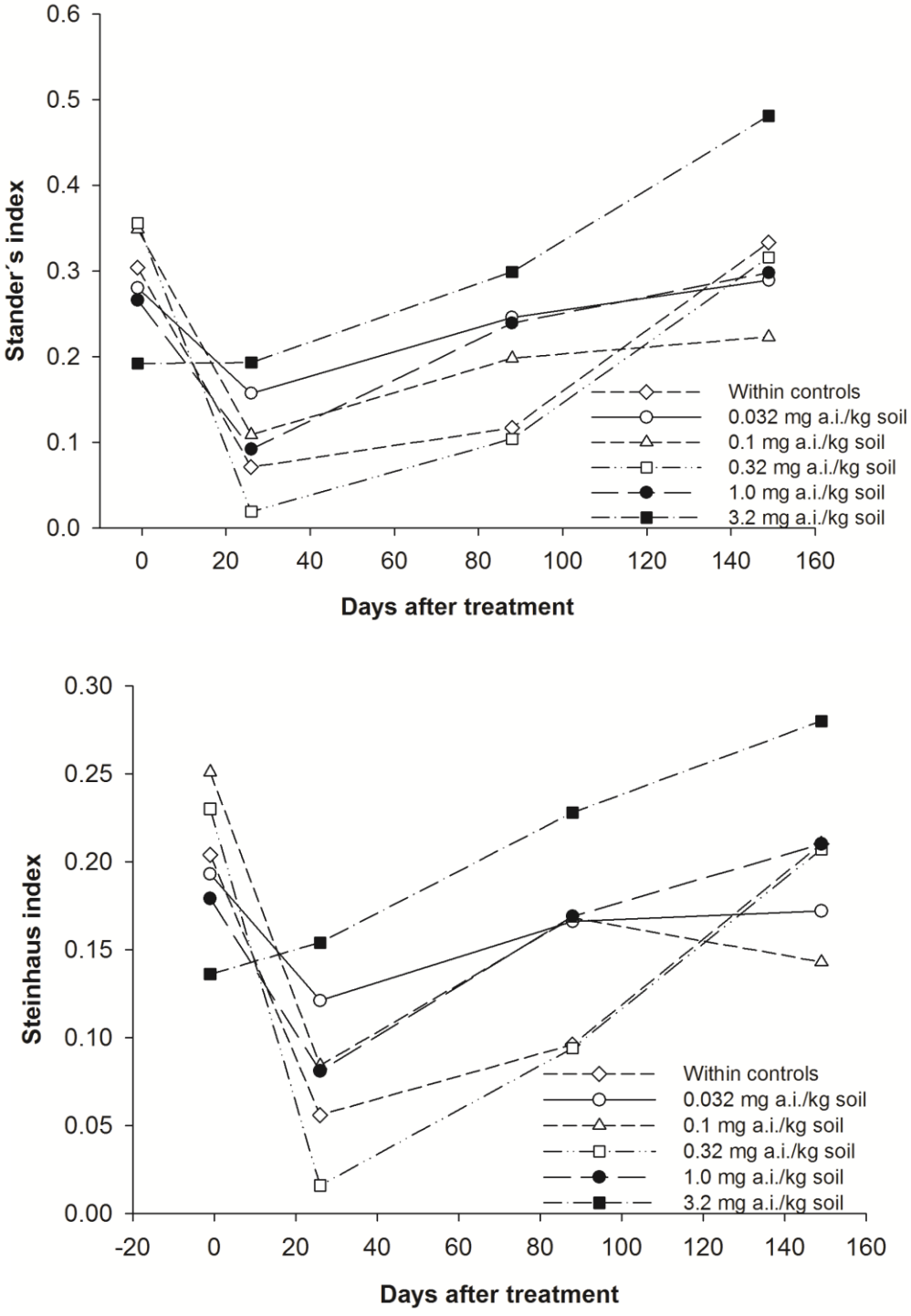


Figure IV-7: Similarity indices oribatids.

One single species *Scheloribates laevigatus* provided more than 50% of the whole community; 19 % and 11 % of the total were determined as *Achipteria coleoptrata* and *Galumna obvia*. Ten species were of minor dominance or occurred exclusively in the treated TME as singletons or doubletons (Figure IV-6). In total, 13 species of oribatids were found in the study (refer to species list Table IV-2). Oribatid mites appeared not to be susceptible at all to soil concentrations between 0.032 and 3.2 mg lindane/kg dry weight soil. None of the endpoints directly measured or secondarily derived, responded to the treatment by significant negative deviations from control level (compare Table IV-2). The NOEC at c_4 after 88 days post treatment for the dominant species *Scheloribates laevigatus* can be ascribed to a promotion of the abundance at the highest treatment level. The PRC analysis did not explain a significant part of the overall variation of the data; nonetheless, further calculations were carried out by default, which did not indicate treatment related effects after qualitative interpretation PCA-sample scores were significantly lower for the highest treatment group 89 days after application (Williams multiple t-Test, alpha one-sided smaller 0.05). The diversity of oribatid communities was not significantly affected by the lindane treatment. The only difference in Shannon index and species richness was observed at the date before application at the highest treatment level c_5 (Figure IV-8).

Table IV-2: Species lists, effects on the abundance and on the communities endpoints for the groups of oribatids, if sampled at the given date and applicable to analyse. 0!: no variance at date, species did not occur; ≥3.2: no effects including the highest treatment; -: statistics not applicable because no dose-response relation given, slope of the function <0.001 ; n.s.: no significant effect concentration, but calculation possible, slope not <0.001; hatched cells: not sampled.

	Days after treatment	-1		26		88		149		351	
	Endpoints (effect measures oribatids)	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}
	PRC [significance of treatment regime] set 5 overall significance: 0.5982		0.255		0.445		0.099		0.253		
	Principal community response (c_{dt}) set 5	≥3.2	-	≥3.2	$0.9^{n.s.}$	1	-	≥3.2	$1.6^{n.s.}$		
	Shannon-index	1	$1.299^{n.s.}$	≥3.2	$1.445^{n.s.}$	≥3.2	-	≥3.2	-		
	Evenness	≥3.2	-	≥3.2	0.895	≥3.2	-	≥3.2	-		
	Taxa richness	1	$1.787^{n.s.}$	≥3.2	-	≥3.2	$24.791^{n.s.}$	≥3.2	-		
Achipteriidae	***Achipteria coleoptrata*** (Linné, 1758)	1	$0.019^{n.s.}$	≥3.2	-	≥3.2	-	≥3.2	$0.077^{n.s.}$		
Camisiidae	***Platynothrus peltifer*** (C.L. Koch, 1839)	≥3.2	-	≥3.2	-	0!		≥3.2	-		
Ceratozetidae	***Ceratozetes mediocris*** Berlese, 1908	0!		0!		≥3.2	-	0!			
	Diapterobates humeralis (Hermann, 1804)	≥3.2	-	0!		0!		0!			
Damaeidae	***Belba (Damaeus) clavipes*** (Hermann, 1804)	0!		≥3.2	-	0!		0!			
Galumnidae	***Galumna obvia*** (Berlese, 1915)	≥3.2	-	≥3.2	-	≥3.2	-		-		
Mycobatidae	***Minunthozetes semirufus*** (C.L. Koch, 1841)	≥3.2	1243	≥3.2	-	≥3.2	-	≥3.2	0.295		
	Punctoribates punctum (C.L. Koch, 1839)	0!		≥3.2	-	0!		0!			
Oppiidae	***Oppia clavipectinata*** (Michael, 1885)	0!		0!		0!		≥3.2	-		
	Oppiella nova (Oudemans, 1902)	0!		0!		0!		≥3.2	-		
Phenopelopidae	***Pelops occultus*** (C.L. Koch, 1835)	≥3.2	-	0!		≥3.2	-	≥3.2	-		
Scheloribatidae	***Liebstadia similis*** (Michael, 1888)	≥3.2	$0.238^{n.s.}$	≥3.2	-	≥3.2	-	≥3.2	-		
	Scheloribates laevigatus (C.L. Koch, 1836)	≥3.2	-	≥3.2	-	1	-	≥3.2	-		
	Total abundance	1	$0.954^{n.s.}$	≥3.2	1921	≥3.2	-	≥3.2	$4.782^{n.s.}$		

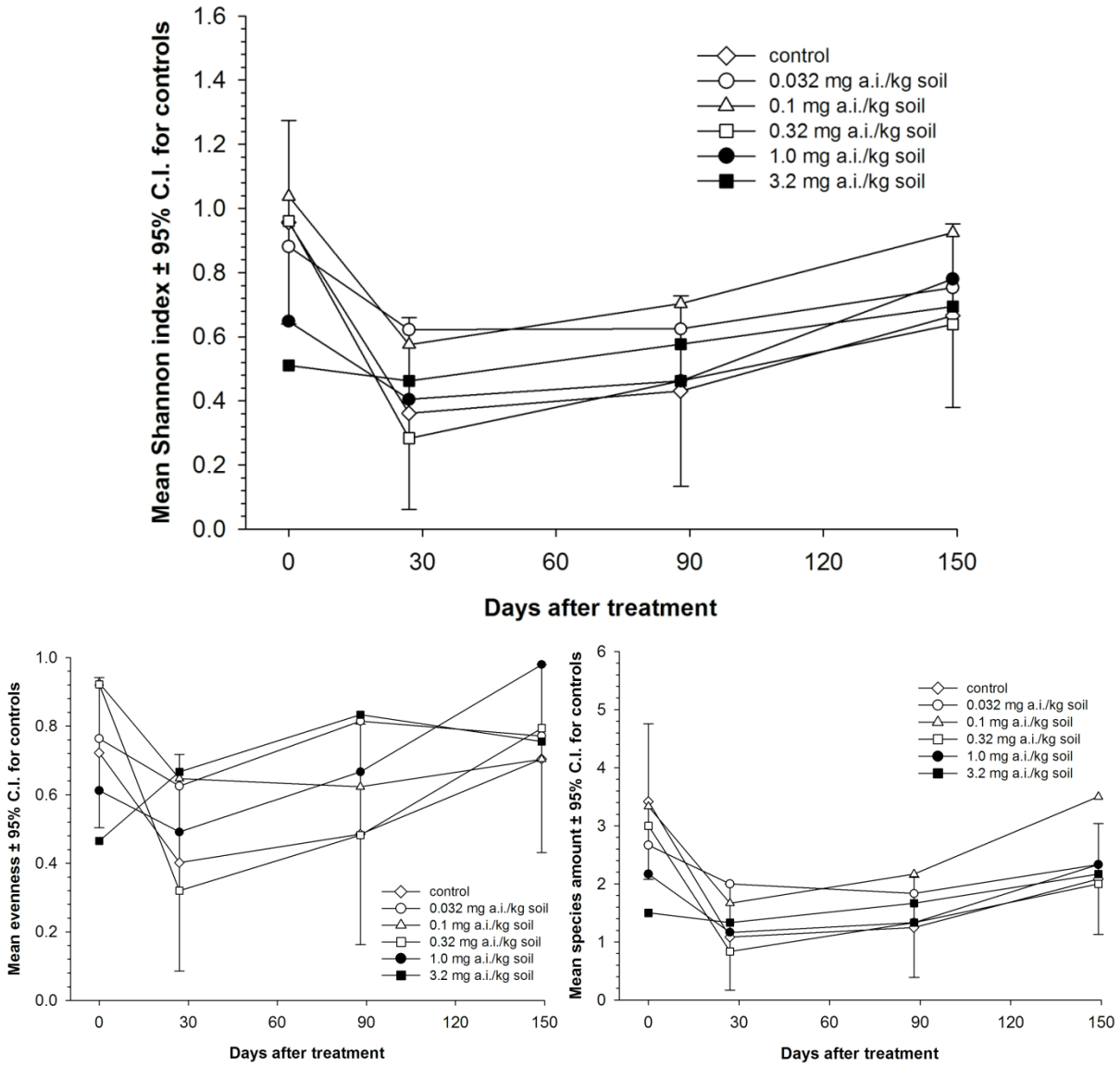


Figure IV-8: Diversity indices of oribatid mites.

In the course of the experiment, the similarity indices (Stander´s and Steinhaus´ index, see Figure IV-7) decrease from about 30 % and 20 %, respectively for the two indices to about 10 % at the first two dates after treatment. It increases in most treatments at 149 days after application to the initial point. There is no dose-related effect seen, but the highest treatment c_5 showed the largest relative deviations during the experimental period, starting from the lowest similarity of all treatment groups at the date prior the application and not following the decreasing trend of the remainder treatment groups. At the end, a relatively uniform community marked by a 50 % and 30% similarity results of the treatment.

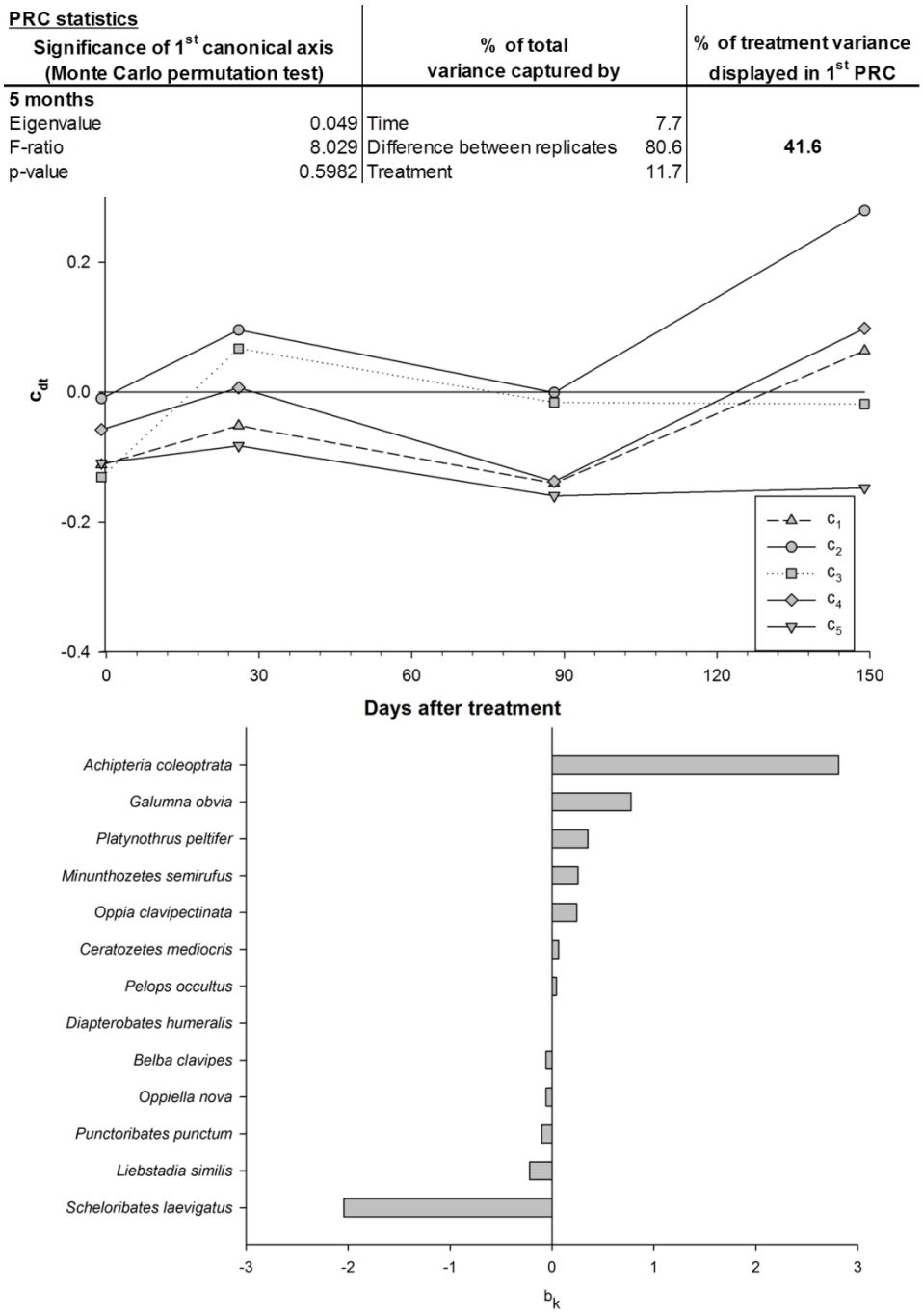


Figure IV-9: Principal Response Curve of oribatids as obtained in the TME dose-response test 5 and 12 months after exposure of lindane in concentrations of c_1 = 0.032, c_2 = 0.100, c_3 = 0.320, c_4 = 1.000, c_5 = 3.200 mg a.i./kg dw soil. The control is set to zero and is represented by the abscissa. The first canonical axis of a Redundancy Analysis (RDA) with time as covariable and lindane treatments as explanatory variables is shown. The ordinate of the PRC diagram shows the canonical coefficients (c_{dt}-values). The significance of the overall treatment regime is given in the table provided with the figure; significance of the treatment regime per sampling date is indicated by Table IV-2 (α<0.05 accepted significant). Significant differences of treatments in comparison to control by applying the Williams test (two-sided, α <0.05) are shown in Table 1. Species with high (decrease of mean abundance in case of treatment related decrease of c_{dt} values) and low (mirror-inverted response) b_k-values are supposed to follow the response pattern in a similar manner as shown by the diagram.

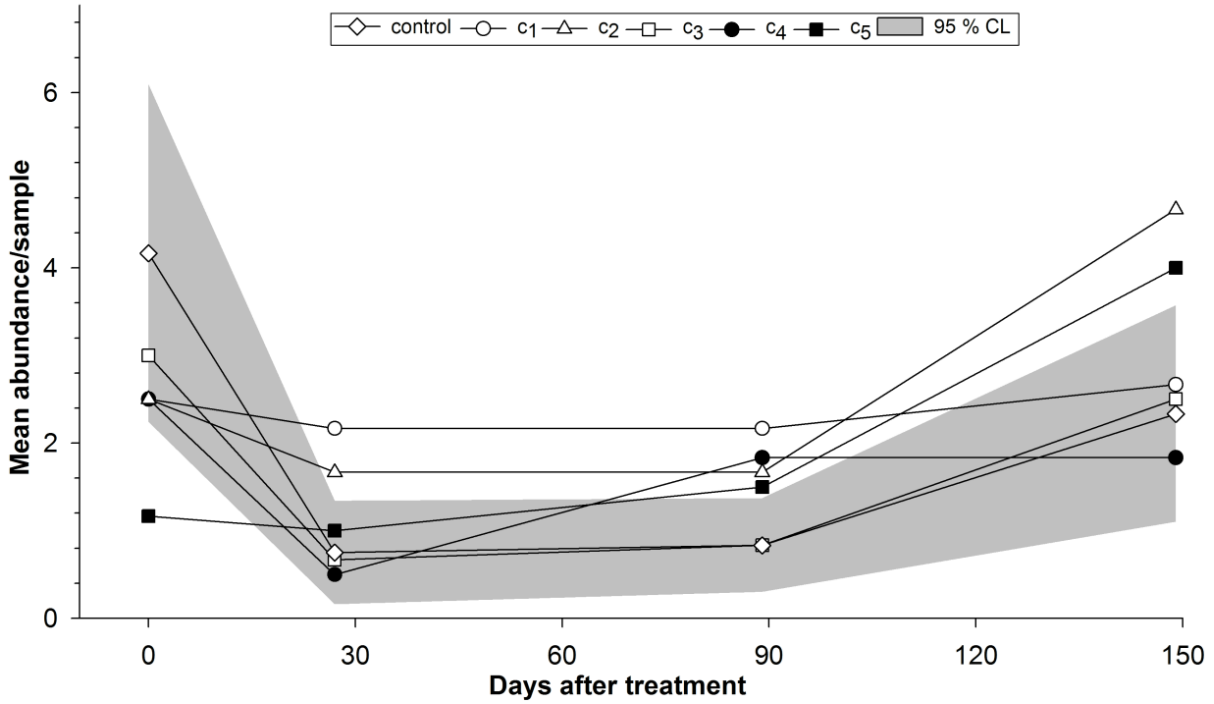


Figure IV-10: Arithmetic mean (± 95 % confidence interval for the controls) of oribatid abundance ($N_{control}$ = 12 $N_{treatment}$ = 6 samples per TME and date) as obtained in the dose-response study.

IV-1.3 Effects on enchytraeids

The lowest abundance of enchytraeids was recorded in June 2006 in the c_1 treatment (group mean of 8 individuals per sample corresponding to 4159 individuals per m^2), while the highest abundance was found in October 2006 in the c_2 treatment (group mean of 57 individuals per sample corresponding to 28860 individuals per m^2). In all treatments, the abundance was considerably higher in October than in May and June (Figure IV-13). Seventeen species of the family Enchytraeidae were found during the experimental period (Table IV-3). One species of the family Tubificidae *Rhyacodrilus falciformis* that phylogenetically belongs to the same class of oligochaetes like enchytraeids was also counted and included in the follow-up analyses. The most dominant species was *Achaeta unibulba*, providing about one quarter of the community abundance. Another 20% of the total abundance belonged to *Marionina communis*. *Fridericia paroniana*, *Fridericia singula* and *Enchytraeus buchholzi agg.* complemented

Table IV-3: Species lists, effects on the abundance and on the communities endpoints for the groups of enchytraeids, if sampled at the given date and applicable to analyse. 0!: no variance at date, species did not occur; ≥3.2: no effects including the highest treatment; -: statistics not applicable because no dose-response relation given, slope of the function <0.001 ; n.s.: no significant effect concentration, but calculation possible, slope not <0.001; hatched cells: not sampled.

Days after treatment	-1		26		88		149		351	
Endpoints (effect measures enchytraeids)	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}
PRC [significance of treatment regime] set 5 overall significance:0.8216		0.755		0.477			0.532			
Principal community response (c_{dl}) set 5	≥3.2	-	≥3.2	-			≥3.2	-		
Shannon-index	≥3.2	-	≥3.2	-			≥3.2	-		
Evenness	≥3.2	-	≥3.2	-			≥3.2	-		
Taxa richness	≥3.2	1343.319$^{n.s.}$	≥3.2	-			≥3.2	-		
Achaeta unibulba Graefe, Dózsa-Farkas & Christensen, 2005	≥3.2	-	≥3.2	-			≥3.2	-		
Enchytraeus buchholzi agg. Vejdovský, 1879	≥3.2	-	≥3.2	-			≥3.2	-		
Enchytraeus bulbosus Nielsen & Christensen, 1963	0!		0!				≥3.2	-		
Enchytraeus minutus agg. Nielsen & Christensen, 1961	≥3.2	-	≥3.2	-			≥3.2	-		
Enchytronia minor Möller, 1971	≥3.2	-	≥3.2	-			≥3.2	-		
Fridericia bisetosa (Levinsen, 1884)	≥3.2	-	≥3.2	-			≥3.2	-		
Fridericia bulboides Nielsen & Christensen, 1959	≥3.2	0.077$^{n.s.}$	≥3.2	-			≥3.2	1177		
Fridericia christeri Rota & Healy, 1999	≥3.2	-	≥3.2	-			≥3.2	-		
Fridericia galba (Hoffmeister, 1843)	≥3.2	-	≥3.2	-			≥3.2	-		
Fridericia isseli Rota, 1994	0!		0!				≥3.2	-		
Fridericia paroniana Issel, 1904	≥3.2	-	≥3.2	-			≥3.2	5.84$^{n.s.}$		
Fridericia singula Nielsen & Christensen, 1961	0!		0!				≥3.2	-		
Fridericia ulrikae Rota & Healy, 1999	0!		0!				≥3.2	-		
Henlea perpusilla Friend, 1911	≥3.2	0.585$^{n.s.}$	≥3.2	-			≥3.2	-		
Henlea ventriculosa (Udekem, 1854)	0!		≥3.2	-			1	-		
Marionina argentea (Michaelsen, 1889)	1	-	≥3.2	-			≥3.2	-		
Marionina communis Nielsen & Christensen, 1959	≥3.2	-	≥3.2	-			≥3.2	-		
Rhyacodrilus falciformis Bretscher, 1901	≥3.2	-	0!				≥3.2	2.1E+11$^{n.s.}$		
Total abundance	≥3.2	-	≥3.2	-			≥3.2	-		

the upper three quarters of most dominant members of the enchytraeid community (Figure IV-14). *F. singula* was not present at all (as adult) in the samples of the first two dates, but became very dominant at the last sampling (20% of control abundance at day 149). In general, there is a clear time-dependent dynamic of the community, resulting in a higher proportion of juveniles and a lower dominance of e.g. *M. communis* at the end of the experimental period. Those results were not attributed to effects of the lindane treatments.

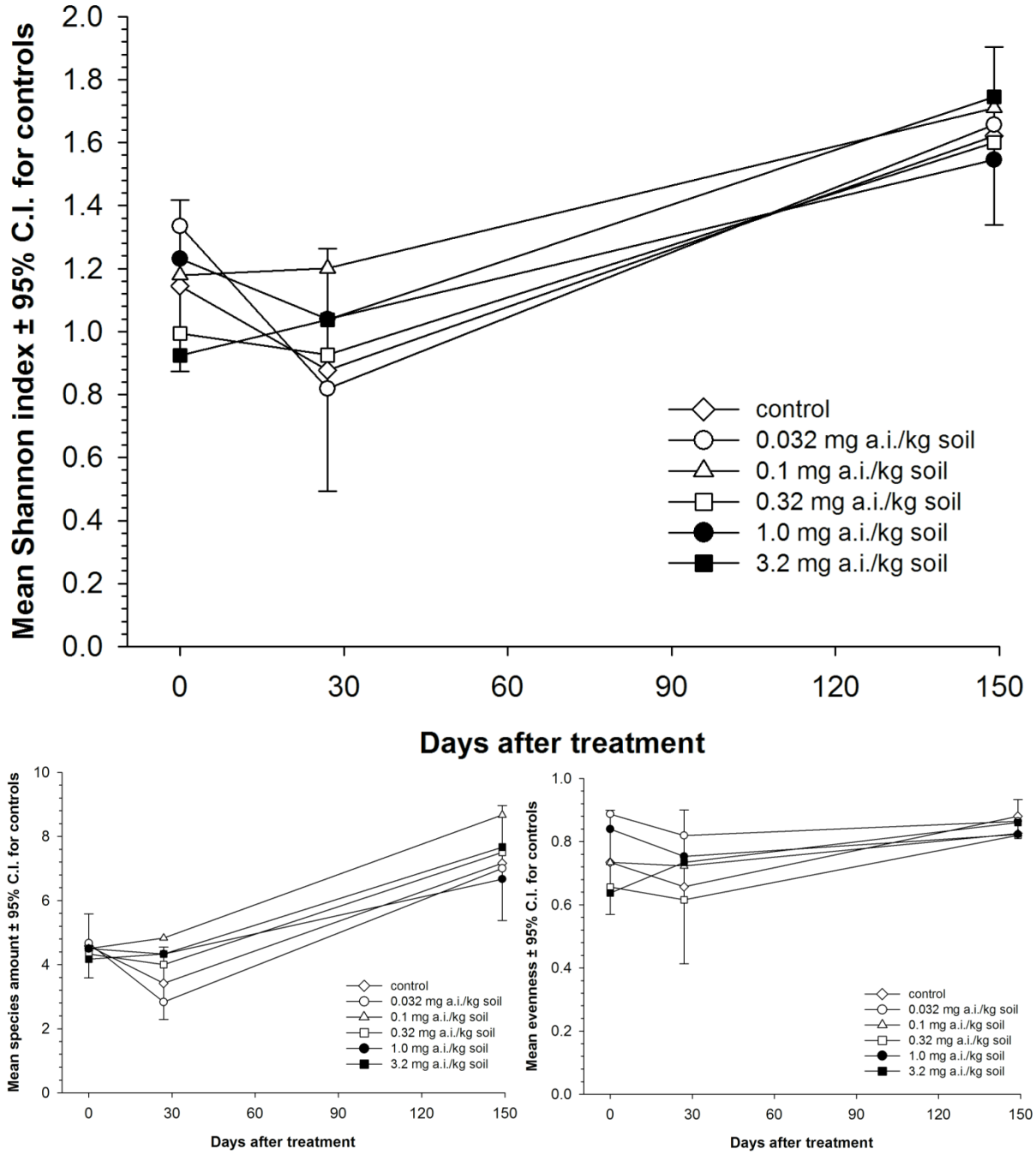


Figure IV-11: Diversity indices enchytraeids.

Neither the abundance of single populations nor the community as a whole as described by PRC-analyses (Figure IV-15) or by diversity indices (Figure IV-11) responded significantly to the treatments. Treatments did not cause initial or long-term effects. NOECs or EC_x-values could not be determined because no dose dependent effects occurred. However, there was no

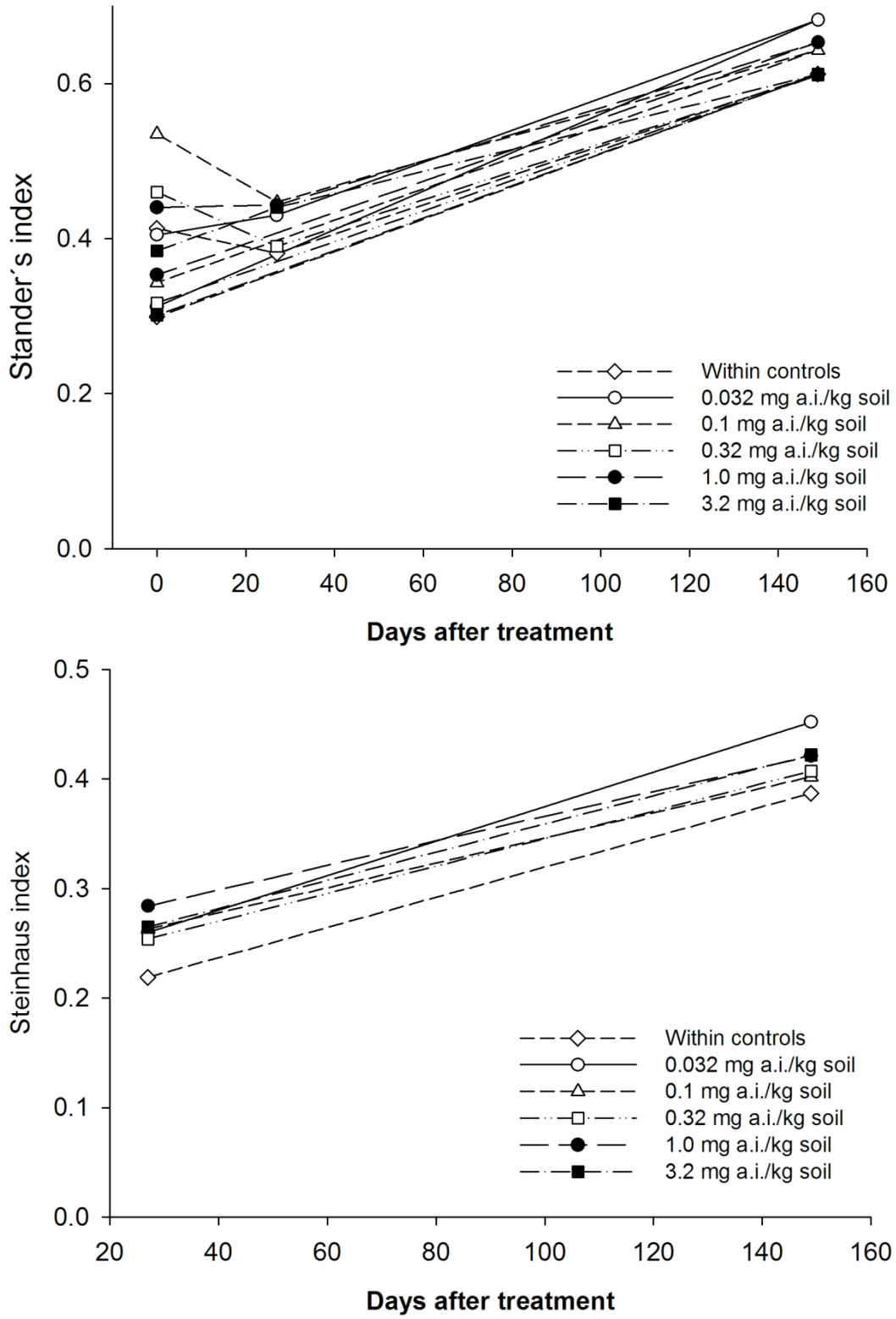


Figure IV-12: Similarity indices of enchytraeids.

indication of instability of the enchytraeids communities that could be caused by isolation for more than five months after coring of soil cores. The similarity indices (shown by Figure IV-12) did not show dose-related patterns.

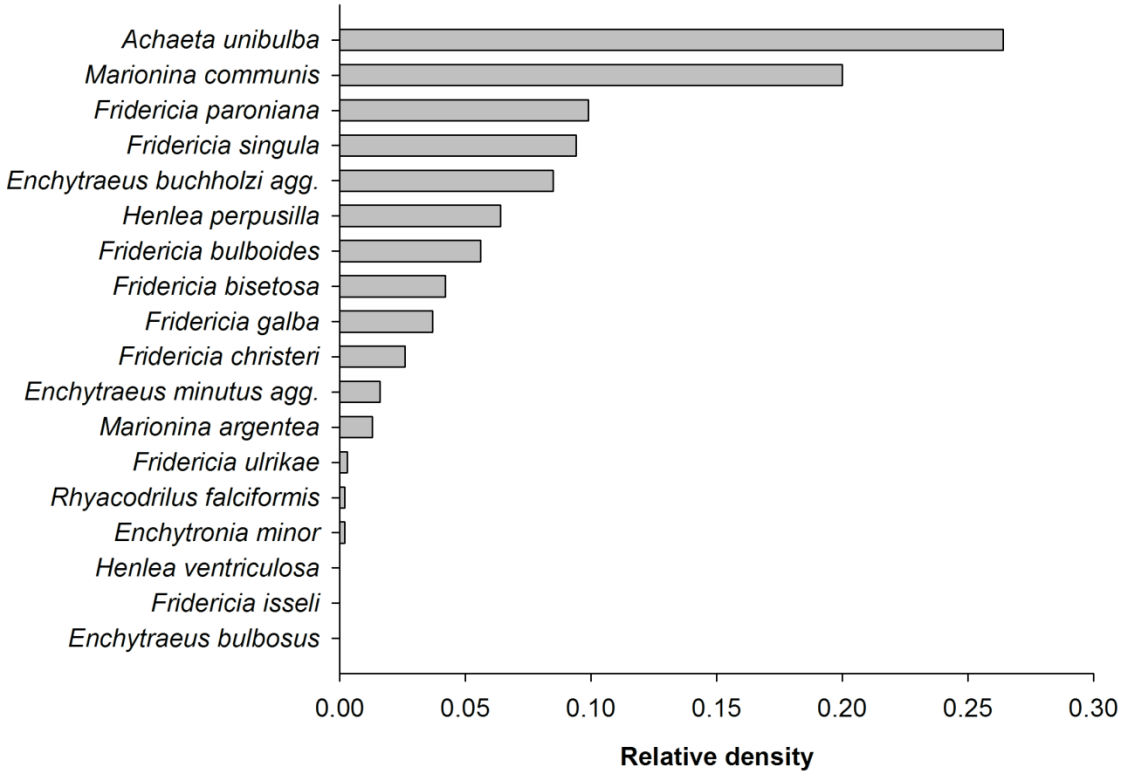


Figure IV-14: Dominance spectrum of enchytraeids. Figure is based on mean control values of all dates

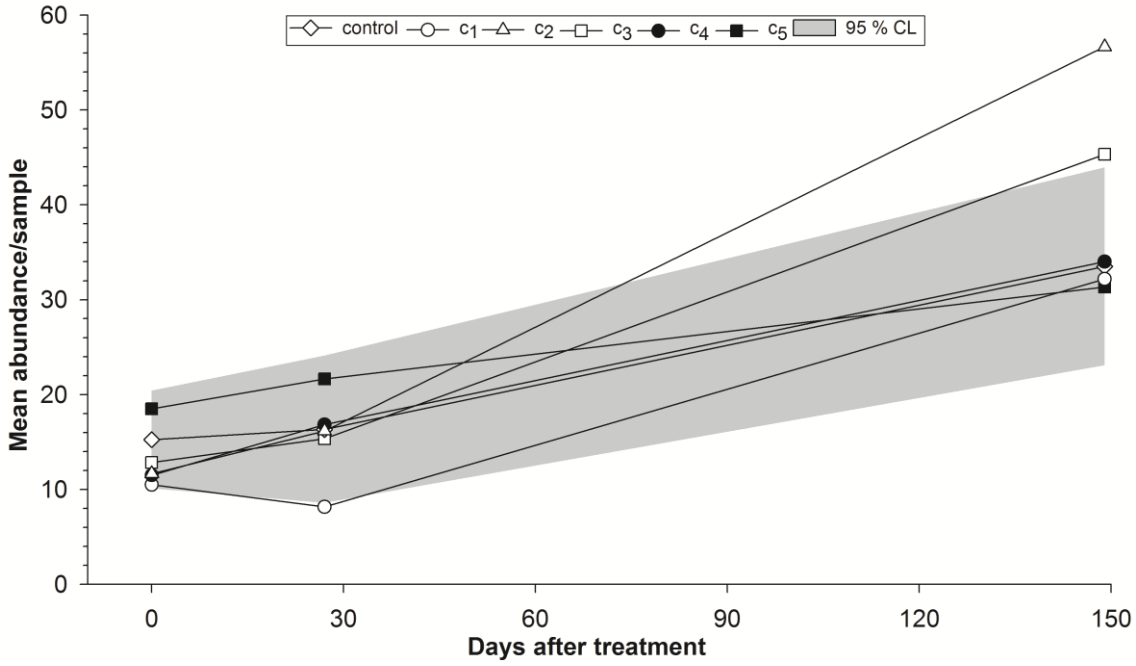


Figure IV-13: A Arithmetic mean (± 95 % confidence interval for the controls) of enchytraeids abundance ($N_{control}$ = 12 $N_{treatment}$ = 6 samples per TME and date) as obtained in the dose-response study.

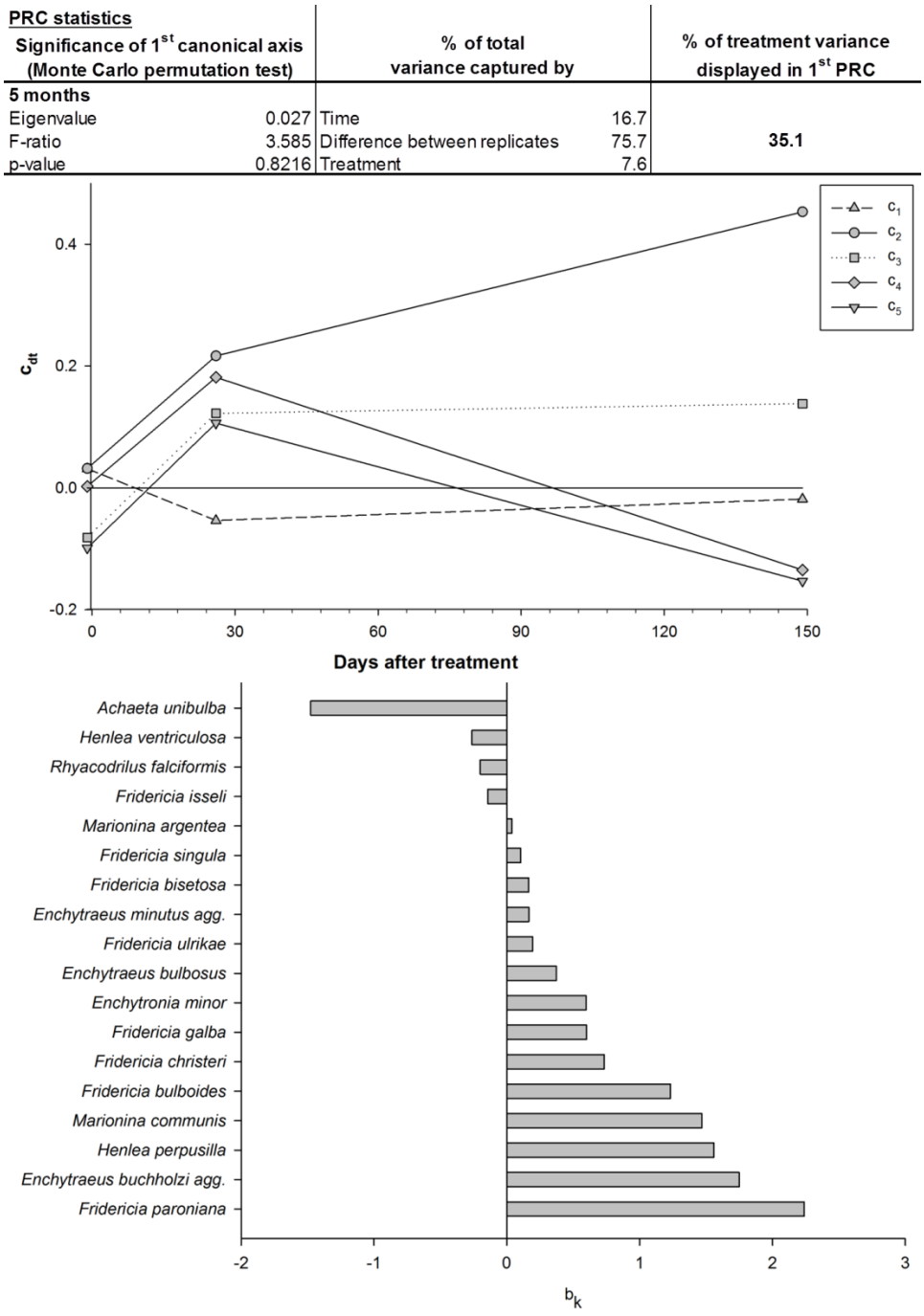


Figure IV-15: Principal Response Curve of enchytraeids as obtained in the TME dose-response test 5 and 12 months after exposure of lindane in concentrations of c_1 = 0.032, c_2 = 0.100, c_3 = 0.320, c_4 = 1.000, c_5 = 3.200 mg a.i./kg dw soil. The control is set to zero and is represented by the abscissa. The first canonical axis of a Redundancy Analysis (RDA) with time as covariable and lindane treatments as explanatory variables is shown. The ordinate of the PRC diagram shows the canonical coefficients (c_{dt}-values). The significance of the overall treatment regime is given in the table provided with the figure; significance of the treatment regime per sampling date is indicated by Table IV-3 (α<0.05 accepted significant). Significant differences of treatments in comparison to control by applying the Williams test (two-sided, α <0.05) are shown in Table 1. Species with high (decrease of mean abundance in case of treatment related decrease of c_{dt} values) and low (mirror-inverted response) b_k-values are supposed to follow the response pattern in a similar manner as shown by the diagram.

IV-1.4 Effects on nematodes

In sum, 29 families of the phylum Nematoda were found (Table IV-4, Figure IV-17). Most of them belonged to the orders of *Dorylaimida* and *Tylenchida*. The latter order provided 75% of all individuals, among them most dominant were *Tylenchidae* (21.3 % of control samples of all dates), followed by *Dolidochoridae* (19.6 %) and *Paratylenchidae* (11.2 %). Prior the application at day -1, several nematode families and the total abundance showed abundances in the predestined treatment TME that differed significantly from the prospective control level (compare Table 1 and discussion). Very few and transient effects on the community, on the families and the trophic groups of nematodes as responses to the treatments could be demonstrated (Table IV-4). The group of omnivores that is known to be particularly sensitive towards chemical stressors was affected by the treatment. Nematode families feeding on microflora (bacterial feeders and fungivores) seemed to be enhanced in the lowest concentration of 0.032 mg a.i./kg dry soil (refer to the tabular appendices).

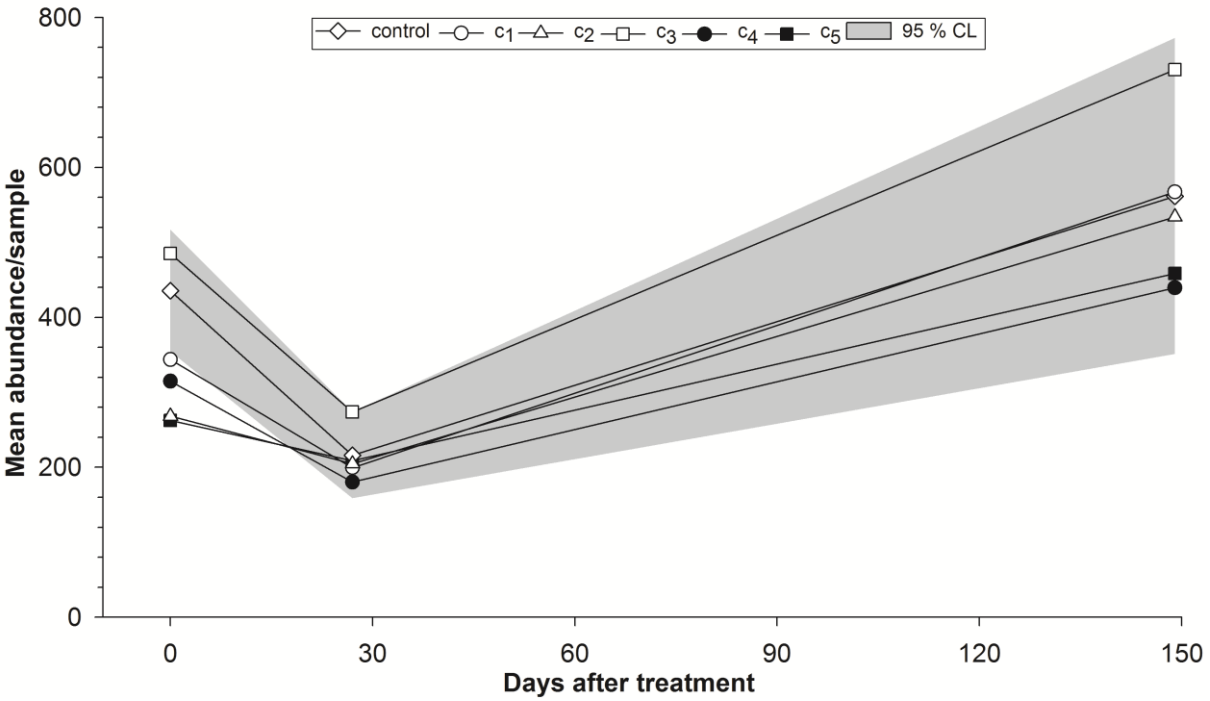


Figure IV-16: Arithmetic mean (± 95 % confidence interval for the controls) of nematode abundance ($N_{control}$ = 12 $N_{treatment}$ = 6 samples per TME and date) as obtained in the dose-response study.

For the group of predators, the lowest variability between the treatments occurred at the first date after the application. For most of the guilds, the variability of controls was highest five months after application. Only delayed adverse effects on the abundance of the family *Hoplolaimidae* (refer to the tabular appendices) and on the evenness of the dominance structure were detected at day 149. Neither the PRC analysis showed a significant influence of the

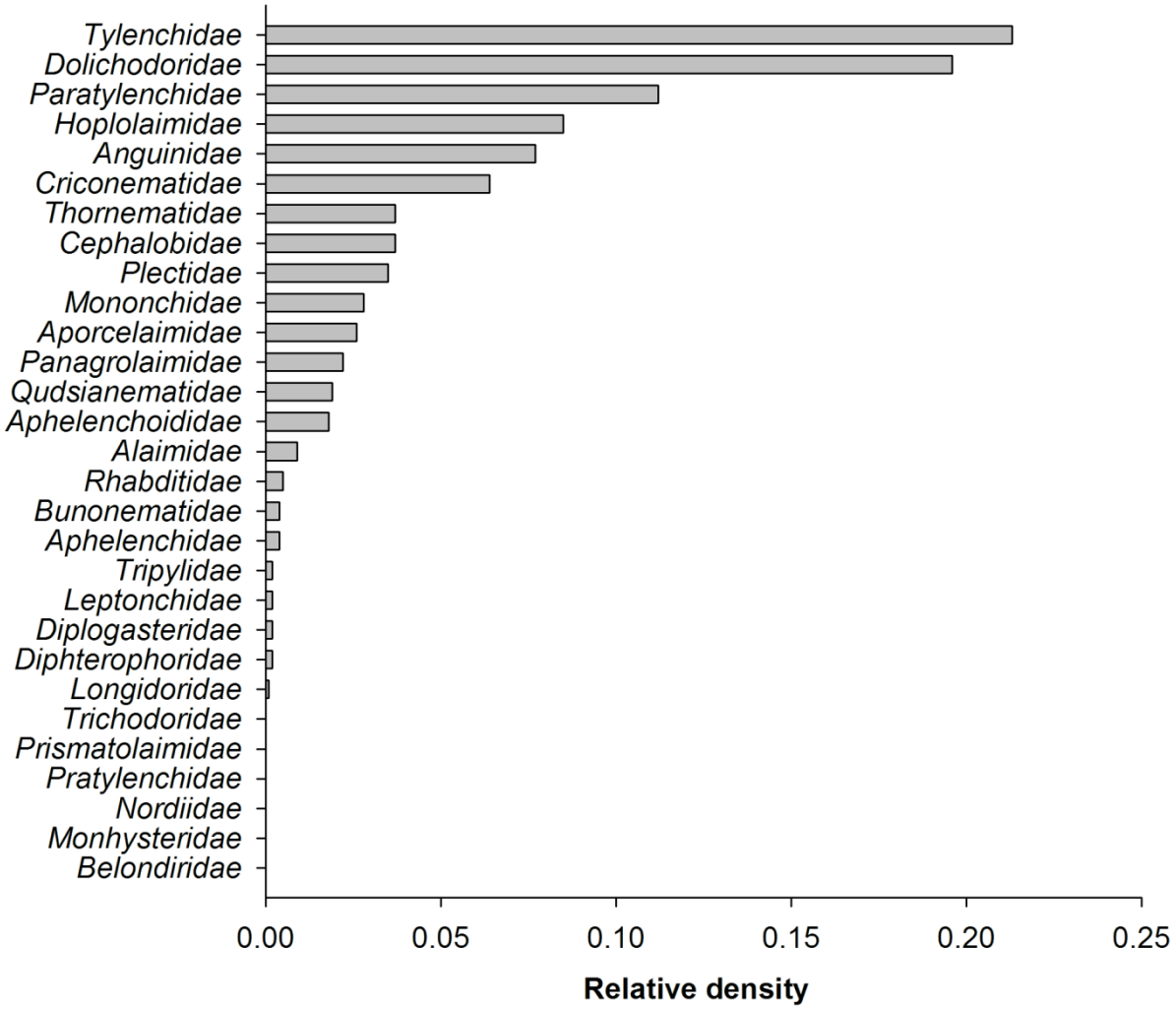


Figure IV-17: Relative densities of nematode families (mean of all controls at all sampling dates).

treatment regime, nor were deviations from the control level at certain sampling dates detected, nor reacted the sum parameters of the maturity indices to the treatment (results not included in Table IV-4).

Table IV-4: Species lists, effects on the abundance and on the communities endpoints for the groups of nematodes, if sampled at the given date and applicable to analyse. 0!: no variance at date, species did not occur; ≥3.2: no effects including the highest treatment; -: statistics not applicable because no dose-response relation given, slope of the function <0.001 ; n.s.: no significant effect concentration, but calculation possible, slope not <0.001; hatched cells: not sampled.

Days after treatment	-1		26		88		149		351	
Endpoints (effect measures nematodes)	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}	NOEC	EC_{50}
PRC [significance of treatment regime]		0.024		0.780				0.376		
set 5 overall significance: 0.6327										
Principal community response (c_{dt}) set 5	≥3.2	-	≥3.2	-				≥3.2		-
Shannon-index	≥3.2	-	≥3.2	-				≥3.2	1.92E+32 $^{n.s.}$	
Evenness	≥3.2	-	≥3.2	-				**1**	6.6+21 $^{n.s.}$	
Taxa richness	≥3.2	1589223 $^{n.s.}$	≥3.2	-				≥3.2		-
Aphelenchidae (Fuchs, 1937)	≥3.2	-	≥3.2	-				≥3.2	0.111 $^{n.s.}$	
Aphelenchoididae Skarbilovich, 1947	≥3.2	0.11 $^{n.s.}$	≥3.2	-				≥3.2	1.417 $^{n.s.}$	
Plectidae Örley, 1880	1	-	**1**	-				≥3.2	8.198 $^{n.s.}$	
Diplogasteridae (Micoletzky, 1922)	≥3.2	-	≥3.2-	-				≥3.2		-
Alaimidae Micoletzky, 1922	≥3.2	5.743 $^{n.s.}$	≥3.2-	-				≥3.2		-
Aporcelaimidae Heyns, 1965	≥3.2	13764	≥3.2-	-				≥3.2		-
Belondiridae Thorne, 1939	≥3.2	-	≥3.2-	-				0!		
Diphterophoridae Thorne, 1935	≥3.2	-	≥3.2-	-				≥3.2		-
Leptonchidae Thorne, 1935	≥3.2	0.295	≥3.2-	-				0!		
Longidoridae Thorne, 1935	1	-	≥3.2-	-				≥3.2		-
Nordiidae Thorne, 1939	≥3.2	-	≥3.2-	-				0!		
Qudsianematidae Thorne, 1939	≥3.2	0.375 $^{n.s.}$	≥3.2-	-				≥3.2		-
Thornematidae Siddiqi, 1969	≥3.2	4.306 $^{n.s.}$	≥3.2	3.6441 $^{n.s.}$				≥3.2		-
Trichodoridae Thorne, 1935	0!		≥3.2	-				0!		
Prismatolaimidae Micoletzky, 1922	0!		0!					≥3.2		-
Tripylidae (De Man, 1876)	≥3.2	-	≥3.2	-				≥3.2		-
Monhysteridae De Man, 1876	1	-	0!					0!		
Mononchidae Chitwood, 1937	≥3.2	-	≥3.2	-				≥3.2	0.899 $^{n.s.}$	
Bunonematidae Micoletzky, 1922	≥3.2	-	0!					≥3.2		-
Cephalobidae Filipjev, 1934	1	3059	≥3.2	-				≥3.2		-
Panagrolaimidae Thorne, 1937	≥3.2	-	≥3.2	-				≥3.2	**0.816**	
Rhabditidae Örley, 1880	≥3.2	-	≥3.2	-				≥3.2		-
Anguinidae Nicoll, 1926	≥3.2	-	≥3.2	2.656 $^{n.s.}$				≥3.2		-
Criconematidae Taylor, 1936	1	-	≥3.2	-				≥3.2		-
Dolichodoridae Chitwood, 1950	≥3.2	-	≥3.2	-				≥3.2		-
Hoplolaimidae (Filipjev, 1934)	1	0.133 $^{n.s.}$	≥3.2	-				**1**	0.178 $^{n.s.}$	
Paratylenchidae Thorne, 1949	≥3.2	0.021 $^{n.s.}$	≥3.2	-				≥3.2		-
Pratylenchidae Thorne, 1949	≥3.2	-	≥3.2	-				≥3.2		-
Tylenchidae Örley, 1880	1	-	≥3.2	-				≥3.2		-
Total abundance	1	536.343 $^{n.s.}$	≥3.2	-				≥3.2		-

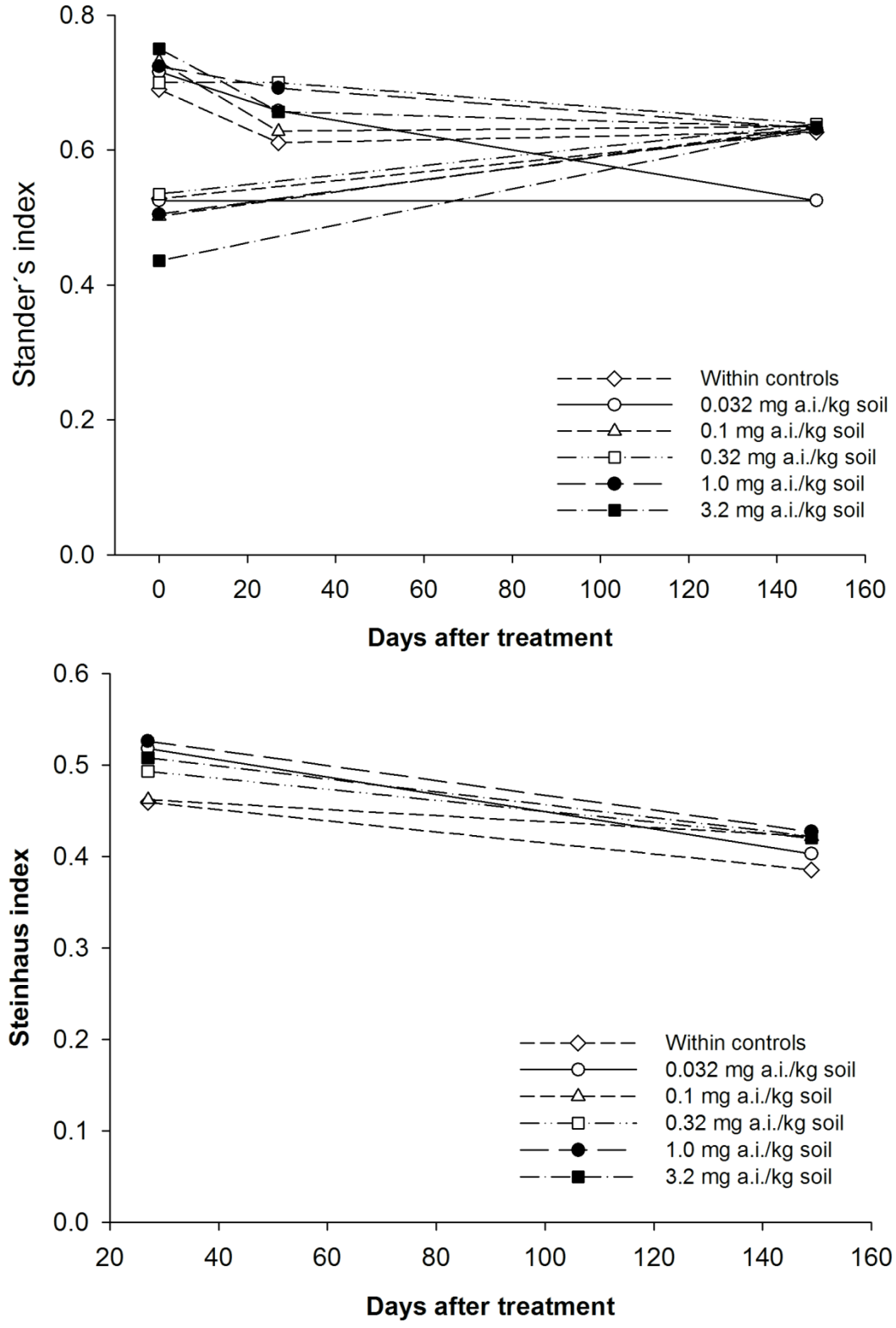


Figure IV-18: Similarity indices of nematodes based on family composition.

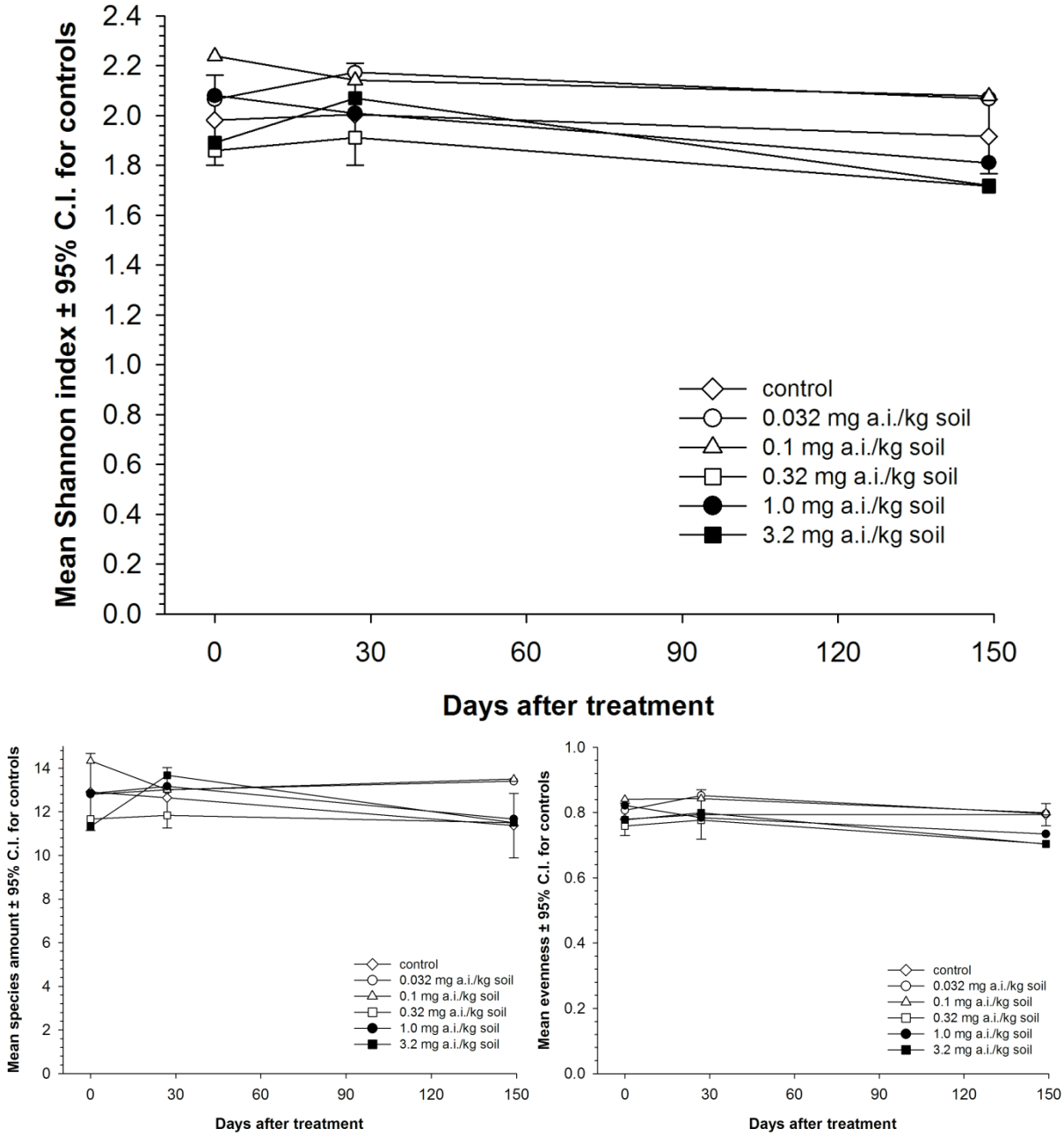


Figure IV-19: Diversity indices of nematodes based on family composition.

PRC statistics				
Significance of 1st canonical axis (Monte Carlo permutation test)		% of total variance captured by		% of treatment variance displayed in 1st PRC
5 months				
Eigenvalue	0.026	Time	8.6	
F-ratio	3.2	Difference between replicates	79.7	**22.5**
p-value	0.6327	Treatment	11.7	

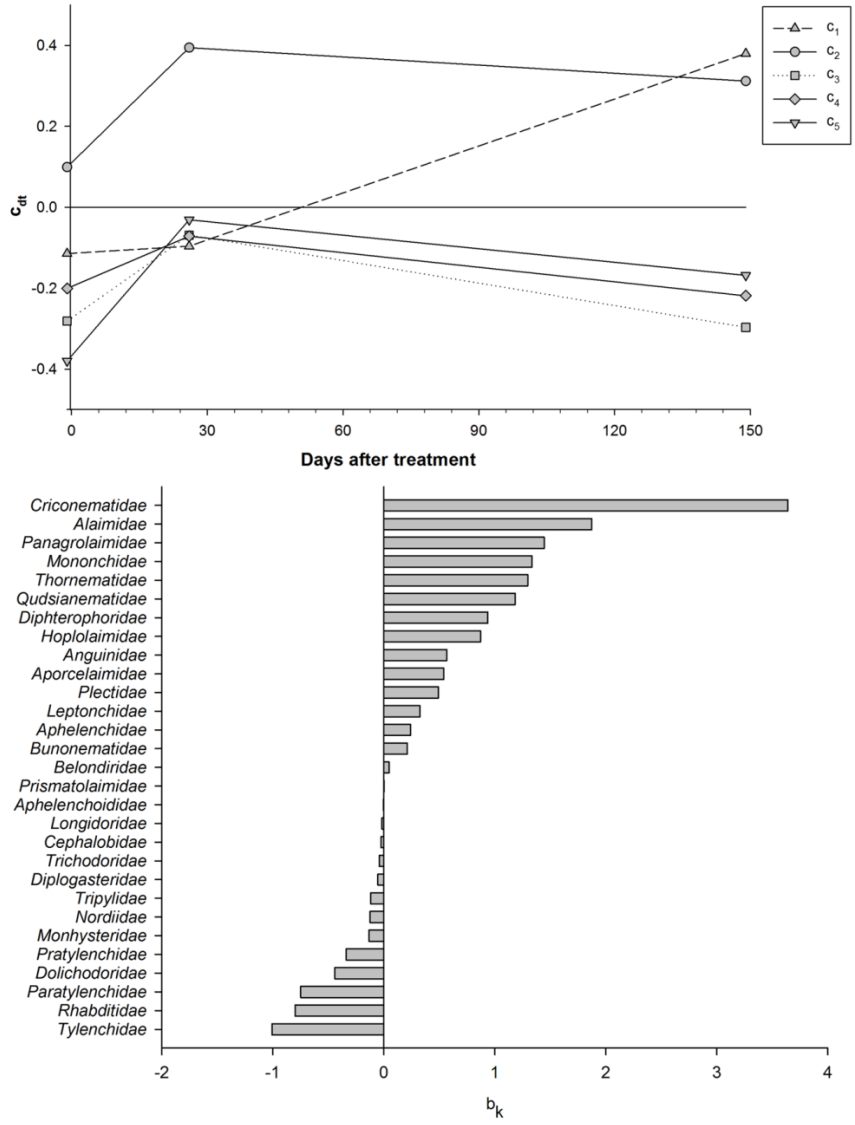


Figure IV-20: Principal Response Curve of nematodes as obtained in the TME dose-response test 5 and 12 months after exposure of lindane in concentrations of c_1 = 0.032, c_2 = 0.100, c_3 = 0.320, c_4 = 1.000, c_5 = 3.200 mg a.i./kg dw soil. The control is set to zero and is represented by the abscissa. The first canonical axis of a Redundancy Analysis (RDA) with time as covariable and lindane treatments as explanatory variables is shown. The ordinate of the PRC diagram shows the canonical coefficients (c_{dt}-values). The significance of the overall treatment regime is given in the table provided with the figure; significance of the treatment regime per sampling date is indicated by Table IV-4 (α<0.05 accepted significant). Significant differences of treatments in comparison to control by applying the Williams test (two-sided, α <0.05) are shown in Table 1. Species with high (decrease of mean abundance in case of treatment related decrease of c_{dt} values) and low (mirror-inverted response) b_k-values are supposed to follow the response pattern in a similar manner as shown by the diagram.

IV-1.5 Effects on fungi

The concentration of ergosterol in the top soil varied independently of the lindane treatment. Its content was determined to be between 2 and 6.5 µg/g dry weight of soil in control and in treated TME at the date before application of the test item. Coefficients of variation of three sampling dates between three replicates for each treatment level ranged between 10 and 50 %. Before application, ergosterol content of control TME was highest and significantly higher compared to c_5 (Mann-Whitney U-test: $\alpha \leq 0.05$; $p = 0.0495$, $N = 3$). At day 26, the ergosterol content of the treated soils was higher at c_5, indicating a slight promotion of fungal biomass. The low treatment c_3 was not affected by lindane (see Figure IV-21).

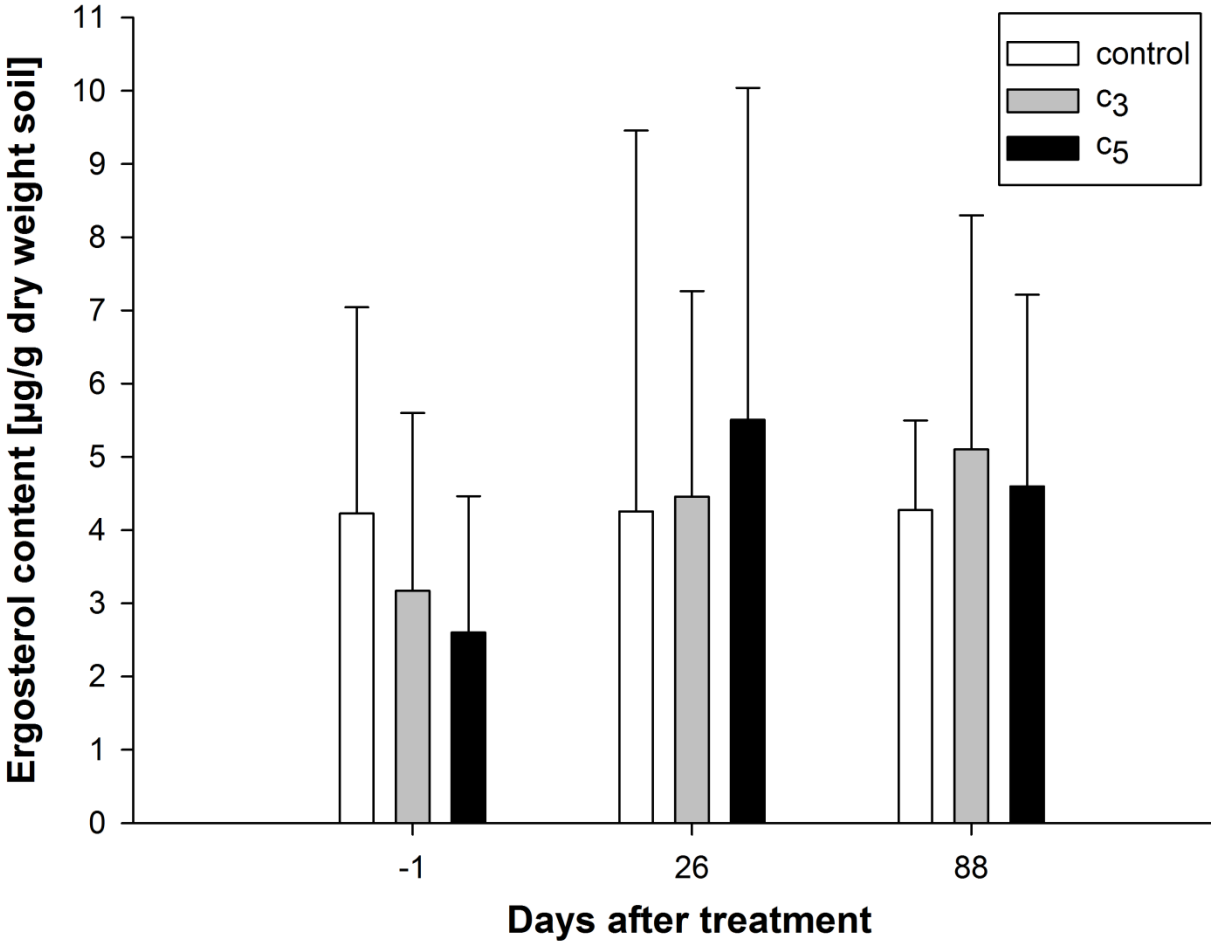


Figure IV-21: Mean ergosterol content of soil ± 95% confidence interval before application of the test substance lindane (day -1), also results 26 days and 90 days after treatment are shown.

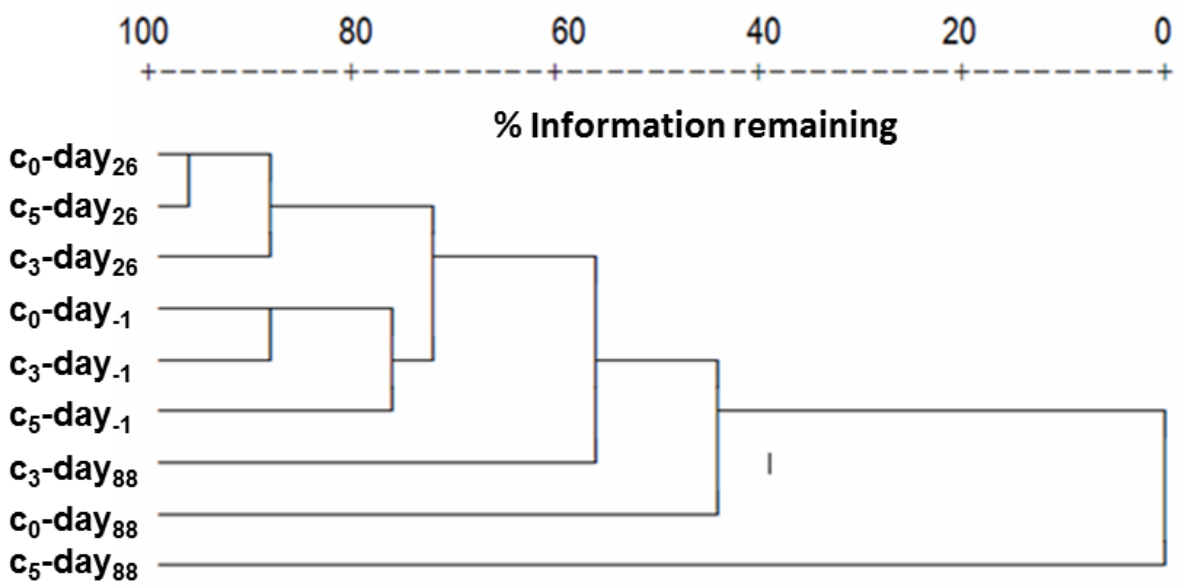


Figure IV-22: UPGMA cluster analysis of DGGE band patterns of PCR products (subsamples unified after amplification). The relative similarity between samples is shown, based on Pearson-correlation coefficients.

The community of fungi consisted of 47 different phylotypes (Table IV-5). None of them was assigned to a certain fungal species, but they were considered 'surrogate species', and thus suitable for subsequent statistical analyses. Some phylotypes were identified that specifically responded to the treatment. B25.9 disappeared completely from both treatments and coincidentally became highly dominant in the control TME at day 26. B8.3, B85.4, B98.0 were not found in the untreated TME, but were detectable at day -1 and day 26 in the treated TME; B24.9 and B27.2 were found the dominant phylotypes of c_5 at day 88, its intensities increased compared to the controls where they were not found at any sampling date. The DGGE method and the consecutive cluster analysis showed no clear treatment related effects on the community of soil fungi. Figure IV-22 represents the result of a cluster analysis, which demonstrates that most pronounced differences could be rather attributed to temporal changes in phylotype composition than to effects of the lindane treatments.

Table IV-5: Relative abundances of fungal phylotypes. 0! = phylotypes did not occur at the given sampling date.

Days after treatment	-1			26			88		
Endpoints (relative abundance fungi)	C_0	C_3	C_5	C_0	C_3	C_5	C_0	C_3	C_5
B 06.6	98	188	114	47	134	46	34	24	9
B 08.3	0!	0!	0!	0!	0!	0!	0!	0!	14
B 10.1	190	100	98	339	343	86	89	58	94
B 12.1	569	619	309	1568	706	813	414	475	767
B 15.2	0!	52	0!	0!	0!	0!	0!	53	9
B 16.4	48	90	299	240	517	201	25	25	33
B 19.2	218	467	401	644	681	710	250	213	213
B 20.9	0!	1	0!	0!	54	42	0!	0!	0!
B 23.2	9	21	110	79	83	120	372	420	383
B 24.9	0!	0!	0!	0!	55	0!	0!	177	788
B 25.9	232	108	374	153	118	289	1215	0!	0!
B 27.2	0!	0!	0!	0!	0!	0!	0!	60	1945
B 28.3	741	0!	0!	122	16	0!	140	201	0!
B 28.9	0!	350	0!	219	0!	24	0!	0!	0!
B 30.5	0!	0!	12	0!	17	0!	0!	0!	0!
B 31.9	0!	101	0!	0!	0!	0!	0!	23	0!
B 33.4	0!	0!	574	0!	14	14	0!	0!	0!
B 34.7	33	22	0!	20	1	0!	0!	4	8
B 37.0	0!	10	5	0	0!	0!	0!	48	6
B 38.7	43	117	49	45	57	21	25	41	386
B 40.3	0!	0!	0!	39	52	12	0!	0!	0!
B 41.2	3	7	20	0!	53	17	3	8	0!
B 43.4	502	171	98	240	200	375	124	168	332
B 46.3	87	85	0!	49	71	0!	55	227	0!
B 47.6	0!	149	535	34	121	0	0!	38	30
B 51.4	55	70	142	342	247	303	119	147	148
B 54.9	172	81	57	76	331	131	399	232	72
B 56.7	650	334	342	384	660	531	884	322	565
B 58.8	15	1	1	3	43	1	23	14	164
B 60.9	110	170	97	0!	0!	0!	0!	413	130
B 62.6	636	417	738	1135	776	854	900	658	781
B 64.3	489	522	413	0!	0!	0!	0!	0!	0!
B 66.4	883	1137	973	958	635.33	1207	304	318	438
B 68.3	952	1291	768	692	946	392	324	723	636
B 70.3	879	1233	599	423	850	568	738	1698	159
B 75.9	205	132	265	199	240	271	183	231	121
B 78.7	276	160	250	106	102	416	128	186	279
B 80.4	250	0!	461	86	173	300	0!	11	255
B 81.5	0!	0!	0!	0!	0!	0!	1	0!	148
B 83.5	0!	0!	0!	0!	1	0!	36	21	0!
B 85.4	0!	0!	0!	0!	9	0!	0!	59	114

IV-1.6 Effects on plant biomass

Growth rates per TME did not differ significantly between controls and treatments. Qualitative observations have shown that vegetation cover was getting denser during the course of the study. This fact results in increasing growth rates per day.

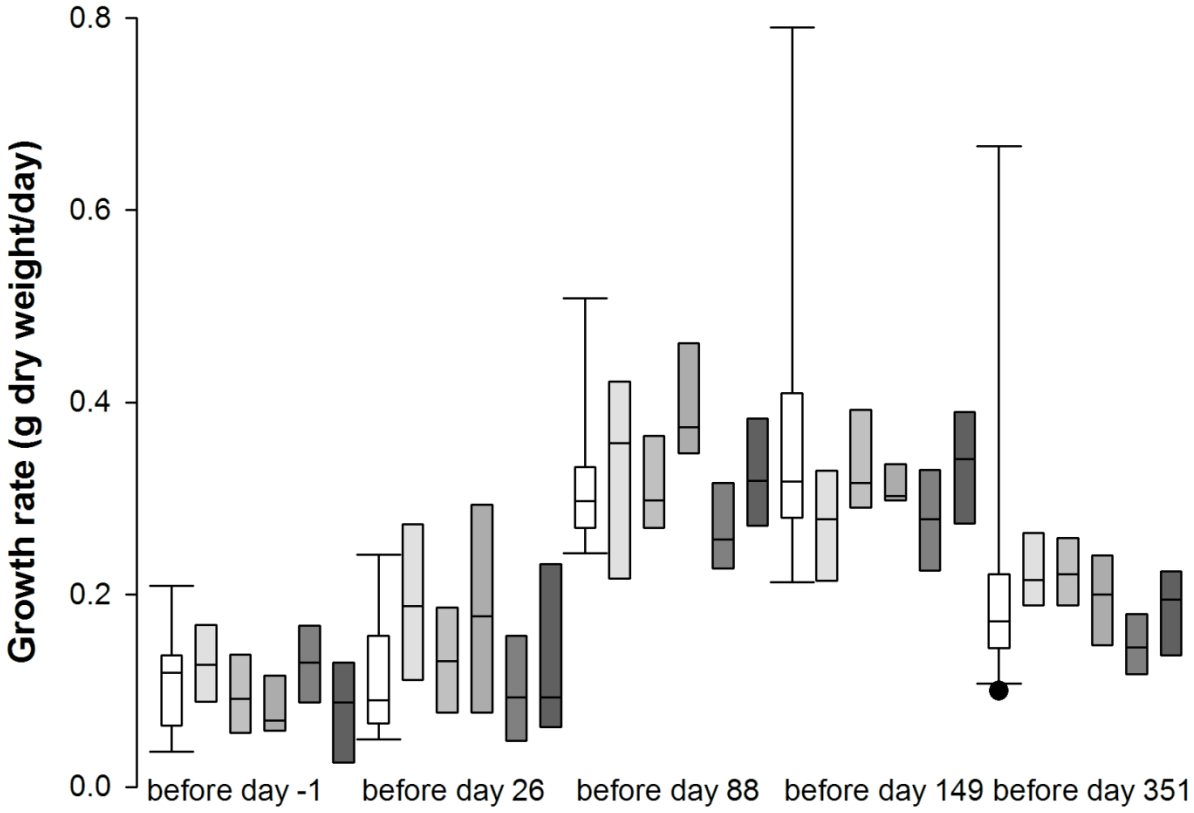


Figure IV-23: Growth rates of plant biomass between two sampling dates in the dose-response test. Increasing intensity of grey shades: lindane concentrations from control to 3.2 mg a.i./kg soil.

IV-2 Discussion

IV-2.1 Expected effects

A single pulse of a lindane treatment at the usual application rates should result, at least at the beginning of an experiment, high concentrations in the upper soil layer that can be assumed sufficient to kill insects and oligochaetes. In the laboratory, the lowest observed effective concentrations (LOEC) for *Folsomia candida* and *Enchytraeus albidus* were between 0.056 and 56 mg a.i./kg dw soil (LOCK *et al.* 2002). Beside of direct impacts intermediate disturbances of biotopes could have promoting effects on the diversity and the evenness of the dominance structure of soil organisms (ROß-NICKOLL *et al.* 2004). STAMOU & ARGYROPOULOU (1995) found the highest species diversity of oribatids along with moderately heavy metal pollution at roadside sites in Greece. In case of lindane, effects were expected to last for several months, because the degradation rates for lindane are relatively low. From field studies, DT_{90}-values in different soils have been reported usually to be less than one year but under certain circumstances, the rates can be prolonged considerably (SWEDISH EPA 2002). Based on the

experience of previous studies (chapter III) we expected transient effects in the range of the tested concentrations of the upper 5 cm soil layer.

IV-2.2 Collembolans

IV-2.2.1 General findings

The total abundance of collembolans in untreated TME was stable over a period of one year, indicating the integrity of the model ecosystems in general. The collembolans turned out to be the only sensitive group of all groups investigated. Transient effects on various endpoints could be demonstrated; especially species of the families *Isotomidae* and *Entomobryidae* were affected by the treatment. Generally, it is known from laboratory standard tests that arthropods show a range of susceptibilities to different active substances of different modes of action. Springtails in particular are relatively susceptible to gamma-HCH compared to oligochaetes. Similar observations were made for a set of other insecticides (e.g. dimethoate, lambda-cyhalothrin, parathion), whereas fungicides such as carbendazim seem to have minor impact on arthropods than on other invertebrates. The toxicity of heavy metals is comparably high for both oligochaetes and arthropods (a synopsis of available literature was compiled by FRAMPTON *et al.* 2006).

IV-2.2.2 Response to the treatments

The most sensitive species belong to ep- or hemiedaphic life forms, namely the predatory *Isotoma viridis* was followed by *S. aureus*, *I anglicana* and *L. lanuginosus*. Not at least due to the active extraction method that prefers highly mobile species over species that live in deeper soil layers these species were dominant in the whole community of collembolans. The susceptibility could be theoretically traced to the higher exposure in the upper soil layers, which has not been chemically analyzed. The community structure of collembolans is one of the most sensitive endpoints after 26 days after treatment (NOEC = 0.32 mg a.i./kg dw soil). The community recovered since the sampling of day 88, indicating only transient effects of the concentrations applied in this experiment. This did not meet previous expectations because in an earlier TME-experiment using nominal concentrations of 10 and 100 mg a.i./kg dw soil, respectively, no recovery at all could be seen.

IV-2.3 Oribatids

IV-2.3.1 General findings

The literature indicated that oribatids could reach densities of up to 10.000-40.000 individuals per m^2 on long-standing meadows, and they were found in numbers of up to 400.000 ind./m^2

in old-grown forest soils providing a beneficial humus structure, and thus being the dominant arthropod group in many habitats (WEIGMANN 2006). In our study, very few species occurred in comparably low densities. Densities of 400-2700 point to the disturbance of a grassland habitat (HUBERT & TUCKOWA 2003). Hence, the term 'undisturbed', which is used to characterize the coring area relates to the application of pesticides during the last decades. However, it could not be derived from the data that further decrease could systematically endanger the integrity of the TME regarding the communities of oribatids. Consequently, the systems were regarded as providing stable oribatid communities over the complete study period, similar to the group of collembolans. The particular low density of oribatid mites compared with those found in other years on the same meadow points to specifically adverse conditions for the reproduction of this group in the specific year. To meet the target of statistical needs for oribatid mites as well as for other arthropod groups it is highly recommendable to use larger TME in the future to provide a greater number of sub-samples at a time. By using sub-samples, the absolute abundances increase and the variation of mean abundances will be consolidated. Thus, the statistical power increases and fluctuations of abundances would be moderated.

IV-2.3.2 Response to the treatments

Until now, oribatids were rarely tested under standardized conditions in the laboratory. FRAMPTON *et al.* (2006) tested the oribatids species *Plathynothrus peltifer* using dimethoate as the test substance. The results showed a contradictory response to dimethoate in comparison to lindane – both pesticides with a comparable mode of action. Dimethoate, acting as inhibitor of cholinesterase activity by binding at the target site as an antagonist (FOLKER-HANSEN *et al.* 1996), caused pronounced negative effects on *P. peltifer* in the laboratory (FRAMPTON *et al.* 2006) while it remained unaffected by lindane - an antagonist of GABA-receptor chloride channel within the nervous system (EUROPEAN COMMISSION 2000b) in the present TME study. In complete contrast, the species showed tendencies for increasing populations in the highest treatments.

IV-2.4 Enchytraeids

IV-2.4.1 General findings

The numbers of enchytraeids sampled in May (day -1) and June (day 26) are rather low compared to other findings at grassland sites. RUTGERS *et al.* (2008) give reference values of 14200 - 20700 individuals per m^2 for grassland on sandy soils in the Netherlands, depending on management. Investigations on soil monitoring sites in Northern Germany provided a reference range of 9000 to 75000 individuals per m^2 for grassland sites (BEYLICH & GRAEFE

2009). Samplings in early summer often result in low animal densities due to drought. The data of the October sampling lie well within the ranges mentioned above. Species number and species composition are within the expectations for grassland sites.

IV-2.4.2 Response to the treatments

Although not showing significant deviations at even the highest lindane concentration as compared to controls the group is known to be sensitive to a whole range of pesticides (DIDDEN & RÖMBKE 2001). Considering the analysis of FRAMPTON *et al.* (2006), it is well known that oligochaetes react less susceptible to insecticides than arthropods and collembolans in particular. In the light of this knowledge, the result of our study was expectable. Expectations will be similar for other insecticides, such as dimethoate and lambda-cyhalotrin. It is possible that while testing of pesticides with alternative modes of action, enchytraeids appear to be the most sensitive group. This group of organisms should be further included in TME studies, especially because they were sufficiently abundant and diverse and therefore as suitable for statistical testing as the group of collembolans (Figure IV-24).

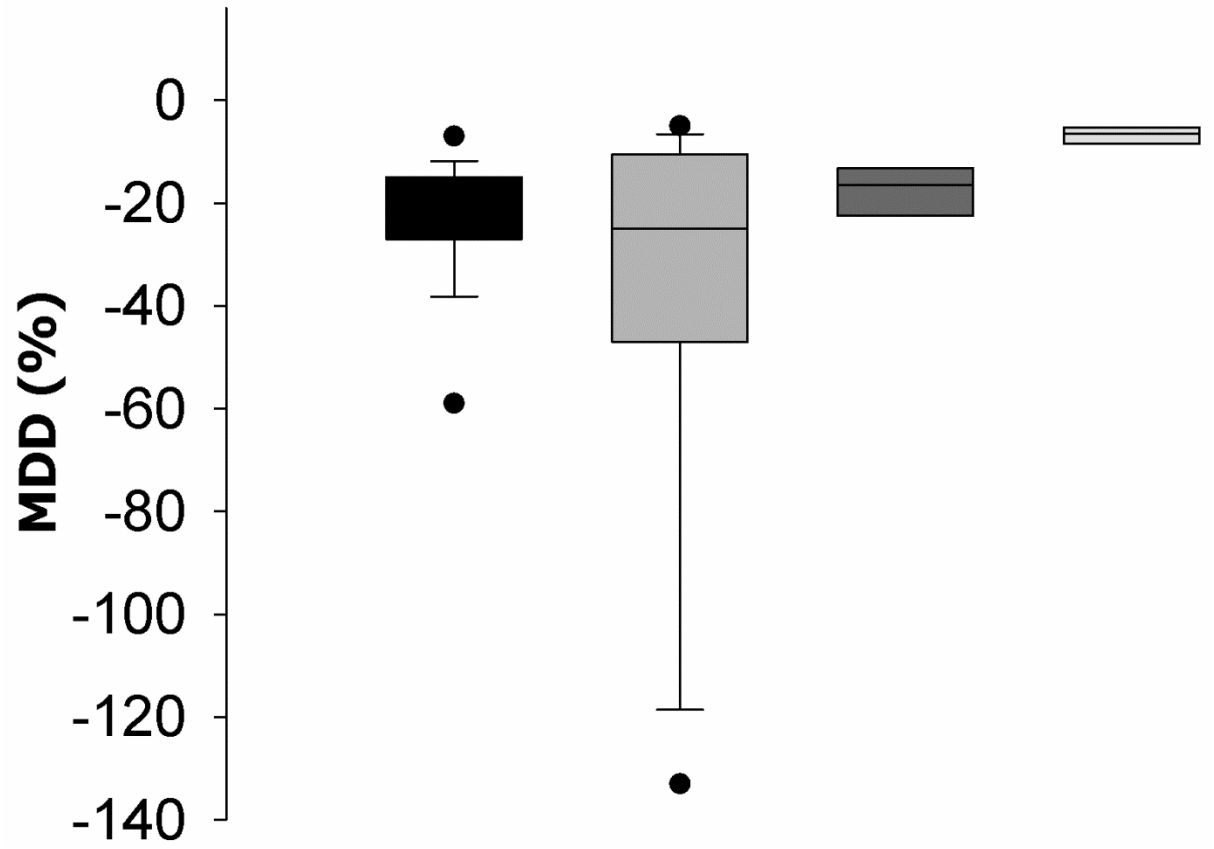


Figure IV-24: Boxplots of the MDD between control and treatment total abundances as percentage of control numbers of the four soil organism groups that were investigated at 18 different sampling dates in the context of TME studies that took place during the years 2005-2007 by a Williams sequential t-Test after ln-transformation of raw data for $N_{control}$ = 20 or 12, $N_{treatments}$ = 10 or 6. Test direction: One-sided lower, significance level: 5 %. Boxes from left to right: collembolans, oribatids, enchytraeids, and nematodes.

IV-2.5 Nematodes

IV-2.5.1 General findings

The community of nematodes in TME was quite diverse; the number of 29 families is typical for terrestrial agro-coenoses (MOSER *et al.* 2004A, BONGERS & FERRIS 1999). For the reason that nematodes representing probably the most abundant group of soil mesofauna, which has due to its intense interactions with other biota an important impact on most groups of the eco-system, it is highly recommended to include this group in an integrative semi-field approach like ours.

IV-2.5.2 Response to the treatment

Putting the fact aside that no clear, significant effects of lindane towards nematodes have been measured, pronounced effects of insecticides to eelworms could not be expected. Admittedly, they are widely known to be sensitive against environmental contaminants (YEATES *et al.* 1983, PARMELEE *et al.* 1993). However, the susceptibility against insecticides is rather low (FRAMPTON *et al.* 2006). The group of TME that was assigned to be applied with the highest concentration c_5 showed significant deviations from the controls before treatment. It is questioned if a rearrangement of the treatment groups could be desirable, but we think it is feasible to use an analysis of the results of a pre-treatment sampling as a criterion for rearrangement. This has to be done with respect to all groups intended for testing and would be suited to further enhance the statistical power. In the present case, effects on nematodes could be masked by the fact that the composition of the communities of the particular treatment groups at the beginning of the experiment was not homogenous. Accounting for relative changes of abundance, the picture is completely different. Figure 8 clearly shows that after setting abundance at day -1 to zero, at day 26 the control group and the treatments abundances between c_1 and c_4 decreased or increased only slightly, whilst the relative change within the c_5-treatment changed by more than 100 %. The relative representation of abundances contributes information on what happened within the community between the sampling dates and crosses out large differences at the date before the application.

IV-2.6 Fungi

IV-2.6.1 Ergosterol contents

The natural variability of ergosterol contents was rather high, but in good accordance with values reported in the literature (DJAJAKIRANA *et al.* 1996). There was no significant effect on fungal biomass, but between day -1 and day 26 the relative increase of ergosterol content in

the highest treatment was by far the clearest of all treatments (Figure IV-21). A promotion of the fungal biomass was also seen in pre-studies using the fungicide tebuconazol (RUMPLER 2007). It is possible that chemically similar precursors of ergosterol were measured that could not be resolved by the HPLC method. A promotion of the fungal biomass could also occur due to minor grazing pressure via collembolan or oribatid fungivores. Since the populations of microarthropods were merely slightly decreased, the influence of those seems unlikely to cause significant effects on the fungal biomass. In case amongst the members of fungal communities are those who have the ability to metabolize lindane (HERBST & VAN ESCH 1991), this mechanism could explain both alterations of the community structure as a whole, and the increased dominance of single phylotypes.

IV-2.6.2 DGGE analysis and fungal phylotypes

The community of soil fungi was recorded using molecular fingerprinting methodologies. Therefore, evidence taken from this part of the study is interpreted differently compared to the animal data. 'Abundance' was measured as relative intensity of bands on the electrophoresis gels and species were characterized as phylotypes that did not necessarily represent true species. For methodological reasons, it is not distinguishable for the experimenter either if one band represents one true species, or a sub-fragment of a gene of a true species belonging to another one at a different position on the gel, or an aggregation of several species. Further analysis by sequencing the DNA code of the single bands would be deliver the necessary information but was not feasible in the current experimental context. However, lots of information on diversity and community structure could be obtained and interpreted, in particular by comparing the three treatment groups. The number of phylotypes was nearly constant over time, which indicates stable fungal communities in TME over time. The informative value of phylotype patterns based on DGGE methodology was suspect of many controversies. For example, diversity could be underestimated by means of co-migration of fragments from different species due to similarity of the compared DNA-region. On the other hand, additional bands could occur due to formation of heteroduplex molecules (FERRIS & WARD 1997). Nevertheless, those problems can be reduced e.g. by identifying DNA-regions with high taxonomic resolution or by optimizing the PCR-conditions. Anyway, many authors do not esteem it as a problem (MURRAY *et al.* 1996). Effects on community structure became obvious only after three months after application. Eight out of 47 phylotypes showed pronounced, mainly promoting effects in response to the treatment with lindane (Table IV-2).

IV-2.7 Plant biomass

In contrast to the first test with the model compound lindane, no fertilizing effect of the self-made formulation could be stated (for discussion of unsuited solvents see dose-response study chapter III-2.5). Qualitative observations have shown that vegetation cover and rootedness became noticeably denser during the course of the experiment.

IV-2.8 Critique of methods and proposal for data interpretation

IV-2.8.1 Classification of effects and definitions of recovery

VAN DER LINDEN *et al.* (2006) proposed a five-part classification system for effects of pesticides for soil communities based on the model of BROCK *et al.* 2006 that was deduced from

Table IV-6: Classification of effects on collembolans based on NOEC-values. Synopsis of two TME studies, conducted separately in the years 2005 and 2006. The study period in both tests was about one year after application of the test item. The effect classes are defined accordingly to VAN DER LINDEN *et al.* (2006). as class 1 = no treatment related effects; class 2 = slight treatment-related transient effects, usually on one or a few isolated sampling dates only; class 3 = clear effects on several consecutive sampling dates, lasting less than 2 months post last application of the test item in the test system; class 4: clear effects on several consecutive sampling dates, lasting longer than 2 months but full recovery within a year post last application of the test item in the test system; class 5 = clear long-term effects, full recovery not within one year post last application of the test item in the test system.

Endpoints Effects of γ-HCH (mg a.i./kg soil)	Dose-response study					Range-finding study	
	0.032	0.1	0.32	1	3.2	10	100
Principle Response	1	1	2	2	2	5	5
Shannon index	1	1	1	1	1	4	4
Evenness	1	1	1	1	1	4	4
Taxa richness	1	1	2	2	2	5	5
Total abundance	1	1	2	2	2	5	5
Brachystomella parvula	1	1	2	2	2		
Entomobrya spec.	1	1	1	2	2		
Desoria trispinata	1	1	1	1	1	4	4
Isotoma anglicana	1	1	2	2	2	5	5
Isotoma viridis	1	2	2	3	3	4	4
Isotomurus palustris	1	1	1	1	1	4	4
Lepidocyrtus cyaneus	1	1	1	1	1	5	5
Lepidocyrtus lanuginosus	1	1	2	2	2	5	5
Lepidocyrtus lignorum	1	1	1	1	1	4	4
Mesaphorura macrochaeta	1	1	1	1	1		
Orchesella spec.	1	1	1	1	1	1	2
Parisotoma notabilis	1	1	1	2	2	4	4
Sminthurinus aureus	1	1	4	4	4	3	3
Sphaeridia pumilis	1	1	1	1	1	2-3	2-3
Lowest Community-NOEC			x			<	
Lowest population-NOEC		x				<	
NOEAEC					x	?	
Similarity (Steinhaus´ Index)	1	1	1	2	2	4	4
Similarity (Standers´ Index)	1	1	1	2	2	4	4

aquatic mesocosm studies. The proposal was also picked up by SCHÄFFER *et al.* (2010). A classification system has some major advantages for the interpretation of the complex results of a semi-field study that includes many different taxa. It can give a good overview of the experimental results, facilitate the comparison of different experiments, and be used to rank transient effects and show the dose-dependency of the effects by a clear arrangement. Table IV-6 shows the results of the TME-range-finding study of the year 2005 (refer to chapter III and the dose-response experiment of the year 2006 for the group of collembolans. This group of taxa turned out to be the most sensitive. Thus, it was taken as an example of the advantages of effect classification. It can be shown that the severity and persistence of effects spans over the two studies continuously. Whilst there were very few clear effects on most endpoints for soil concentrations between 0.0032 and 3.2 mg a.i./kg dry soil, no recovery took place for the principal response of the whole community and the diversity indices, as well as for the total abundance and several species. There was a steep increase of the effect between the two experiments. This finding can serve in future TME experiments as a rationale for the choice of effective concentrations in case lindane would become a standard reference substance.

IV-2.8.2 Statistical methodology

It was found worthwhile to analyse the results and consequences of the PRC analysis for the group of collembolans more intensely in a slight digression. This is meant to be beyond the strict requirements of the stepwise statistical procedure proposed in the literature as sketched below.

- Test the significance of the first principal component of a partial redundancy analysis by Monte-Carlo permutations tests by permuting the whole time series. Proceed only with step 2 if the precondition of a significant first principal component is fulfilled.
- The same permutations tests have to be performed at each of the sampling dates on several redundancy analyses separately, to test for significant differences between the treatments on the communities at the single sampling dates. Proceed with step 3 for the datasets of the significant sampling dates.
- Calculate the community NOEC by applying separate Williams tests on the sample scores of a Principal Component Analysis.

The procedure was originally described by VAN DEN BRINK & TER BRAAK (in a series of related papers, e.g. VAN DEN BRINK & TER BRAAK 1998) and applied in various publications in the broader context of ecotoxicological community studies. By completely repeating all steps of PRC calculations for certain sub-sets of the whole dataset, we realized that the outcome and the final consequences of our experiment was not independent of the sub-set chosen

(*set 5*: data used until 5 months after application of the test item, *set 12*: data used until 12 months after application, Figure IV-2). While investigating the results, it became obvious that specific differences between the two datasets hamper the detection of effects because the significance of the first canonical axis did not reach the default level when applied to set 12. (Figure IV-5) What is the reason and how would it be possible to overcome the weaknesses of a step-wise procedure that largely depends on the total variation in the dataset? It is questioned if it is statistically sound to relinquish on the consecutive procedure or to let allow for the interpretation of results that do not fulfil the prior steps currently defined as prerequisites of further analyses. We list some possible reasons and interpretations for the discussion in the scientific community.

The analysis of single sampling dates resulted in the same significances of the treatments and NOECs for both datasets because the corresponding data remains the same except for the last sampling date one year after application. The minor differences of the PRC c_{dt}-diagram even at the corresponding sampling dates base on the differing total standard deviation in species data TAU given by the CANOCO program. The original sample scores are multiplied by TAU, which is 0.64 for set 5 and 0.57 for set 12, resulting in slight differences in c_{dt}-values. The question remains why the PRC of set 5 is significant but not of set 12? The worst case would be that with increasing total variation due to progressing time the detectability of effects will be decreased systematically. Alternatively, is as much non-treatment related variability added to the dataset as necessary to hide the effects? Consequently, a power analysis for multivariate statistical methods is urgently stipulated but it demands much computational power because of the inhomogeneity of the univariate distributions underlying the multivariate dataset. In the arena of aquatic semi-field test guidance, the demand for information on the specific power of an analysis is already formulated (e.g. OECD 2006). As the final consequence, the detectability of initial effects on community level is lowered by either increasing variability of the dataset or by blurring the initial response of the community with onward duration of an experiment. The strict criteria of a step-wise statistical procedure should not apply for TME-data when the regulatory acceptable concentration in soil should be deduced. In higher-tier semi-field studies like aquatic mesocosms or TME, the Regulatory Acceptable Concentration (RAC) is defined as the No Observed Ecologically Adverse Effect Concentration (NOEAEC) divided by the relevant safety factor.

Recently, in the arena of aquatic semi-field experiments using experimental data of mesocosm studies new methods beyond the PRC have been proposed and criticized. LIESS & BEKETOV (2011) found long-term effects by using a new, trait-based approach $SPEAR_{mesocosm}$ and uni-

variate statistics (ANOVA and corresponding post-hoc tests) that were not indicated by the multivariate PRC-method. The immediate response and objection of VAN DEN BRINK & TER BRAAK (2011) opened the floor for further discussions on the detection of subtle and long-termed effects on the structure of community under chemical stress. It was proposed to use further axes of the multivariate analyses to detect the effects of low dosages on predominant taxa groups rather than reducing the variability of a dataset by a priori classification. Our perception is that the statistical methodologies for the analysis of community effects are far from being exhausted. Additional to the improvement of the statistical methodologies, it is necessary to establish more relevant test systems.

V

Beyond substance related effects

The preceding chapters reviewed the history of TME studies, described the conceptual approach of this thesis, exposed the methodology of TME studies in great detail and analysed the effects of the model compound lindane on populations and communities and of soil organisms in TME. By focusing on the effects of the toxic compound and by mainly using statistical methods that were currently used for the analysis of model ecosystems for the aquatic risk assessment, the considered test system was seen under a quite narrow angle of mere regulatory ecotoxicology. The following section should provide a basis for a general discussion of the characteristics of a soil ecosystem that determine the limits of statistical analyses. Questions that arose during intensive discussions with internal and external experts at international conferences and workshops will be reasoned. Especially the international SETAC Workshop on the future use of terrestrial semi-field methods in Coimbra in the year 2007 gave many thought-provoking impulses and was helpful to sort out non-relevant directions of further investigations. The following chapter is structured by three experimental phases '*experimental design*', '*experimental period*' and '*analysis of results'*, and it discusses the *representativeness* of TME for the situation in the field.

- *First*, the *experimental design* has to be developed and adjusted to both the specific research question and the characteristics of the test system. An intensive field screening should deliver robust estimators of the expectable variability in the field and an optimized sampling strategy of TME soil cores (chapter V-1).
- *Second*, the test system should be stable over a certain *experimental period*. For this, the temporal stability of the test system should be demonstrated to avoid systematic artefacts due to effects of isolation other aspects of the test systems properties (chapter V-2).
- *Third*, for the *analysis of the test results*, the variability of the experimental units (TME) should be known in order to assess the relevance of effects and the limits of effect detection. In the following, data of all studies in the field and in TME that had been conducted between the years 2004 and 2007 (chapter II-1.1) were used to investigate the different questions (chapter V-3).

- *Fourth*, the *representativeness* is analysed by comparing the TME collembolan communities with the communities of the coring site. Differences are assumed to be due to transport and storage effects after TME coring, and should be at least partly ascribable to climatic differences between the two areas of origin and the experimental storage site, respectively. Furthermore, a comparison between the TME species inventory with the typical agricultural species in Central Europe has been undertaken (chapter V-4).

V-1 Proposition of an experimental design

Prior the TME experiments, the coring area was sampled intensively for microarthropods with soil cores (the methodology was described in chapter II-1.6.1) in a random grid design. The resulting data were used to estimate the general suitability of the coring area with respect to the overall abundance and the species richness on the one hand; on the other hand, the results were used to identify the best strategy of coring the TME considering the spatial distribution of soil animals. It was questioned whether the coring area could provide homogenous experimental units in TME studies. In the unwanted case that the distribution of the considered taxa groups would be heterogeneous, an optimal coring strategy should be deduced. Descriptors of community traits (total abundance of microarthropods, diversity measures and community structure of collembolans) were used to describe the *variability* on the coring area, as well as geospatial models of taxa distribution were used to describe the *heterogeneity*. The terminology and the procedure followed the definitions provided by ETTEMA & WARDLE (2002). It was hypothesised that an untreated off-crop area should provide higher species diversity than cropped arable land. The strategy that is demonstrated in the following is meant to be a general template for a concept of designing a TME experiment. The mesofauna of 189 soil samples was extracted, counted, and the total abundance of oribatids and collembolans was analysed. Additionally, collembolans were determined to species level. The following analyses were focused on small- and large-scale variability of total abundance and species composition on the coring area, comprising differences between the sample locations (the sampling grid is shown in section II-1.6.1).

V-1.1 Vegetation cover

The vegetation cover on the coring area in Monheim/Rhine was homogenous and did not show clear gradients of humidity. It was dominated by *Holcus lanatus*, *Bromus hordeaceus* and other weed species, accompanied by herbs (*Taraxacum officinale*, *Ranunculus repens*). It can be attributed to the class of *Molinio-Arrhenateretea* (tall oat grass meadows), which are classified as intensively managed, artificially built, mown at least twice a year and to be found usually on 'normal soils' (nomenclature after DIERSCHKE 1994). It cannot definitely be assigned to a single plant community (phytocoenosis) because it lacks typical character species. In the following, it is therefore referred to as a 'disturbed tall oat grass meadow', which is expected as a characteristic phytocoenosis at a fresh-to-moist cultivated grassland site.

Table V-1: Vegetation cover as percentage of the total grass-scrub layer on the TME-coring area. Empty cells indicate that the species was not found in the particular survey. Total cover on the surveyed areas 95 %.

Plant species	Survey No.		
	1	2	3
Bromus hordeaceus (L.)	20	20	20
Holcus lanatus (L.)	60	60	60
Taraxacum officinale (Wiggers)	5	5	5
Arrhenatherum elatius (J. et C. Presl)	5	5	10
Dactylis glomerata (L.)	10	5	1
Poa trivialis (L.)	5	5	3
Ranunculus repens (L.)	3	3	3
Alopecurus geniculatus (L.)	5	-	5
Cirsium arvense (Scop.)	1	-	-
Trifolium spec. (L.)	-	-	1
Geranium dissectum (L.)	2	-	-
Rumex obtusifolius cf. (L.)	1	1	-
Senecio vulgaris/sylvaticus (L.)	2	-	-
Heracleum sphondylium (L.)	1	-	-

V-1.2 Abundance of microarthropods

The mean number of both collembolans and oribatid mites was equal on the first small-scale sampling points of the *ss1*-patch and the large scale sampling points *bsc* (design explained in chapter II-1.6.1). The most abundant patch regarding the collembolans was by far *ss2* (descriptive statistics provided by Table V-2); the abundance was three-times higher than on *ss1* and *bsc*. According to these large differences between the patches and the whole grid and realizing that the range of collembolan abundance was 2-3 times higher than for oribatids, the results indicate a much higher variability of collembolan numbers. The abundance of oribatid mites was constant regarding small- scale and big-scale samples. Gamasid mites as well other organisms (mainly larvae of dipterans, coleopterans and lepidopterans) were equally distributed between small- and big-scale samples (for an overview of descriptive parameters and the results of the Student's t-test see Table V-2).

Table V-2: Descriptive statistics of the mesofauna abundance of 189 soil cores sampled on the coring area 'Altjudenhof' during the pre-screening campaign (MacFadyen extraction only). Rare species are assumed to be slightly underrepresented in small-scale samples, due to lower sample number. Mean values (within taxa groups) sharing the same letters are not significantly different (Students t-Test on untransformed data; two-sided, $\alpha \leq 0.05$).

	Parameter	*Collembolans*	*Oribatid mites*	*Gamasid mites*	*Others*
BSC (N=93)	Minimum	1	0	0	0
	Maximum	131	10	5	12
	Mean	27.5[a]	1.2[a]	0.5[a]	1.2[a]
	Std. Deviation	23.5	2.1	1.0	2.1
	Coefficient of Variation	85	164	197	169
	Sum	2563	118	47	116
SS1 (N=48)	Minimum	5	0	0	0
	Maximum	106	12.0	6.0	23
	Mean	27.7[a]	1.6[a]	0.7[a]	1.8[a]
	Std. Deviation	16.6	2.0	1.1	3.4
	Coefficient of Variation	60	126	162	190
	Sum	1332	78	34	86
SS2 (N=48)	Minimum	5	0	0	0
	Maximum	356	11	2	7
	Mean	96.9[b]	1.6[a]	0.6[a]	1.7[a]
	Std. Deviation	78.7	2.4	0.7	1.7
	Coefficient of Variation	81	141	122	95
	Sum	4653	80	29	84

V-1.3 Community structure of collembolans

In sum, twenty-five collembolan species were found, belonging to nine families. About 96 % of the total individual numbers belonged to the family Isotomidae, mainly epedaphic species. Ten most common species contributed more than 1 % to the total abundance; out of them,

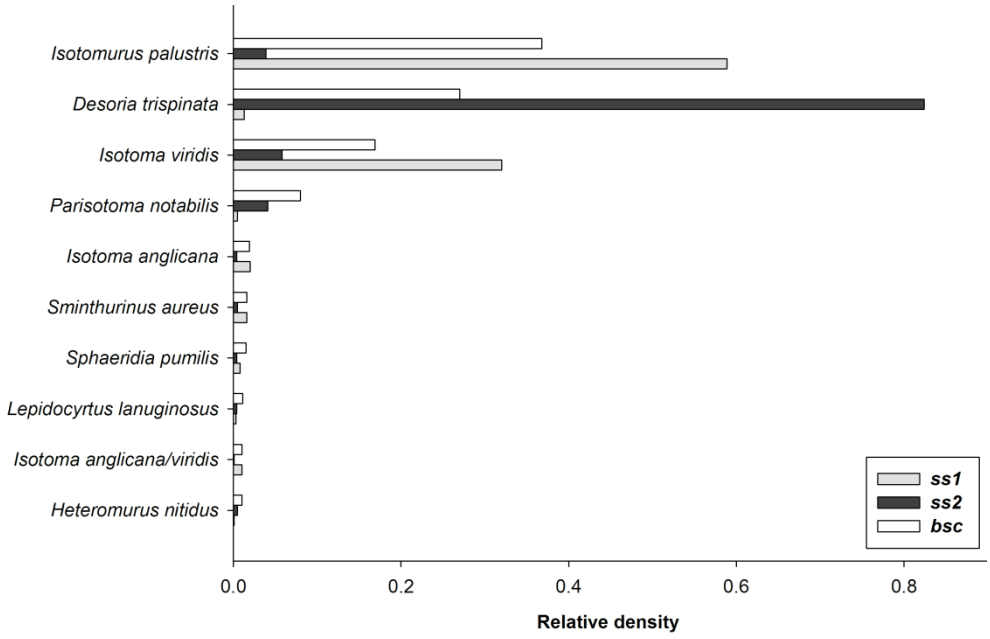


Figure V-1: Comparison of dominance spectra between small-scale and large-scale samples on the coring area. Shown: species that contribute at least 1 % of the total abundance.

Desoria trispinata, *Isotomurus palustris* and *Isotoma viridis* contributed nearly 90 % of all individuals. The samples of the large-scale grid (*bsc*) showed a comparatively balanced distribution of species. On the two small-scale patches, other species than the three mentioned above became dominant: On small scale-patch 1 (*ss1)*, *Isotomurus palustris* contributed 59 % and *Isotoma viridis* another 32 % to the total abundance, while *Desoria trispinata* contributed only 1.3 %. The picture on the small-scale patch 2 (*ss2*) was completely different: *Desoria trispinata* contributed 82 % to the community. Figure V-1 illustrates the changing proportions that contribute the dominant species to the total abundance. It was due to the strongly dominant species *D. trispinata* that the diversity measures were on *ss2* lower than on *bsc* and *ss1* (Figure V-2). The diversity on *ss2* was marked by a low degree of evenness compared to the other patches. The difference between *bsc* and *ss1* was mainly due to a reduced number of species on *ss1*. Since the sampled area of *bsc* was much larger compared to *ss1* and *ss2*, a higher number of rare species was found (refer to the tabular appendices). The diversity index is lowest on ss2 because of the mass occurrence of one single species *D. trispinata* (Figure V-1, Figure V-2).

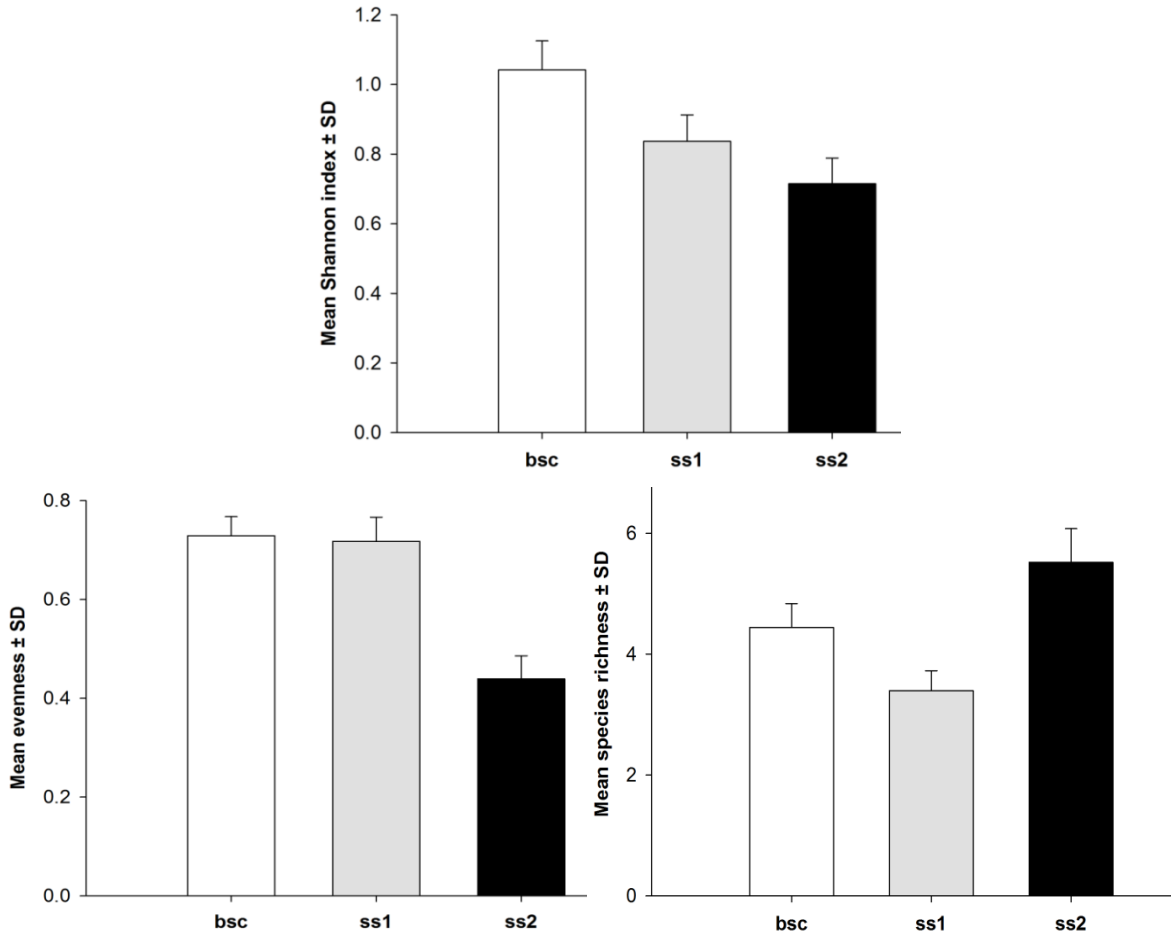


Figure V-2: Diversity on both the large-scale grid and the small-scale patches. Shannon index values depend on the species richness and the evenness of the species distribution in the community.

V-1.4 Patchiness

A statistical classification of all samples taken on the coring site by the specific species composition by means of a cluster analysis (Ward's method, squared Euclidian distance measure, results not shown for reasons of stringency) did not show systematically differences between the small- and the large-scale patches. It was concluded that a merely statistical methodology would not be suitable to describe the explicitly space-dependent distribution of species. Therefore, a geostatistical approach was chosen and the data were analysed by variographical and kriging methods.

The degree of the patchiness of a distribution of organisms in a geographical area can be described by indices that allow for a comparison of different species and areas. Large values of

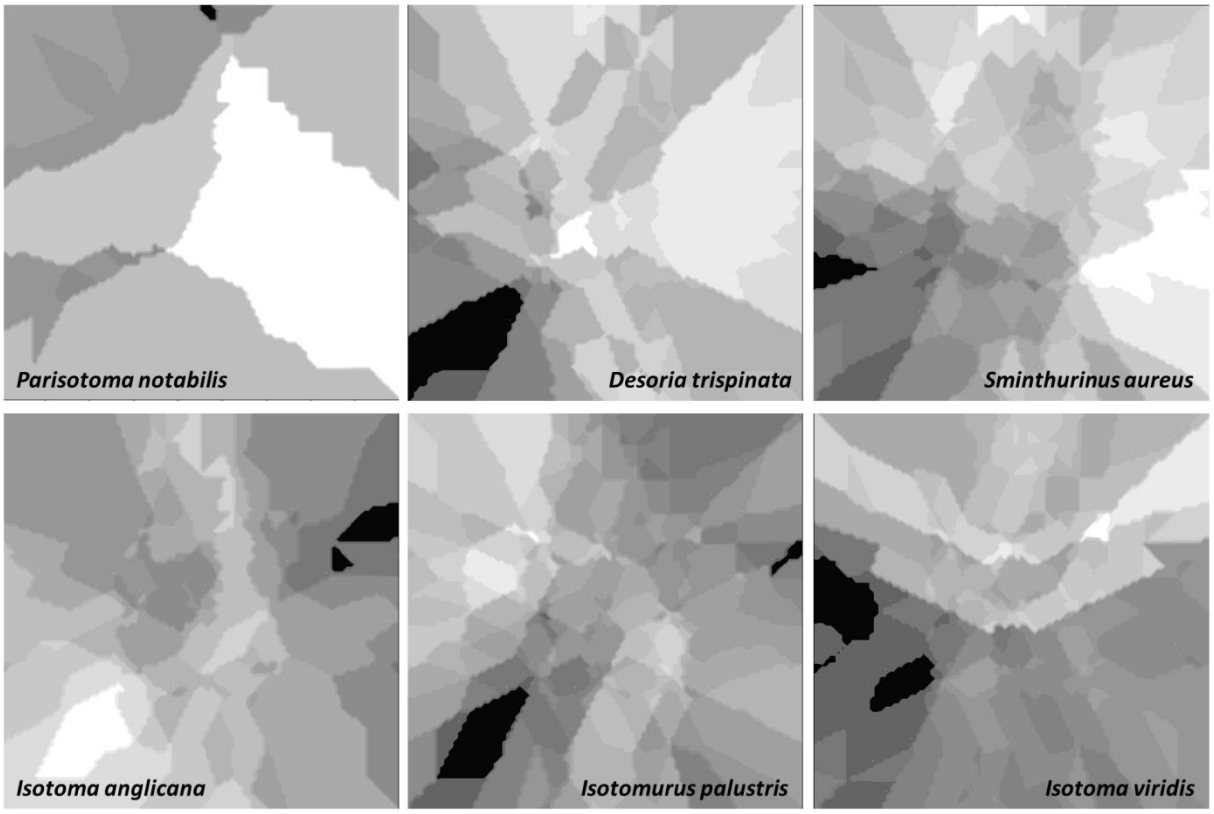


Figure V-3: Kriging diagrams of the abundances of the most dominant collembolan species on the small-scale patch *ss1* showing the interpolation between sampling points. Greyscale indicates the relative density of a species (ln-abundance); light colours indicate low densities while darker colours indicate high abundance. Each square's edge length is 1.4 m.

an index of patchiness indicate a high degree of aggregation of the species (Table V-3). Measured values for *liop* (method refer to chapter II-4.7) of above one refer to a patchy distribution of the specific taxon. That applied for most of the single species at least on *bsc*, *ss1* or *ss2* a patchy distribution. However, for the total abundance no aggregation of the collembolans could be seen. Exceptions from the patchy patterns were *I. palustris* (*ss1*), *I. viridis*

(*ss1+ss2*), and *D. trispinata* and *P. notabilis* (*ss2*). This finding was unexpected, since it was anticipated that most species would be patchily distributed over the area regarding the relatively large scale of the investigation compared to the organisms' radius. It is assumed that the remaining rare species, i.e. those that contribute less than 1 % to the total abundance of the community, can be called patchy per definition. *D trispinata* showed a high degree of aggregation on *bsc* and *ss1* that is in sharp contrast to its regular distribution on *ss2*.

As can be seen in Figure V-3, on *ss1* the four species *D. trispinata*, *S. aureus*, *I. viridis* and *I. palustris* showed an absolute maximum in the middle-lower left side of the sampling grid. In the upper and the right sector, they were relatively rare, according to the relative ln-scale adjusted to the individual range of numbers of each species. *S. aureus* and *P. notabilis*, occurring in very in low numbers, were nearly absent in the middle-right. *I. anglicana* showed a reversed image compared to the four species mentioned above, suggesting competition between the species for some resources on a small scale of few centimetres. The range of autocorrelation for all these species on *ss1* was between 6 and 90 cm (Table V-3). On *ss2*, similar patterns were observed, but no systematic differences between the species could be deduced

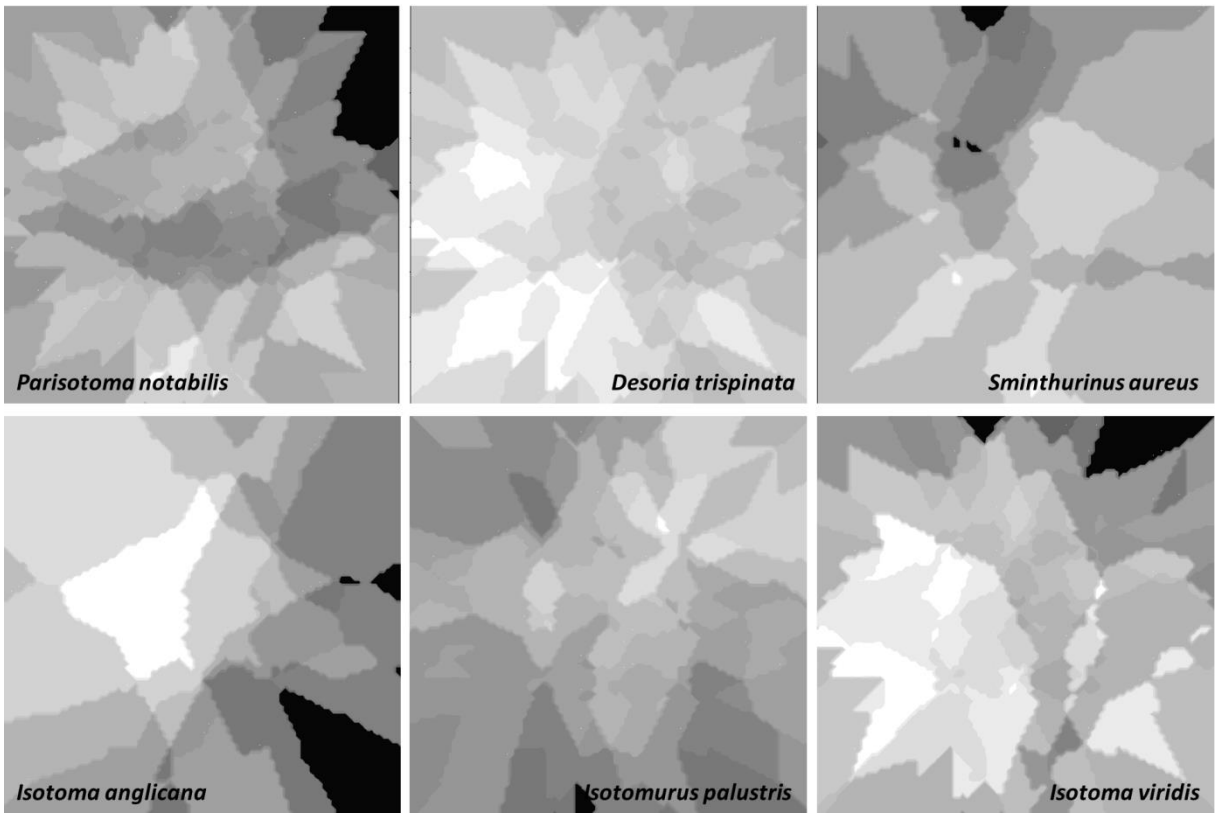


Figure V-4: Kriging diagrams of the abundances of the most dominant collembolan species on the small-scale patch *ss2* showing the interpolation between sampling points. Greyscale indicates the relative density of a species (ln abundance); light colours indicate low densities while darker colours indicate high abundance. Each square's edge length is 1.4 m.

by the kriging analyses (Figure V-4). The ranges on *ss2* were between 11 and 66 cm (Table V-3).

V-1.5 Best-fit coring strategy

The results of the geospatial analyses depend on the lag-distance chosen for the calibration of the model, which is the mean distance between two sampling points taken from a frequency distribution. Results turned out to be quite ambiguous regarding the possible best-fit-models (Gaussian, exponential, spherical and linear). As one of the most important results of the analysis, the range (i.e. the distance above which no autocorrelation occurred) of the total abundance on *bsc* was between 16 centimetres for *I. viridis* and 940 cm for *I. palustris*. Significant regression fits were found only for *D trispinata* and *I. palustris* (ranges of 343 and 940 cm, respectively, see Table V-3).

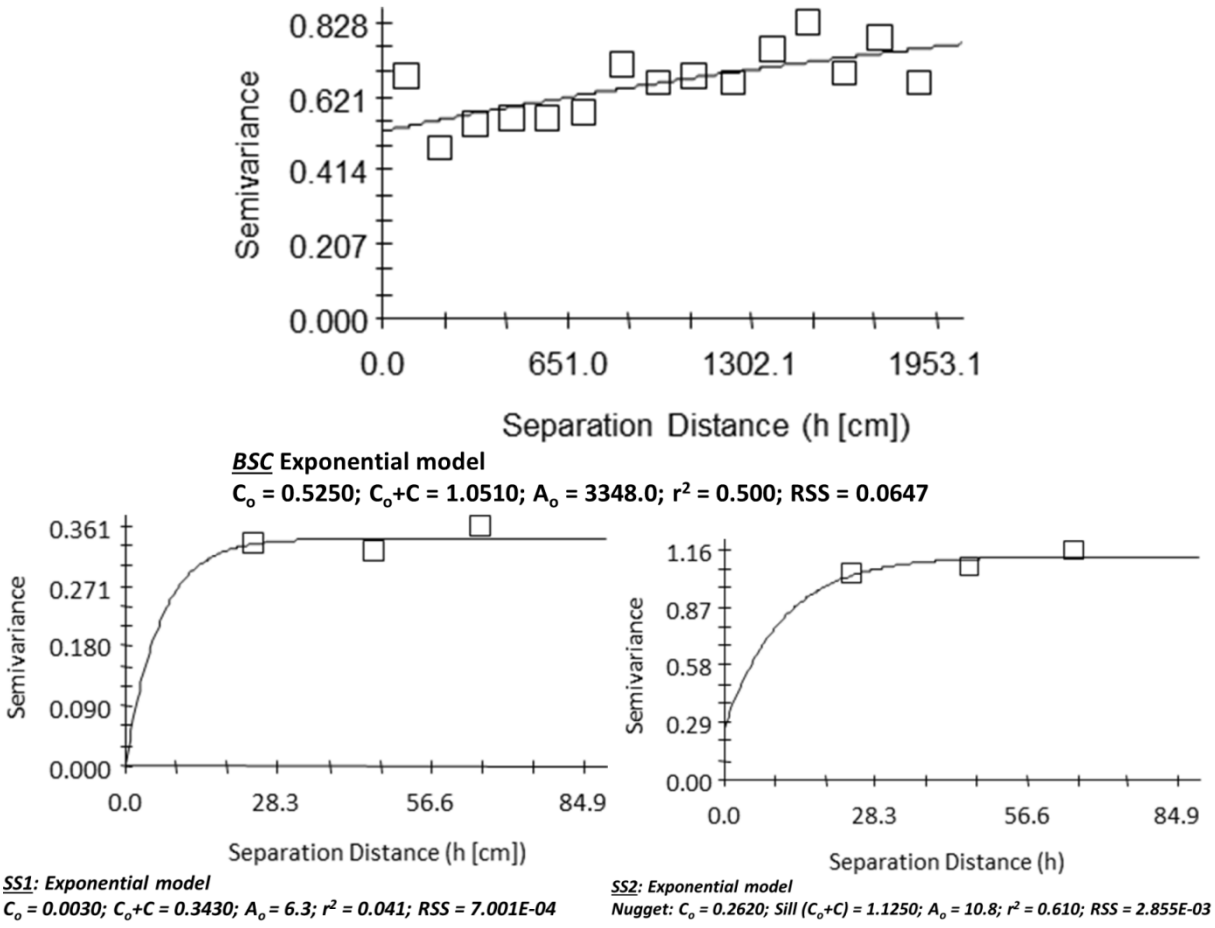


Figure V-5: Isotropic semi-variograms of collembolans' total abundance on the TME coring site near Monheim a. R. Classified input data (squares) and modelled semi-variance (lines). The abbreviations of the model parameters are explained by section II-4.7.

V-1.6 Gradients

Beyond the descriptions of the small-scaled patchiness of the collembolan distribution, the question raised if there were environmental gradients across the coring area, determining the patterns of occurrence of the organisms. Because of the interpolation of the point samples on the *bsc*-sampling grid by ordinary kriging analyses (Figure V-6 ff.), gradients became obvious for some of the dominant species. As shown by Figure V-6, the total abundance of collembolans over the whole area appeared to be homogenous.

Figure V-6: Kriging diagram of total abundance (per square meter) of collembolans (data: *bsc*).

Table V-3: Results of variographical analyses and of the calculations of the index of patchiness after Lloyd (XIAO *et al.* 1997) Grey shaded entries are considered relevant for further interpretation. Explanation of the geostatistical methods could be found in chapter II-4.7).

	Endpoint	Patchiness		Variography				
		liop	*bfm*	*nugg*	*sill*	*rge*	r^2	*rss*
BSC (N=93)	Total abundance	0.70	*exp*	0.53	1.05	3348	0.500	0.065
	D. trispinata	6.10	*exp*	7.33	16.21	343	0.624	35.500
	I. anglicana	5.98	*sph*	0.01	7.08	118	0.079	8.170
	I. palustris	0.59	*gau*	3.04	6.39	940	0.667	10.100
	I. viridis	1.06	*exp*	0.64	6.31	16	0.000	12.500
	P. notabilis	3.61	*exp*	1.44	13.17	37	0.137	9.050
	S. aureus	5.95	*lin*	7.35	7.35	1893	0.017	10.100
SS1 (N=48)	Total abundance	0.38	*exp*	0.00	0.34	6	0.041	0.001
	D. trispinata	5.30	*sph*	0.22	7.06	33	0.841	0.059
	I. anglicana	2.96	*sph*	0.05	9.27	27	0.072	0.270
	I. palustris	0.38	*sph*	0.50	8.24	40	0.993	0.013
	I. viridis	0.88	*gau*	2.22	6.55	90	0.931	0.083
	P. notabilis	12.60	*sph*	0.00	2.78	24	0.000	0.019
	S. aureus	4.12	*sph*	0.50	8.24	40	0.993	0.013
SS2 (N=48)	Total abundance	0.72	*exp*	0.26	1.13	11	0.610	0.003
	D. trispinata	0.82	*exp*	0.35	1.62	11	0.682	0.005
	I. anglicana	6.26	*sph*	0.01	6.22	24	0.000	0.533
	I. palustris	1.00	*lin*	9.33	9.33	66	0.412	0.020
	I. viridis	0.78	*exp*	1.75	7.56	18	0.948	0.054
	P. notabilis	0.83	*sph*	0.20	7.56	31	0.998	0.000
	S. aureus	4.62	*lin*	7.77	7.77	66	0.814	2.080

Abbreviation	Description
liop	Lloyd's Index of Patchiness
bfm	Best fit model
exp	*Exponential*
sph	*Spherical*
gau	*Gaussian*
lin	*Linear*
nugg	Nugget
sill	Sill
rge	Range [cm]
r^2	R-squared
rss	Residual sum of squares

However, the area on the bottom right side in x-direction was dominated by high abundances of the epigaeic *D. trispinata, which is thus*. The gradiental occurrence of this species can be seen in Figure V-8. An ecological interpretation of this finding remains speculative, because no environmental measurements were available. The evidence should be taken from personal

Figure V-7: Kriging diagram of *I. anglicana (*abundance per sample*)* (data: *bsc*).

observations: the meadow was mowed shortly before the sampling, and the mulch was not removed.

From the literature, *D. trispinata* is known to prefer high contents of organic material (TANAKA 1970). High amounts of rotten grass were dispersed in this part of the sampling grid, so this explanation was assumed to be well-founded. Further interpretation of the pattern observed for *D. trispinata* could be based on the distribution pattern of the competing species *I. palustris*. It has shown the oppositional preferences on the area, i.e. it was mainly found in the bottom-left of Figure V-10. It is implicated that some kind of exclusive competition took effect on these two similar species.

I. palustris prefers humid habitats and is therefore more dominant in the left of Figure V-10, since there was a negative slope of the meadow from the country lane to the ditch (Figure V-10). In conclusion, two gradients of species distribution could be stated: The most dominant species *D. trispinata* and consequently the total abundance increased from the left to

Figure V-8: Kriging diagram of *D. trispinata (*abundance per sample*)* (data: *bsc*).

Figure V-9: Kriging diagram of *S. aureus (*abundance per sample*)* (data: *bsc*).

right on the coring area. In contrast, the abundance of *I. palustris* decreased accordingly. Other species showed divergent distribution patterns over the sampling grid. *P notabilis* (Figure V-11) occurred more patchily and sporadically than *I. anglicana* (Figure V-7). The latter had its main occurrence in the left side of the area.

A 'gradiental' species distribution could be stated as well for *S. aureus* and *I. viridis*, both of them showing absolute minima of occurrence in the left or right side of the sampling area (Figure V-9, Figure V-12).

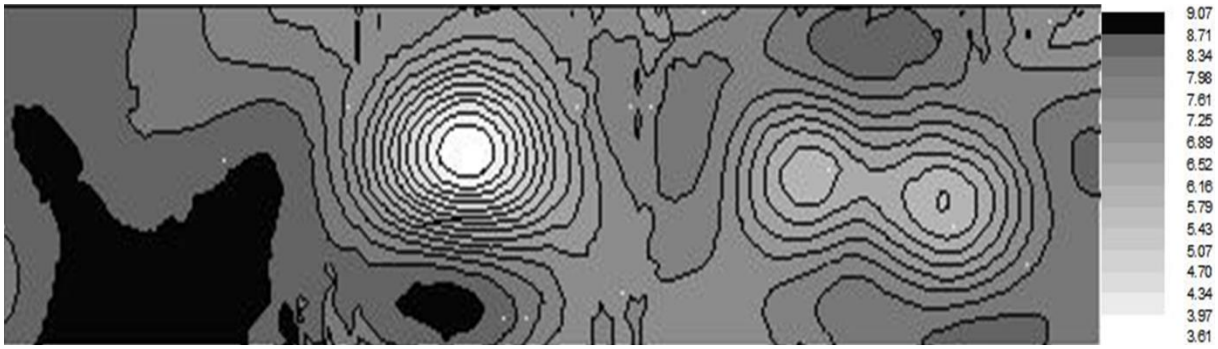


Figure V-10: Kriging diagram of *I. palustris (*abundance per sample*)* (data: *bsc*).

Figure V-11: Kriging diagram of *P. notabilis (*abundance per sample*)* (data: *bsc*).

Figure V-12: Kriging diagram of *I. viridis (*abundance per sample*)* (data: *bsc*).

V-1.7 Concluding 'proposition of an experimental design'

A preliminary screening of the site at which soil cores are intended to be cored in a TME study should serve as an estimator of the general suitability for the purposes of an ecotoxicological effect study. A coenosis of soil organisms should hold a sufficient overall abundance as well as a minimal diversity to reflect different sensitivities and reaction patterns (see chapters III-1.2.2, III-1.3.2 of the dose-response study).

The abundances of collembolans were sufficiently high for statistical predications (compare Table V-2) but for the oribatid mites mean numbers of less than two individual per soil sample were very low. It was then decided to enhance the yield of the extraction for the latter group by applying consecutive extraction steps by combining MacFadyen and flotation techniques. The results of those experiments are not amongst the main topics of this thesis, since the flotation as a passive extraction provides information on both dead and living individuals (see the compilation of extraction techniques in section II-2.4.1). Later it was decided rather to optimize the MacFadyen extraction than to combine several methods with several biases that partly hamper the ecotoxicological interpretation of the results.

The overall number of twenty-five species of collembolans was within the range of the literature at the lower end of the recorded species numbers, not surprisingly regarding the small number of sampling dates that could not properly reflect the seasonality of the community of collembolans. THEIßEN (2009) found estimated ('jack-knife'-estimators) species numbers between 39 and 69 species on grassy field margins of different German landscapes within two years of extensive studies. RUSEK (1998) described the range of collembolan local biodiversity as between 3 and up to 60 species in different ecosystems. For the purposes of ecotoxicological studies, the meadow was concluded as suitable for further investigations as providing a sufficient number of species covering the most important grassland species.

As the distribution of soil organisms is generally assumed aggregated (CHALUPSKY & LEPS 1985), this study hypothesized that the degree of aggregation highly depends on the scale. The sampling design allowed for the description of the distribution on a very small scale of 1.4 x 1.4 meters, as well as on a larger scale of 10 x 40 meters. It turned out that certain collembolan species as well as the superior taxonomic group 'Collembola' were randomly distributed over the small-scale patches *ss1* and *ss2*, but also on the large-scaled samples *bsc*. The total abundance did not show a patchy distribution in any case. The discrepancy between the single species, which are mostly patchily distributed and the total abundance, which did not reveal a patchy distribution could be explained by the different preferences of the single species. On the level of the whole taxon those differences blur. This delivers a further argument to deter-

mine organisms to species level in general and the microarthropods in particular. As indicated by the vegetation cover and the plant species composition of the meadow, it was previously concluded that even the whole area provides homogenous environmental conditions. For single species, on the larger scale the distribution was clumped, there were centres of high and low density. This could be due of either competitive exclusion of similar collembolan species that takes effect on the large-scale, or to some weaknesses of the study design that did not allow for significant segregations of taxon-specific patches. The findings of the variographical analyses had serious implications on the future sampling strategy. After the pre-study, it was decided to core the soil cores as small-scaled as possible. This can (theoretically) lead to lowered variance in case of sub-sampling the TME. For TME studies at the Institute of Environmental Research, it was decided that a minimum coring area of about five per five meters for about 55 units could be realised, without unacceptable disturbance of soil structure nearby the borehole. It was concluded that on a large-scale, the avoidance of excess heterogeneity would be feasible; whereas most of small-scale heterogeneity would be included in each of the TME-units. The issue was discussed at the SETAC-PERAS workshop on semi-field methods for the environmental risk assessment in soil in Coimbra 2007 and it was recommended that the 'coring operation should be conducted over a narrow area' (SCHÄFFER *et al.* 2011).

V-2 Temporal stability of TME

Usually each TME approach, whether used as higher-tier semi-field systems in the context of ecological risk assessment or as an experimental tool to address basic ecological questions should be operable on a long-term. Thus, the stability of a (model-) ecosystem should be ensured. It is here stated that it could be described by some selected and meaningful indicators. The basic ecological concepts of ecosystem stability were described in chapter I-4.3.2; from those prerequisites, the following indicators were derived. To decide whether the TME biocoenoses could be concluded to be stable over time, four criteria have to be met (mainly deduced from BEGON *et al.* 1998).

- Abundance

  The total abundance and the abundance of single species should not decrease gradually or catastrophically, at least not systematically within the considered time-period. The idea behind the 'abundance' criterion for stability was based on the assumption that adverse conditions for the reproduction of soil arthropods or limitations by decreasing area within TME would lead to decreasing numbers.

- Diversity

  Species richness, evenness and the interaction diversity (here expressed and calculated as Shannon's H' indicator of diversity) should not decrease significantly and the community structure should not be altered greatly. It was assumed that the obvious lack of predators (we did not find neither gamasid mites nor others like ground beetles or wolf spiders in none of the TME studies) would benefit specific species, probably r-strategists. This effect could shift the whole community, which would be indicated by the 'similarity' criterion.

- Similarity

  The similarity of the composition of TME communities over time after disturbance (here: TME coring) was estimated by different distance measures and quantitatively analysed by ordination methods. For the decision about the stability of TME as a self-contained system, at least biannual data should be interpreted to estimate if clear changes were caused by seasonal fluctuations or by isolation or other undesired and inherent effects. However, the direction of seasonal changes is not supposed to be determined, while changes due to isolation were assumed to tend towards lower or higher parameter values and could not be presumed in advance a study. It is not known whether the grassland system of the coring area was at its final equilibrium state at the time of coring or if changes observed in TME were due to on-going natural dynamics.

- Soil removal

  In our experimental setup, the TME soil cores have been subsampled sequentially. This caused necessarily a loss of habitable space of the upper five centimetres for soil organisms. The impact of the removal of soil has been investigated as the variation explained in a partial redundancy analysis.

V-2.1 Abundance criterion

The analysis of the abundance criterion shows either if the total control abundance of collembolans and oribatids has been systematically affected by the mere duration of a TME experiment, or if the effects of long-lasting isolation and competition have had effects on the level of the collembolan populations. Previously the first experiments started, it was assumed that most likely many of the differences occurring were due to seasonal differences.

V-2.1.1 Abundance of taxa

The abundance of both taxa was not negatively affected by the time after coring over the

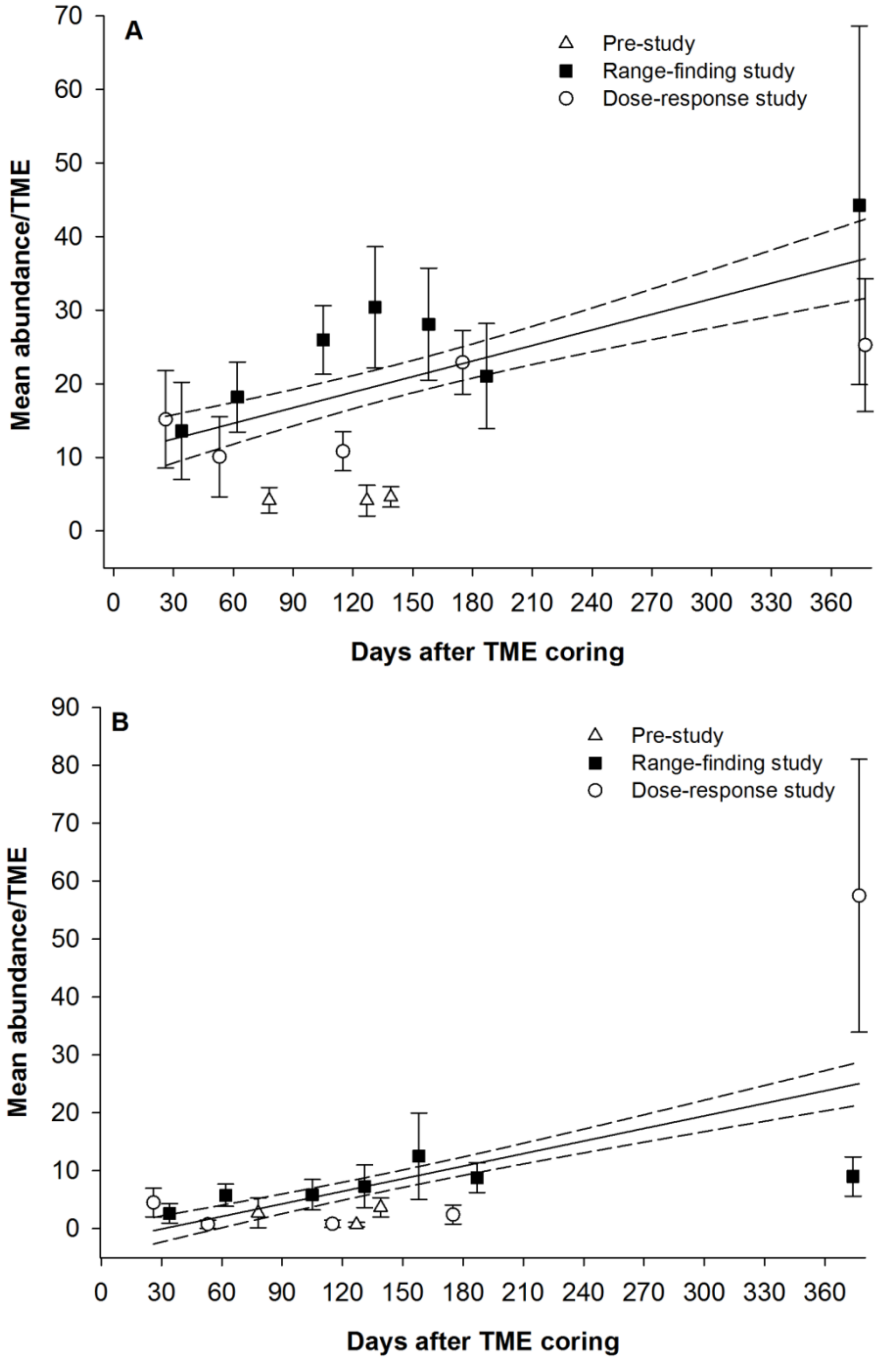


Figure V-13: Total collembolan (part A) and oribatid (part B) abundance (mean values per TME ± 95 % C.I.in case several sub-samples were taken from each of the TME) and its linear correlation (straight line) and its 90 % confidence bands (broken lines) to the days after TME coring. Linear regression analysis showed clearly that the predictive power of the time after coring for the abundance is very low (R^2 = 0.23 for oribatids and 0.12 for collembolans, respectively).

sampling period (Figure V-13). Abundance was unusual low in the pre-study, since the study was conducted in late winter/early spring immediately after ice break.

This is true for both collembolans and oribatids. This fact made a considerable contribution to the trend to increasing numbers with progressing time. During the course of the experiments, and especially over all experiments, there was no systematically decrease of total abundance

observable. The linear regression, which explains a small, but significant part of the total variance for total collembolans and oribatids, i.e. the coefficients of determination R^2 were very low for both taxa groups, illustrated the trend rather than a rule. The results even contrasted the worst-case expectation by increasing abundances. In particular, at dates one year after coring, the abundance of oribatids reached its all-time maximum. Since most of the individuals of oribatids at the last sampling date of the dose-response study were juveniles, it is stipulated for future studies to determine the age and species structure of each taxon has to be determined as deep as possible. Otherwise, the results could lead to misguiding interpretations.

V-2.1.2 Abundance of populations

As shown before, the total abundance of collembolans and oribatids was stable over a period of up to one year. Even a tendency to increasing numbers with longer test duration was observed. To understand these findings, it is necessary to gain a deeper insight into the populations that were mainly forming the overall response. Figure V-14 shows several species specific response patterns to isolation. It was not possible to distinguish clearly between effects of seasonality and the effects of isolation. The parts **A, D** and **F** of Figure V-14 show constantly decreasing abundance with continuous test duration, either indicating a negative answer to isolation or showing a significant peak of population density in spring, which was not reached yet in the following year. Individuals of the genus *Entomobrya* (part **C** of Figure V-14**)** were nearly exclusively found during the dose-response study in 2006. *S. aureus* (part **I** of Figure V-14) occurred in very low numbers in general; only at very few occasional sampling dates, it was found abundant. *P. notabilis* (part **H**) showed increasing numbers during the course of the range-finding study, whereas it was found rarely at other sampling dates. *L. cyaneus* as a dominant species (part **G** in Figure V-14**)** reached an absolute maximum of population density repeatedly in mid-summer; the species was found far less frequent in spring and autumn. *D. trispinata* (part **B)** is known to be an opportunist of high amounts of organic matter (as discussed before in chapter III-1.2.2) and showed a mass reproduction at a single sampling date. Finally, *I. viridis* (part **E**) was the only species constantly occurring during the whole study period, and during the whole vegetation period, respectively; it occurred with highly variable counts between the three different studies. The possible reasons for the patterns observed were up to more or less speculative interpretation. The density of predators (gamasid mites) was very low in the systems and generally on the meadow used as the coring site (results not shown), the regulation of population density could be suspended. It is not very likely that the meadow system at hand is highly influenced by top-down regulation, because also in field samples the density of predators was low.

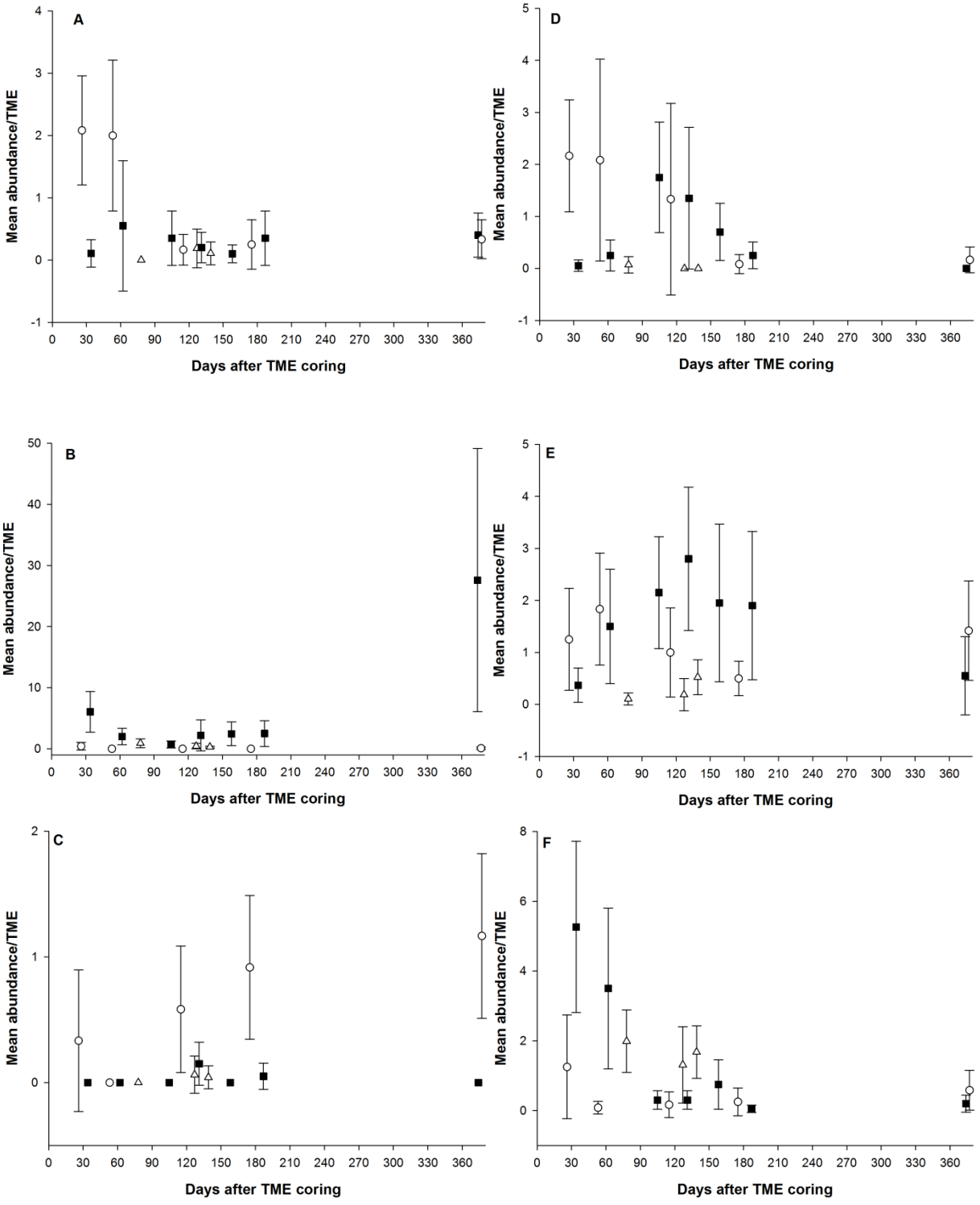


Figure V-14: Mean abundance per TME sample ± 95 % C.I. of A: *B. parvula*, B: *D. trispinata*, C: *Entomobrya spec.*, D: *I. anglicana* E: *I. viridis*, F: *I. palustris*, G: *L. cyaneus*, H: *P. notabilis*, I: *S. aureus*. Attend the different scales of y-axes. Triangles: pre-study 2005, filled squares: range-finding study 2005; circles: dose-response study 2006.

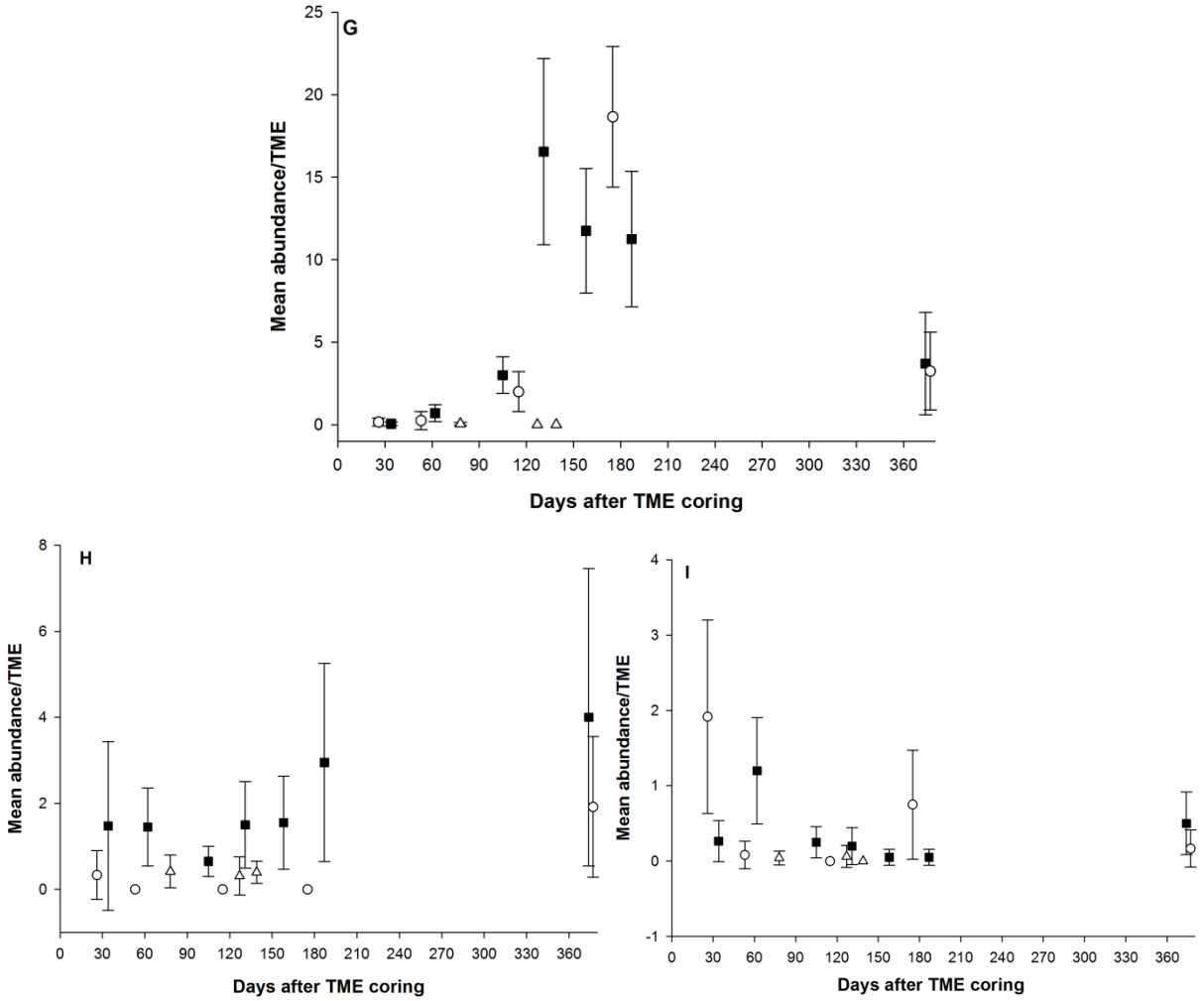


Figure V-14: continued

As indicated by Figure V-13 neither the number of collembolans nor the number of oribatid mites decreased systematically over time. There was an increase at the end of the experimental period most likely due to phenological effects. The spring temperatures were increasing rapidly in this period, providing more and more optimal reproduction conditions for the microarthropods. This result let the TME appear to be stable over time and still reacting to different environmental conditions. It is possible but not provable that the community would follow normal phenological succession. For this, in future studies complementary field samples have been taken to compare natural phenology with TME development and deduce isolation effects from those results (chapter V-4).

V-2.2 Diversity criterion

An indicator of stability besides total numbers per taxon group was the species diversity. Figure V-15, A-C show the Shannon diversity index, the evenness of the community structure and, as the simplest measure, the number of species found per TME as the mean of several

sub-samples in case more than one sample was taken from each TME (compare the sampling scheme of chapter II-1.6).

All indices were stable and constant over time and did not show any dependency of the time after the first sampling (indicated by small coefficients of determination of the regression lines). The Shannon-index, as a combination of evenness and species richness, slightly increased with increasing study duration, but the slope of the regression line was nearly zero. The same fact was observed for the other two parameters. The evenness was slightly decreas-

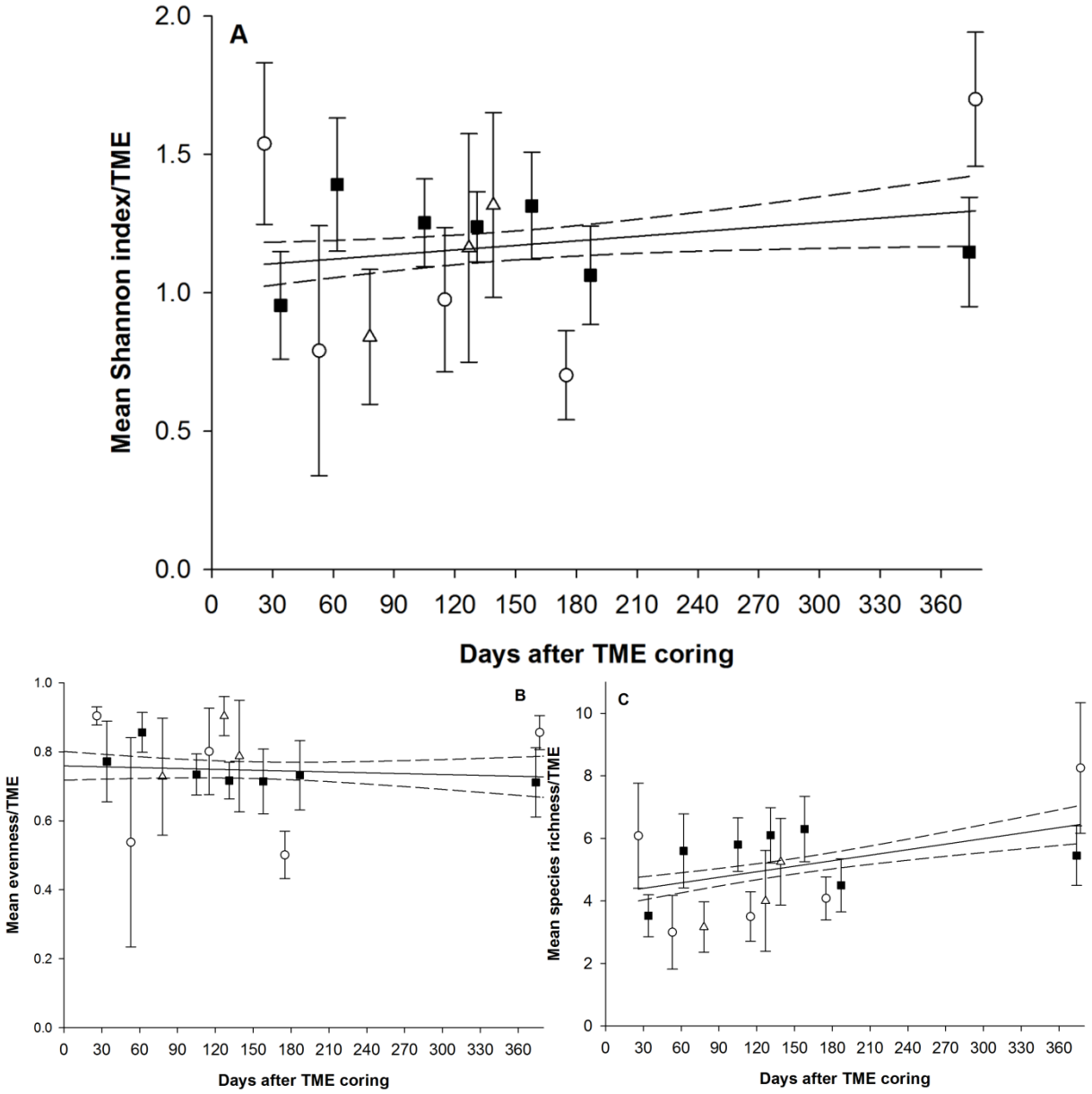


Figure V-15: Shannon-index (A), evenness (B) and species richness (C) of collembolan communities as dependent on the date after coring the TME at the field site. Shown: index of each control TME (means of sub-samples in case more than one at time was taken from a TME, compare the sampling schemes of chapter II-1.6). Coefficients of determination R^2 (A): 0.01, (B): 0.002, (C): 0.07. Triangles: pre-study 2005, filled squares: range-finding study 2005; circles: dose-response study 2006. The regression lines give the linear trend (straight) and the 90 % confidence bands (broken lines).

ing, the species richness was slightly increasing, but slopes were negligible. It can be stated that there was no systematic decline of the diversity of collembolan communities due to isolation effects. On the other hand, a systematic increase of the diversity could be postulated possibly occurring due to immigration of additional species or due to a more even distribution of the species within the collembolan community (lack of predators).

The meadow ecosystem representing the coring area was left undisturbed regarding the application of plant protection products and intense management measures for several years. It was mown regularly (approx. 2-3 times per year, personal communication Bayer CropScience). It is assumed that a very stable and relatively diverse community of soil organisms was established in TME. This was shown in later studies for enchytraeids and nematodes as well, but at this point constraint to the arthropod mesofauna. In companion with the slow reaction of soil organisms towards disturbances due to constant conditions within the soil matrix and the minor impact due to TME soil coring, it is very unlikely that soil communities would answer immediately (in terms of weeks) to the isolation. The relationship between the diversity and the stability of a community was discussed in some detail by IVES & CARPENTER 2007. They stated that there are many existing and contrasting concepts of stability in the history of ecology. Most of them mentioned in IVES & CARPENTER'S review paper were developed using terrestrial plant systems that have been more easily accessible to experimental manipulation and analysis than soil ecosystems in order to derive finally the general rules (e.g. the alternative stable state theory of SCHEFFER *et al.* 2001). In soil, the unifying principles of ecological theory, such as predator-prey relationships should also be valid, but are suspected to be less often adopted and applied (FIERER *et al.* 2009). The dynamic of the diversity measures is assumed more stable than seasonal or even faster temporal fluctuations of the abundance. Thus, the stability of the species diversity (e.g. in terms of the Shannon-index in Figure V-15) is a more reliable indicator of the stability of the test system. It has not been concluded that a lack of predators could induce changes in communities of microarthropods in the system. However, the density of mesofauna predators was very low (see numbers of gamasid mites in the appendix).

V-2.3 Similarity criterion

Indices of similarity between two complex samples, like the Bray-Curtis- index (aka Soerensen-index), compare the species composition of two communities and generate results that allow for a comparison of the similarity as a relative distance between the samples. In combination with multivariate regression methods (e.g. polar ordination) one can focus on the most relevant part of the distance matrices (compare chapter II-4.6.1 in the materials and methods section for further explanation of the methodology). Figure V-16 shows the results of a polar ordination that has used the distance matrix of the pair-wise similarity of the species composition of two samples. The data originated from the untreated controls of the three TME studies (pre-study, range-finding study, dose-response test). Arrows point to the next sampling date within each of the three independent studies. The mean similarity of collembolan communities, expressed as sample scores of the polar

Table V-4: Days after coring in the three TME studies used in Figure V-16.

Days after TME coring	Study
78	Pre
127	
139	
34	Range-finding
62	
105	
131	
158	
187	
374	
26	Dose-response
53	
115	
175	
377	

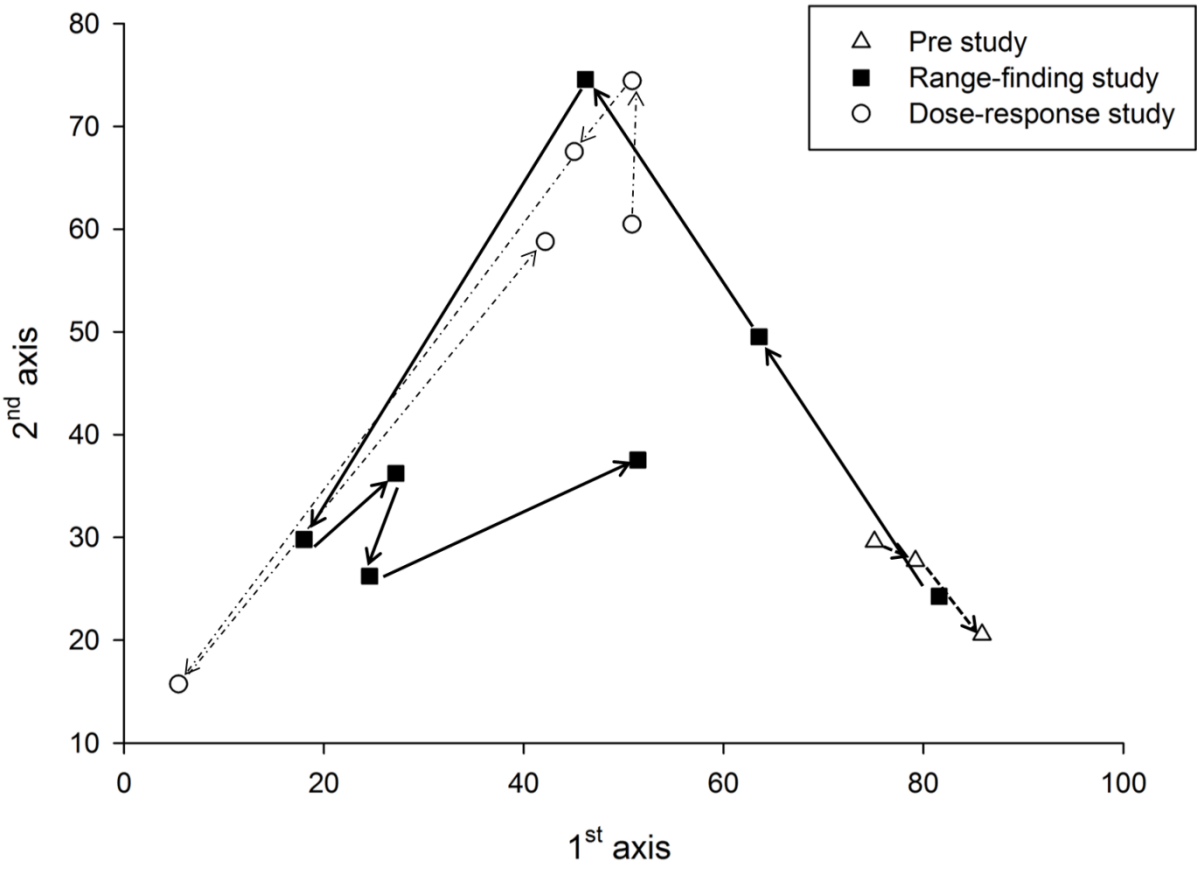


Figure V-16: Mean sample scores of the collembolan community of the first two axes of a polar ordination (similarity measure: Soerensen (Bray-Curtis), variance regression, projection geometry = euclidean). Variance explained by 1st axis: 21.4%, 2nd axis 8.9%.

ordination, and while focusing on the axes of the distance matrix that explain the largest portion of variance in the dataset, was not strictly one-directional. It followed a seasonal trend, caused by alternating dominance of specific species during the course of the year (for the raw data refer to the tabular appendix). Since the first, horizontal axis is more important than the second axis as defined by its explanatory power of 21.4 % versus 8.9 % for the second axis, the main changes of the communities have to be followed-up on that axis. During the relatively short study period of the TME pre-test of 79 days in early spring, the changes in species composition were very small. The same evidence applied for the results of the range-finding study, showing only slight deviations on axis 1. However, during the course of the dose-response study, similarity changed clearly. It was striking that the relative shifts of the sample scores could be described as an oscillation along the x-axis. One year after coring the similarity of the sampled communities of collembolans was very high, indicated by a position very close to the origin one year before (Figure V-16). The results of the multivariate analysis showed seasonal changes in community composition but no systematic shifts that could be induced by the sub-sampling or the duration of the isolation of the communities.

V-2.4 Impacts of soil removal

A partial Redundancy Analysis was applied to the available control data (for explanation of the method see II-4.6.4) to calculate the part of the total variation that can be attached to the influence of soil removal or to temporal variation during the study. It was expected that the variation between samples be mainly due to natural variation rather than variation due to experimental issues. By applying partial RDA to the species data, it was tested if the removal of soil and the duration of the study exert influence on the community composition in TME.

As Table V-5 shows, all the combinations except of the soil removal in the pre-study have

Table V-5: Results of a partial RDA of the collembolan data of the pre-study 2005, the range-finding study 2005 and the dose-response study 2006. The percentage of variance (of species data) explained by the factors 'days after start of the experiment' (DAS) and 'percentage soil removal' were obtained by consulting the canonical eigenvalues of the analysis. The significance of the first canonical axis was tested by applying Monte-Carlo permutation tests (499 permutations under full model) on the whole dataset, followed by an F-test-like procedure to calculate a p-value. n.s.: non-significant, if not indicated p was << than 5 %.

Study	All	Pre	Range-finding	Dose-response
Factors	% variance explained			
■ DAS	3.9	10.3	8.7	9.8
■ DAS+ percentage soil removal	11.9	14.5	17.2	27.6
■ Percentage soil removal	6.1	2.1$^{n.s.}$	11.4	12.5

significant impact on the species composition. Regarding the high variability of the species dataset, it can be assumed that both factors 'time' and 'soil removal, partly inter-correlated, have the same influence on the communities of collembolans. About 10 % of the total variation accounted for each of the factors, which is considered acceptable. In particular, the results of the pre-study indicated that there was little influence of the soil removal on communities of collembolans. Thus, it was decided prior the range-finding study and the dose-response study that a sequential sampling design would be applicable. As the experiments over the period of one year have shown, this assumption was confirmed.

V-2.5 Concluding 'temporal stability'

Two possible hypotheses could mainly describe the empirical findings of shifting communities or systematically decreasing abundances in the TME studies. One possible reason could be the limitation of the communities to a relatively small area in a TME-system: ***fragmentation***. The other hypothesis is that a long period of ***isolation*** would affect the composition of the soil communities. SCHNEIDER *et al.* (2007) discussed six possible mechanisms that could be responsible for the detrimental effects of habitat fragmentation and isolation with respect to experiments in model ecosystems. Both effects were likely to act in TME, because they were isolated from the site of their origin and from the surrounding areas at the experimental site in Aachen, and they were fragmented to an increasing degree by the destructive and sequential sub-sampling.

The mechanisms were described as follows, the squared brackets list the relevant indicators used in this thesis influenced by the mechanisms.

- Reduced number of microhabitats [abundance, diversity, similarity]
- Reduced immigration rate (species with low mobility suffer less from isolation in general) [abundance, diversity, similarity]
- Demographic stochasticity reduces rare species more than frequent ones [diversity, similarity]
- Edge effects and increased variation in biotic and abiotic factors increase the risk of extinction [abundance, diversity, similarity]
- Top trophic levels may suffer from extinction, food chain length may vary [not necessarily detected]
- Generalists may suffer less than specialists due to flexible life history traits [diversity, similarity]

It is principally not possible, by the data at hand, to distinguish clearly between both hypothe-

ses, but answering both positively would presume that the critical minimum area for stable populations of most collembolan and oribatid species would have been fallen below its specific limit. In fact, only 10 % of the total variance could be explained by the soil removal during the pre-study; the regression model (Table V-5) did not statistically significantly explain this portion for the pre-study but for the range-finding study and the dose-response study. The basic dilemma becomes obvious, while considering the fact that on the other hand the confirmation of an 'isolation theory' would be a welcome proof of the prevention of immigration into the TME. This is also assumed essential for the integrity of the test systems and the dilemma illustrates the possible inconsistencies that appear during the development of a complex test system. A further reason for a decreasing integrity of the test systems, herein indicated by the four indicators 'abundance', 'diversity', 'similarity' and 'impact of soil removal', could be found in a decreasing available area of each TME because of the destructive subsampling. The results of the polar ordination did not show clear, one-directional changes of similarity. If TME would disintegrate with proceeding time after coring, the similarity criterion would hypothesise a directed trend towards increasing dissimilarity. The endpoint similarity reflects directly the identity of the communities under concern, whereas abundance and diversity measures abstract from the species level to higher levels of information. It can be stated that the observed changes were seasonal, and the deducible trends could be circular. The integrity of the communities and thus the stability of TME systems as wholes were affected neither by the sequential sampling that reduced the living space nor by the isolation of the communities.

V-3 Limitations of effect detection in terrestrial model ecosystems

The variability of organism related ecotoxicological endpoints in soil is generally high (compare introducing section I-4.1 and the results of this chapter). Consequently, the detection of effects of plant protection products in particular and of basic patterns of distribution of animals in general is hindered. In the following, the prospective power, expressed as the MDD between treatments and control groups, is assessed. From the analysis of the TME data, recommendations of future experiments and the interpretation of readily available experiments are inferred. The current aquatic risk assessment scheme on the level of the European Union is based on no observed effect concentrations (NOEC) or lethal concentrations (LC_x) (EURO-

PEAN COMMISSION 2002b) to derive safe concentrations from ecotoxicological first and higher-tier test systems. These parameters are usually estimated using statistical methods that call preconditions of the data (e.g. normal distribution and/or homoscedasticity) in addition to uncertainty or safety factors that account for the degree of uncertainty while extrapolating from an ecotoxicological study to the real world. When thinking about an appropriate safety factor, a great number of uncertainty components have to be addressed. The 'Guidance Document on Aquatic Ecotoxicology' (EUROPEAN COMMISSION 2002b) stipulates to take intra- and inter species and laboratory variation into consideration. In addition, uncertainties evolving from the extrapolation from short to long term testing and from single species in the laboratory to field ecosystems have to be taken into account. In the context of the elaborations at hand, a TME is considered to cover a huge number of different sensitivities including very sensitive species, furthermore it is assumed to be very near the field situation (compare chapter V-3.3). Thus relatively small safety factors would be recommendable for a refined risk assessment after the standard tests with soil organisms with high safety factors (5-10 for soil and terrestrial assessments (EUROPEAN COMMISSION 2002a). The assessment of TME data is currently leaned against the aquatic guidance on the interpretation of mesocosm data i.e. focused on the time to recovery of the communities to the control level and the derivation of rather NOEC than EC_x as effect measures (OECD 2006). The following chapter investigates the detectability of effects on soil taxa, populations and communities as observed in TME, presumed that the statistical standard methodology is applied to the TME available. It is discussed if alternative testing methods could improve the risk assessment more than merely enhancing the scientific soundness of the analyses (which is not a bad idea, indeed). In three sections, firstly the prerequisites of the data available, then secondly the resulting detection limits and lastly possible improvements of a TME dataset are exposed in great detail. The MDD and the necessary number of replicates to reach the desired level of certainty of the experimental outcome and the power of the test design are shown. The baseline variation in typical datasets that were derived from TME studies is described as being the first prerequisite of sound test systems and as the main reason for hampering the statistical discrimination of toxic treatments from controls. The variability in TME is then compared to the variability of the corresponding field site. Furthermore, the benefits from optimising the methodology in the course of consecutive studies are demonstrated. Mainly pooling of samples and the effects of data transformations were tested. The possibilities to optimise the coring strategy in the planning process of a TME study have been already discussed in the chapter V-1. One can either homogenize the experimental conditions one can increase the number of replicates, whose

effectiveness is elucidated in the following section V-3.2. Another option is to choose alternative statistical methods (V-3.3) or data transformations that is a controversial issue and subjected to discussion. Most of the measures in section V-3.2 should lead to lower the variability or to sharpen the detection limits and lead thus to a better effect detection. In the best case, all opportunities will be used to improve the result of an ecotoxicological or ecological test. It is very often asked if the TME provide a sufficient number of organisms to detect effects. It is assumed as a widespread rule of thumb that higher abundances would lead principally to a lower variability. This paradigm is questioned in section V-3.2.2. In conclusion, suggestions for future improvements in statistical testing and risk assessments are offered in the 'concluding remarks' (V-3.3) to this chapter.

V-3.1 Baseline variation in the field and in field-born TME

The coefficient of variation (CoV) is a widely-used measure of variation in cases where measurements of sampling dates or taxa groups with differing means and standard deviations are intended to be compared (KÖHLER *et al.* 2002). For standard ecotoxicity testing a variation less than 20-10 % is often desired, e.g. the guideline for testing the toxicity for soil microor-

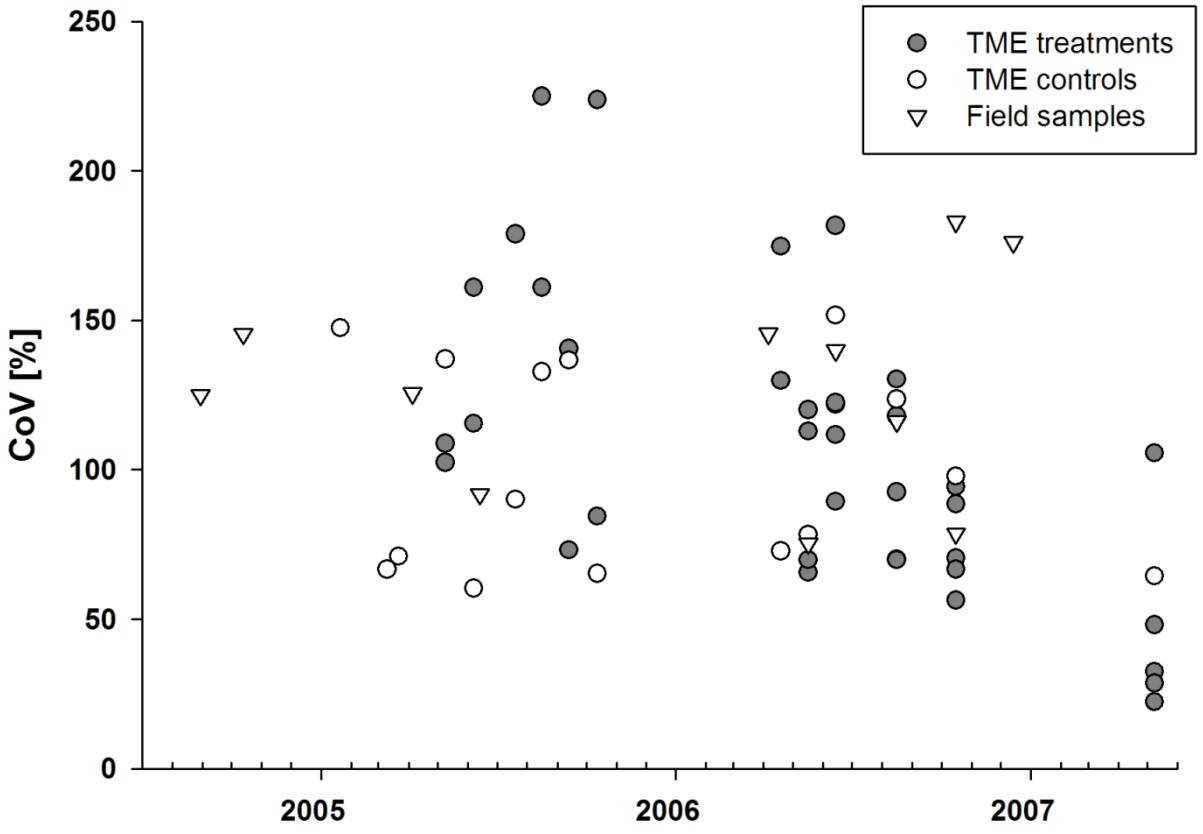


Figure V-17: Coefficients of variation of TME and field samples of *oribatid mites* in the course of approx. 2.5 years. For the description of the experiments, see chapter II-1.6. Treatments varied between 0.032 mg a.i./kg soil dry weight and 100 mg a.i./kg soil dry weight (spray application on short-cut grass cover). Untransformed data.

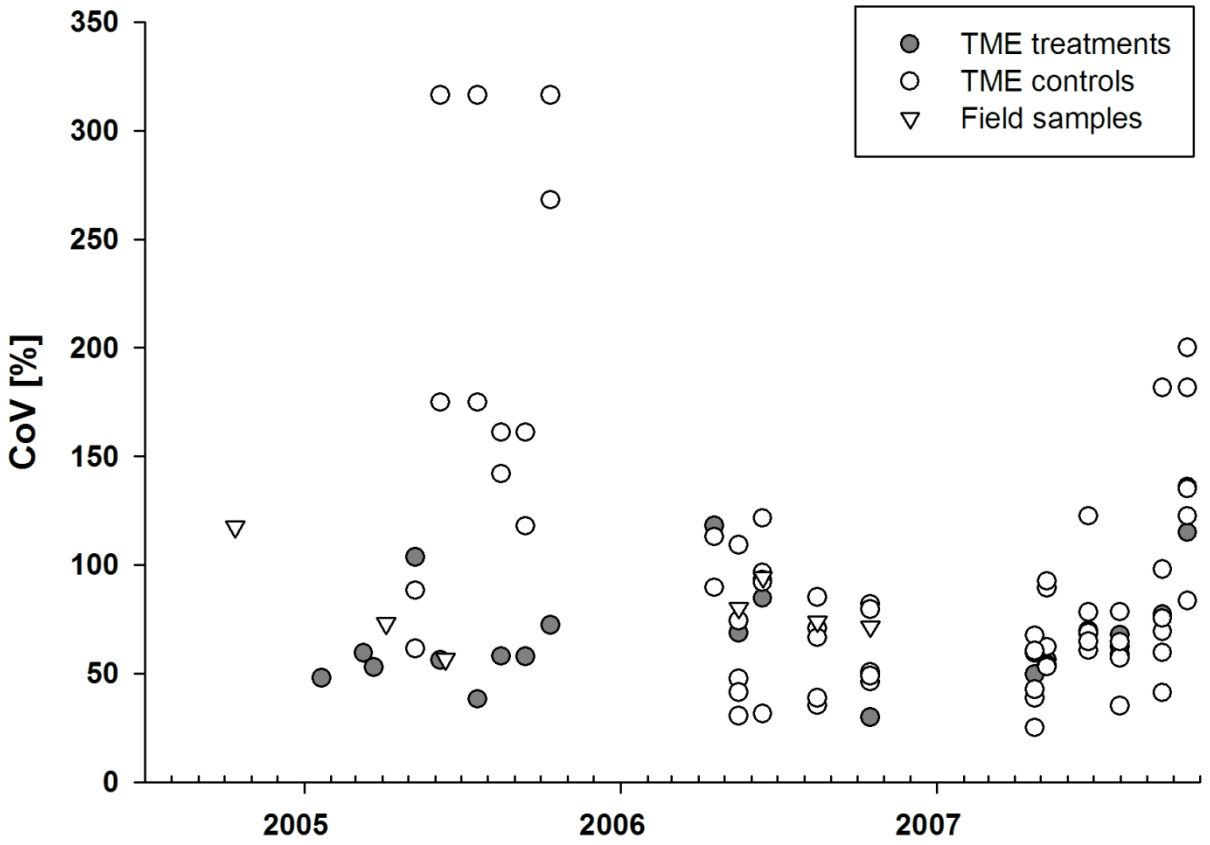


Figure V-18: Coefficients of variation of TME and field samples of *collembolans* in the course of approx. 2.5 years. For the description of the experiments, see chapter II-1.6. Treatments varied between 0.032 mg a.i. lindane/kg soil dry weight and 100 mg a.i. lindane/kg soil dry weight and high amounts of an undisclosed test substance (spray application on short-cut grass cover). Untransformed data.

ganisms by the ability to turn organic compounds into carbon dioxide stipulates variation between replicate control samples of not more than 15 % (OECD 2000).

The variation in *oribatid* density in controls, field samples and the TME treatments was between 20 % and 230 % CoV, however it is considered as quite high (Figure V-17). For the field samples, the mean variation was higher than in TME treatments and untreated TME. The highest CoV were measured within the treated TME replicate groups. The high variation was mainly due to the influence of highly affected treatment groups with many zero values. This was particularly the case for the 2005 range-finding study. On average, *collembolans* CoV were slightly lower compared to the average variation in oribatid numbers (Figure V-18). This group occurred in relatively low numbers (refer to the chapters III-1.2, IV-1.1). Pronounced effects on the abundance of collembolans in most treatment groups at many sampling dates caused extreme outliers of the variation observed. The treatment data in the dose-response study seemed to be less variable. That is mainly due to the pronounced effects that were caused by the two highest concentrations in the dose-response study (refer to chapter IV-1.1).

For the other two groups of organisms that were investigated in the TME studies at hand, *enchytraeids* and *nematodes*, less data were available. Because no field samples were taken, the relative amount of variation was not known for the field, (see study plan Table II-1). For the group of enchytraeids, the variation ranged from 20 to 100% CoV (Figure V-19). There was no indication that the variation was much higher for within the treated TME compared to controls as a rule. Nematodes were on average the least variable animal group, regarding the spatial scale of the investigations this finding has to be discussed. In particular, after pooling of 2 sub-samples in TME-2007, the variation was constantly between 20 and 80 % CoV. Indices of variation set the general conditions for subsequent statistical testing. They used to be highly variable in time by themselves and depended partially on the effect of a toxic treatment. For example, in the studies using the model substance lindane, the variation between replicates increased clearly with increasing treatment level (see an example given by Figure V-21:). The description of the expectable variation between replicates in TME sets the frame for further data analyses and assessments. In the following chapters, implications for the detection of

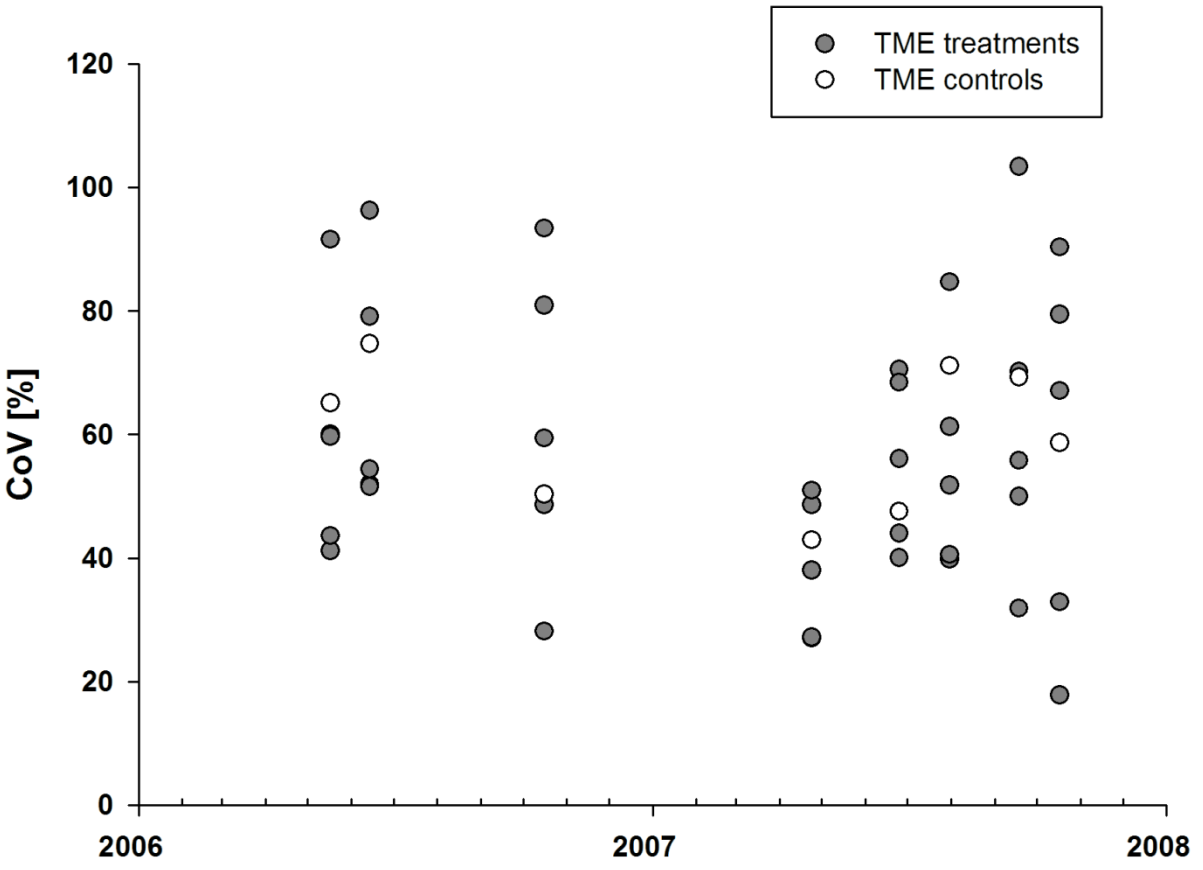


Figure V-19: Coefficients of variation of TME and field samples of *enchytraeids* in the course of approx. 2 years. For the description of the experiments, see chapter II-1.6. Treatments varied between 0.032 mg a.i. lindane/kg soil dry weight and 100 mg a.i. lindane/kg soil dry weight and high amounts of an undisclosed test substance (spray application on short-cut grass cover). Untransformed data.

effects in a regulatory context will be discussed.

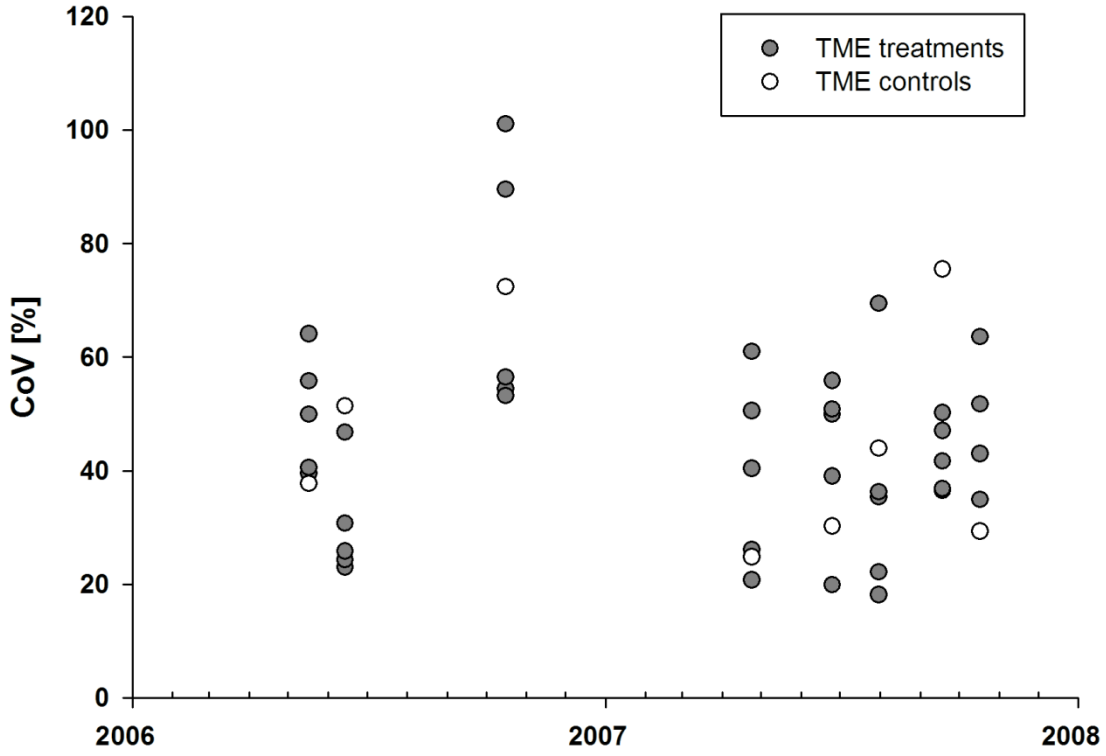


Figure V-20: Coefficients of variation of TME and field samples of *nematodes* in the course of approx. 2 years. For the description of the experiments, see chapter II-1.6. Treatments varied between 0.032 mg a.i. lindane/kg soil dry weight and 100 mg a.i. lindane/kg soil dry weight and high amounts of an undisclosed test substance (spray application on short-cut grass cover). Untransformed data.

V-3.2 Narrowing the limits of effect detection by prospective power analysis

The mere variation observed as described above as 'baseline variation' is an appropriate estimate of the actual state of a system for purposes of study planning, but in the field of ecotoxicology it has to be set in the context of a control vs. treatment comparison. This can be done by introducing yet another parameter, the Minimum Detectable Difference (MDD, method described in chapter V-3.2.5). This parameter depends on the statistics that will finally be applied to the data. The most common test in comparing treatment and untreated controls in an ecotoxicological dose-response test design is the multiple t-test after Williams, because of its high sensitivity to detect differences and the assumption of monotonous treatment related response function. A similar test is the multiple t-test after Dunnett. Usually, some basic conditions are widely agreed: the a-priori power to detect effects (beta-error) is set to 80 %; the probability of falsely assuming differences is set to be 5 % (alpha-error). For study-planning

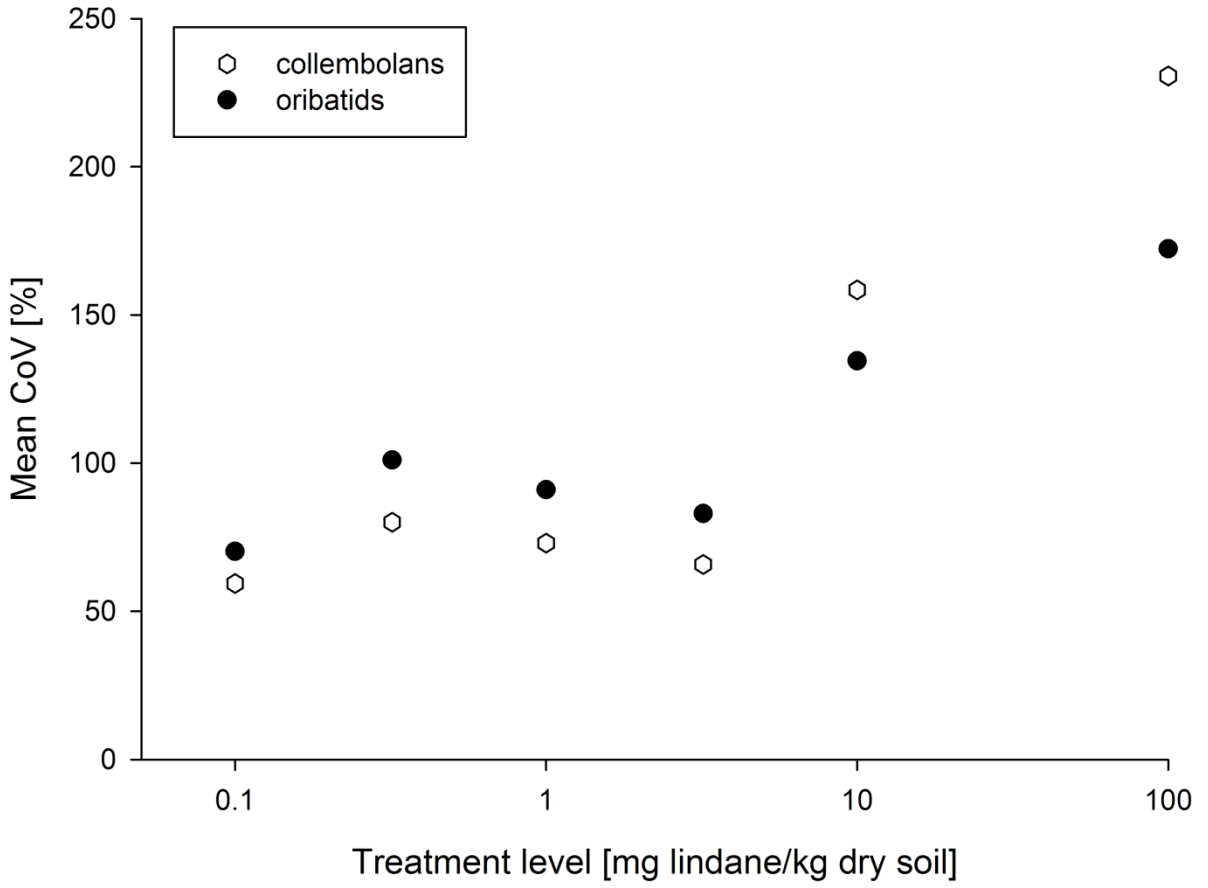


Figure V-21: Relation between the variation of replicates of different treatment levels of the insecticide lindane and the respective treatment level. Data taken from the dose-response study 2006. CoV of untransformed counts. Filled circles: collembolans; empty diamonds: oribatids.

purposes, it is necessary to know something about the sample size that is sufficient for the special goals of the study at hand and about the range of effect sizes that can be detectable under certain circumstances (analysed in chapter V-3.2.1). The previous chapter has shown the prevailing circumstances that define the potential and the limits of the TME methodology as it was used during the consecutive TME studies subjected by this thesis. Several actions could be conceived to be taken in order to reduce the amount of variability that was hampering the detection of toxic effects of pesticides towards soil organism communities. Some of the most promising approaches are listed below. The following sections aim to look further into the details of the meliorating impact of different measures.

The *number of replicates* could be extended, which is always a good strategy to improve the explanatory power of a study in a statistical regard. The theoretical effects of a higher number of replicates are demonstrated in chapter V-3.2.

The sampling effort could be intensified. This resembles a similar measure as to increase the number of TME replicates. A higher mean number of animals per replicate could lead to lower variance in the dataset (compare chapter V-3.2.2). In follow-up experiments, two sub-

samples of each TME were pooled for nematodes and microarthropods (results of the TME-2007 experiment, effects can be seen in chapter V-3.2.3). The pooling could be extended until all possible samples in a TME would be sampled at one time. For our studies, it was considered essential not to abandon the sequential sampling design.

In the following sections, it is firstly investigated if there was a clear trend in the data at hand that showing a close relationship between the mean number of a taxa group or a population and the respective variation to decide about further steps towards a higher sampling effort, which directly results in higher costs of the total study. The relation is shown by some correlation analyses in chapter V-3.2.2 'Relationship between mean and variation'. The theoretical effect of pooling two or several sub-samples is demonstrated in chapter V-3.2.3. The baseline variation can be minimised by building as homogeneous experimental units as possible. The implication of the natural patterns of distribution of soil organisms for the coring of TME soil cores is addressed by section V-1.5 Best-fit coring strategy that deals with the problem of small- and large scaled gradients on the coring site and makes recommendations on how to avoid excess variation at the very first stage on a TME experiment. The reader may refer to this section of the thesis. The baseline variation could be also minimised by improving the methodology whilst sources of variation in the course of sample preparation can be eliminated. In the course of the research and development projects, many endeavours have been made to achieve improvements regarding the homogeneity of organism counts by e.g. standardising the sample processing. The effects of this strategy are demonstrated by contrasting the results of different consecutive TME-studies (section V-3.2.3). The combined effect of a superior TME soil coring strategy and a way of sample processing towards standardisation could be demonstrated in this chapter. The data pool could be 'tuned' by transforming procedures. A common transformation takes the logarithm of the counts in addition of a constant factor to avoid zero values.

V-3.2.1 Number of replicates

Figure V-22 represents a realistic estimate of the necessary number of replicates, based on a multiple comparison test after Dunnett with thresholds for the first order error rate $\alpha = 0.05$ and the second order error rate $\beta = 0.2$. In a dose-response design the number of replicates or the control group should be as twice as large as for the treatment groups. In Figure V-22 the number of replicates for the control group is given. Here, a study design of five groups and one control was simulated. The total number of TME in a study with the respective design could be calculated by

Total number of TME = Number of control replicates + 5 x (number of control replicates / 2).

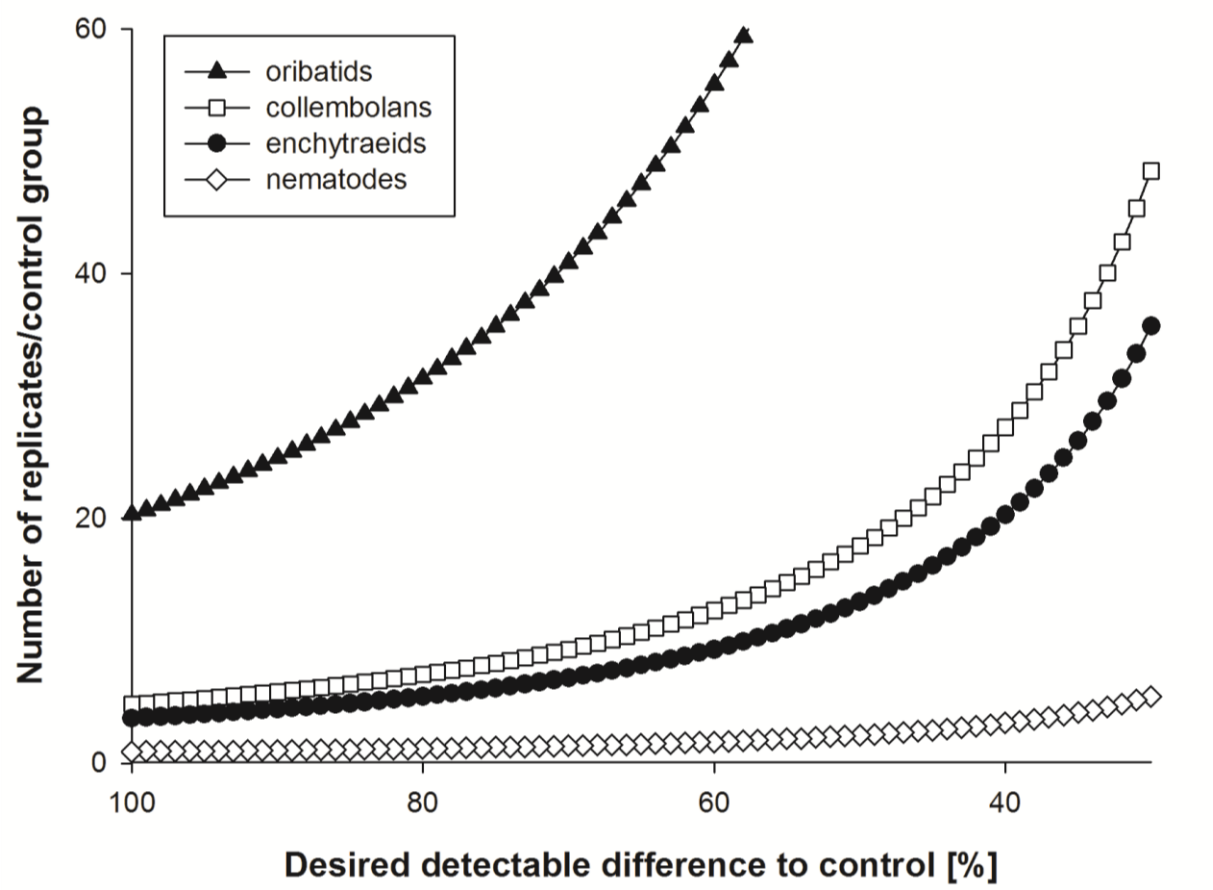


Figure V-22: Desired difference and number of sufficient replicates based on mean CoV that were observed in the TME dose-response study 2006. Data of total collembolan abundance. Test-statistics: multiple comparisons with a control, Dunnett's multiple t-Test.

The description of the statistical method is given in chapter II-4.3.6. It is considered difficult to increase the power of a dose-response design by increasing the number of replicates while searching for significant differences in order to find a LOEC/NOEC.

As for all other analyses of variance, it is obvious that for the group of oribatids the largest number of replicates would be necessary. In the current compromise test design (between dose-response and range-finding study) it will be impossible to detect effects on oribatid mites on average. If there is the 'optimal' date with lowest amount of variance in total abundance of the taxon, the system can detect very huge effects that are unambiguous even without statistical testing. The replicate number for enchytraeids and collembolans is nearly identical because coincidentally mean variation is the same for both groups.

V-3.2.2 Relationship between mean and variation

The 'law of large numbers' states in general: a sample mean converges to the true mean of the parameter distribution with increasing sample size (described in many common textbooks as in KÖHLER *et al.* 2002). This rule is often mixed and expanded to the dogma that a collection of large parameter values should vary less than one of small numbers with the same sample

number. For the necessities of ecological research, this means that it should be aimed at samples with high abundances in order to reach lower limits of detection. It can be questioned if it is possible by expanding the sampling effort to gain substantially higher numbers with less variation and thus enable to detect more subtle effects of a toxic compound on species counts or at higher taxonomical levels.

Furthermore, Figure V-23 clearly shows for the groups of collembolans, oribatids, enchytraeids and nematodes that only a slight decrease of the variability could be expected by encountering higher abundances. The statistical analysis of the linear regression showed that for collembolans, enchytraeids and oribatids the dependent variable 'CoV' could be significantly predicted by the independent variable 'mean'. Only for the group of nematodes, this finding did not apply. However, a very small proportion between 0.2 and 22 % of the variability of CoV could be assigned to the increase in abundance. Admittedly, the analysis of the data set by linear regression appears not to be adequate regarding the distribution of counts that resembles most a Poisson-distribution with many zero values.

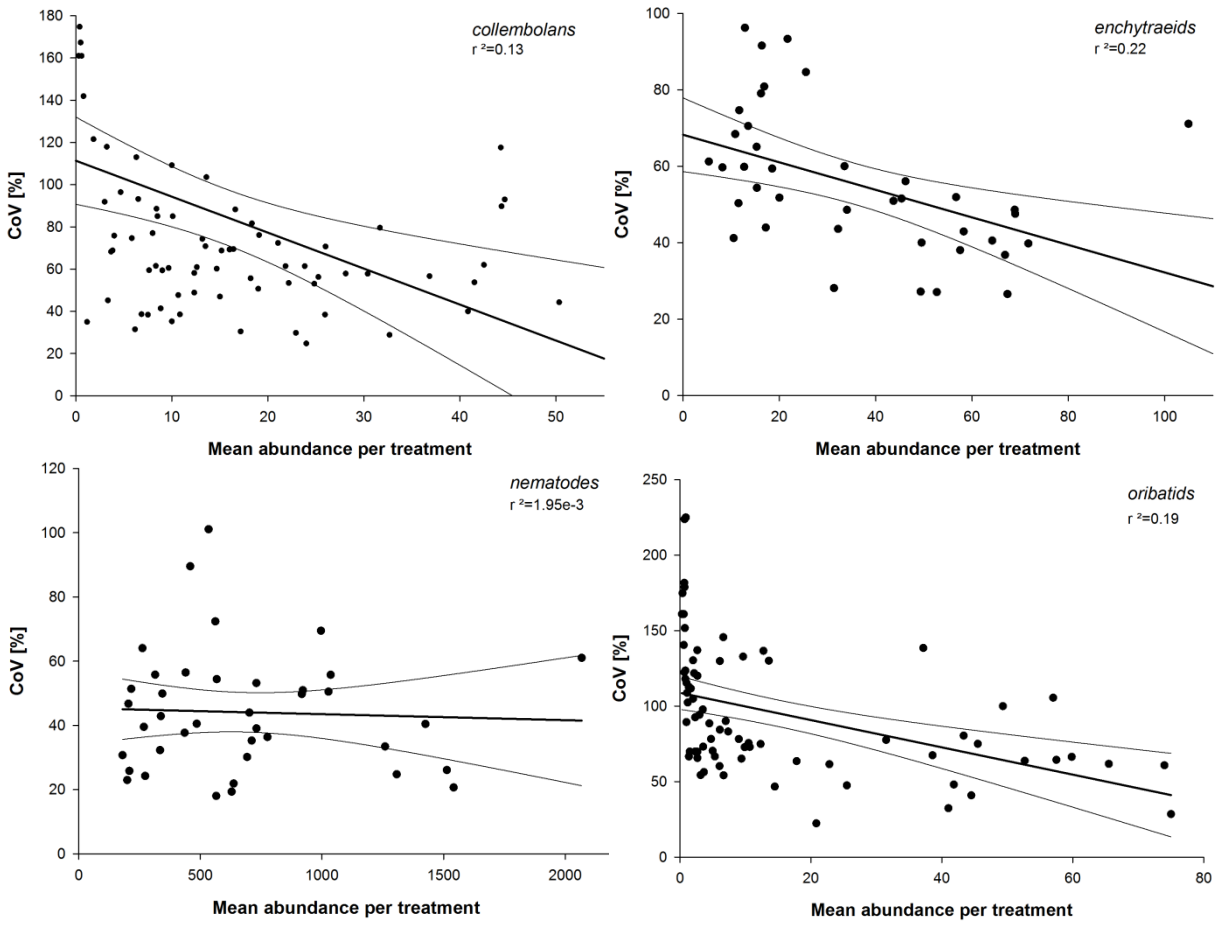


Figure V-23: The relation between CoV and the mean abundance supplemented with a simple linear regression to illustrate the general trend. The R-squared 'coefficient of determination' gives a goodness-of-fit measure for the linear regression.

V-3.2.3 Improvement through standardisation

Since the year 2005 has marked the starting point of our TME studies, it has also been the initial for the development and improvement of the whole methodology. This comprises e.g. the extraction of soil organisms, the sophistication of the study design and the experiences and the qualification of the co-workers. The following chapter is intended to show an abstract 'sum of improvements' by a comparison of the CoV over the years, including the latest dose-response study of the year 2007. The CoV of the figures below were calculated regardless of referring to control or treatment samples. The boxplots are aimed to depict merely the overall variation within the consecutive studies.

For the interpretation of the figures Figure V-24, Figure V-25, Figure V-26, it has to be considered that the boxplots of the pre-study 2005 show distributions of CoV values that were calculated separately for both the full- and the partially sampled TME, using the averaged total abundance for each TME. This approach results in very low values, because up to seven samples were pooled prior the analysis (refer to chapter II-1.1 for a description of the pre-

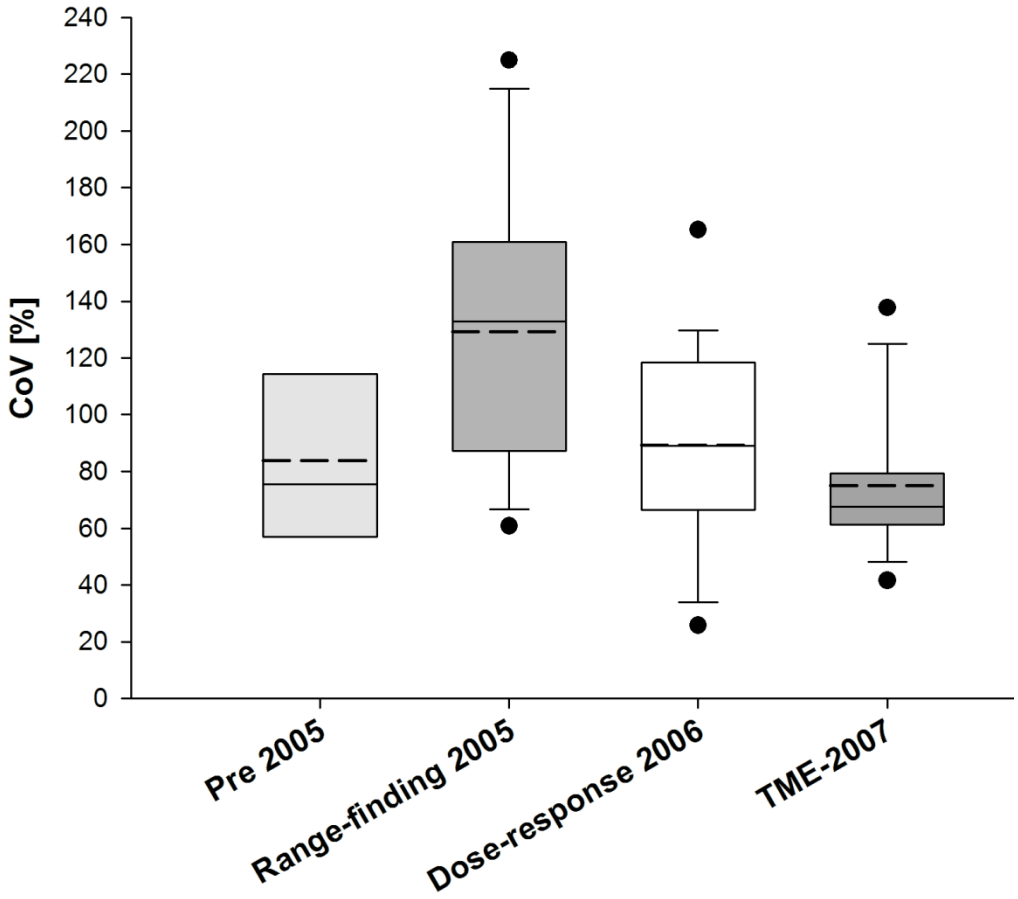


Figure V-24: Boxplot distribution of the coefficients of variation for the *total oribatid abundance* in TME samples (all treatments as separate CoV). Dashed line: Mean of distribution, solid line: median of distribution, whiskers: 10/90 % percentiles, dots: 5/95% percentiles outliers, box boundaries: 25/75 % percentiles. Untransformed data.

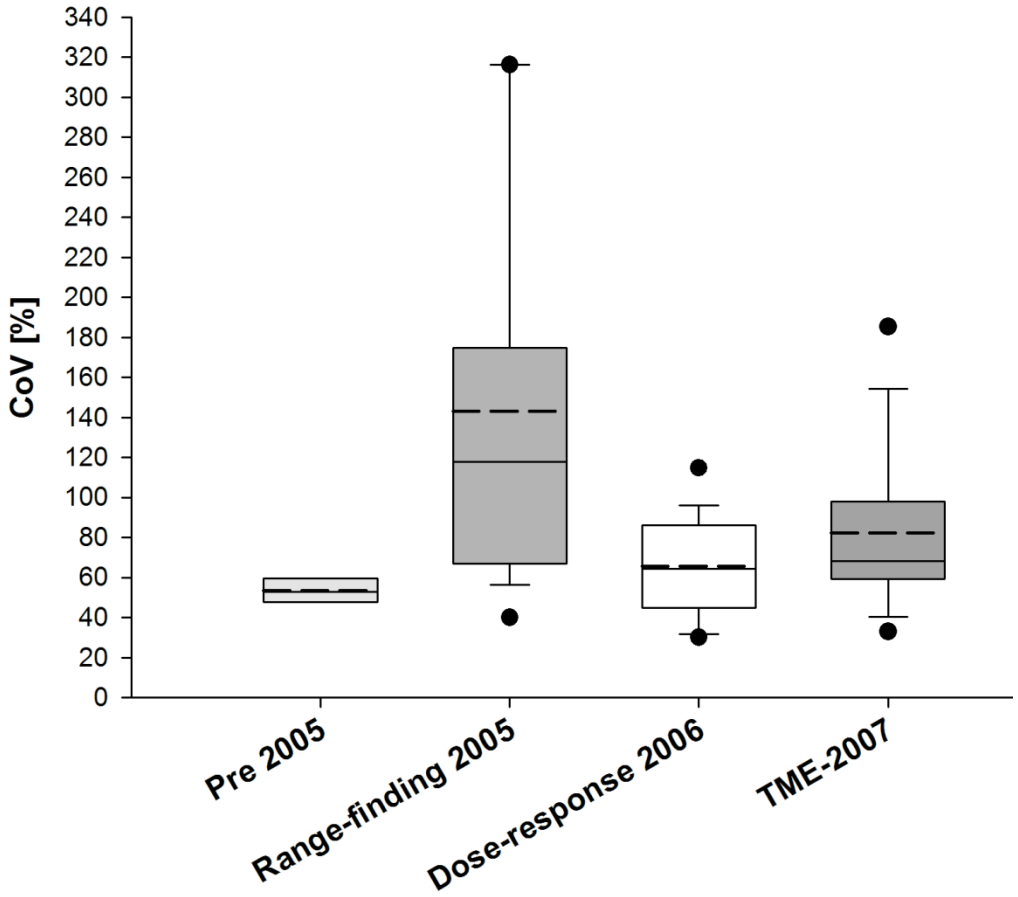


Figure V-25: Boxplot distribution of the coefficients of variation for the *total collembolan abundance* in TME samples (all treatments as separate CoV). Dashed line: Mean of distribution, solid line: median of distribution, whiskers: 10/90 % percentiles, dots: 5/95% percentiles outliers, box boundaries: 25/75 % percentiles. Untransformed data.

study design). This result illustrated the potential for improvements by fully sampling the TME at a certain sampling date and will be discussed as a preferential way to improve the results of a TME study ('pooling of subsamples'). The sampling dates are not covering complete growing seasons.

The March samples of e.g. the pre-study were marked by very low abundances, while in other studies the first samples have been taken henceforward May. The general patterns for oribatids and collembolans were similar, but the mean CoV was considerably higher for oribatids, as also shown in chapter V-3.1. The variation in both groups was largest during the range-finding study, in which extreme values in highly affected treatments occurred. This led to a widespread range of coefficients of variance. For the group of microarthropods, methodological improvements in the course of the studies exhibited effects.

In the dose-response test 2006, the TME-coring and the extraction methods (data of the experiments are not part of this thesis) were optimized according to the findings described in chapter V-1.5. In the TME-2007 study, the TME had been enlarged to a diameter of 465 mm and two sub-samples of soil microarthropods per TME have been pooled. This led to the lowest

variation observed in the course of the TME studies at hand (data not shown). Generally, in the course of the development of the TME method, the mean coefficients of variance of both collembolans and oribatids decreased.

In addition, the range of the percentiles (25 and 75 %, 10 and 90 % percentiles) was clearly lowered. For enchytraeids and nematodes, solely data of the dose-response study 2006 and the TME-2007 study were available (Figure V-26) because the two organism groups were not sampled in the earlier studies. The pooling of two sub-samples in 2007 was applied on nematodes but not for enchytraeids. Improvements for this group most probably base on the experience of the processors or a homogenous soil conditions.

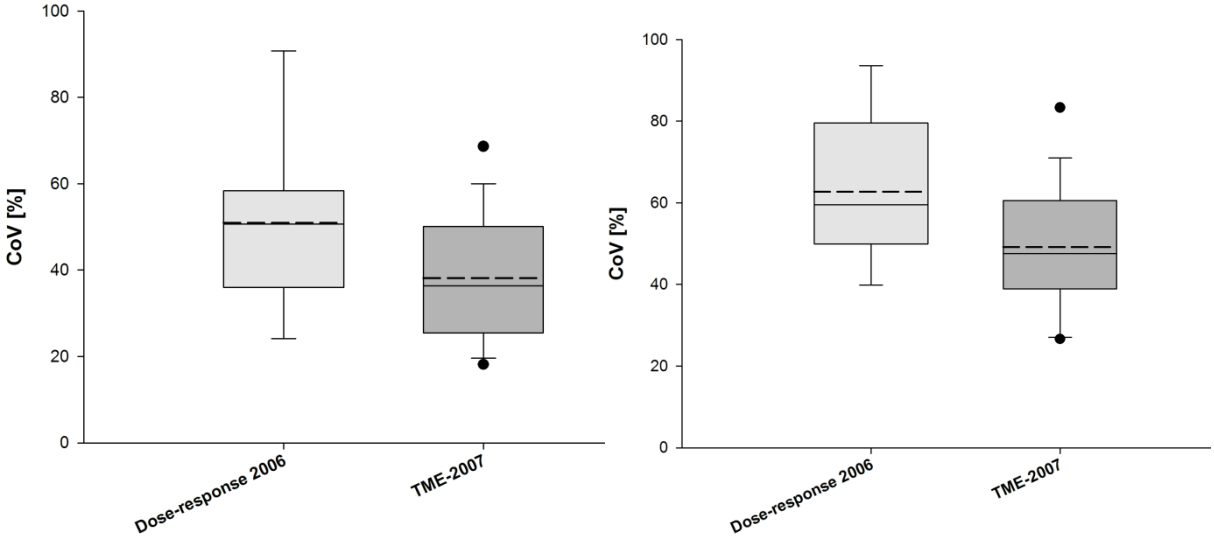


Figure V-26: Boxplot distribution of the coefficients of variation for the *total nematodes* (left) *and enchytraeids abundance* (right) in TME samples (all treatments as separate CoV). Dashed line: Mean of distribution, solid line: median of distribution, whiskers: 10/90 % percentiles, dots: 5/95% percentiles outliers, box boundaries: 25/75 % percentiles. Untransformed data.

V-3.2.4 The pooling of sub-samples

Even though the attention was not focused on the comparison between intra- and inter-TME variation (as aimed by the pre-study of the year 2005, overview given by Table II-1, data not shown), it could be seen that roughly half of the total variance of a 'fully-sampled' TME-design as employed in the pre-study could be ascribed to the intra-TME variability. This leads to the assumption that the total variance in the dataset that finally hampers the effect detection limits could be minimised by taking more than one sub-sample out of each experimental unit. To achieve this goal without sacrificing the concept of a sequential sampling and the follow-up of each of the TME-replicates over time, a complete change of the construction of a TME would be necessary. This means a considerable investment and should be based on a theoretical cost-benefit analysis. For this purpose, an artificial dataset was build, by re-arranging the

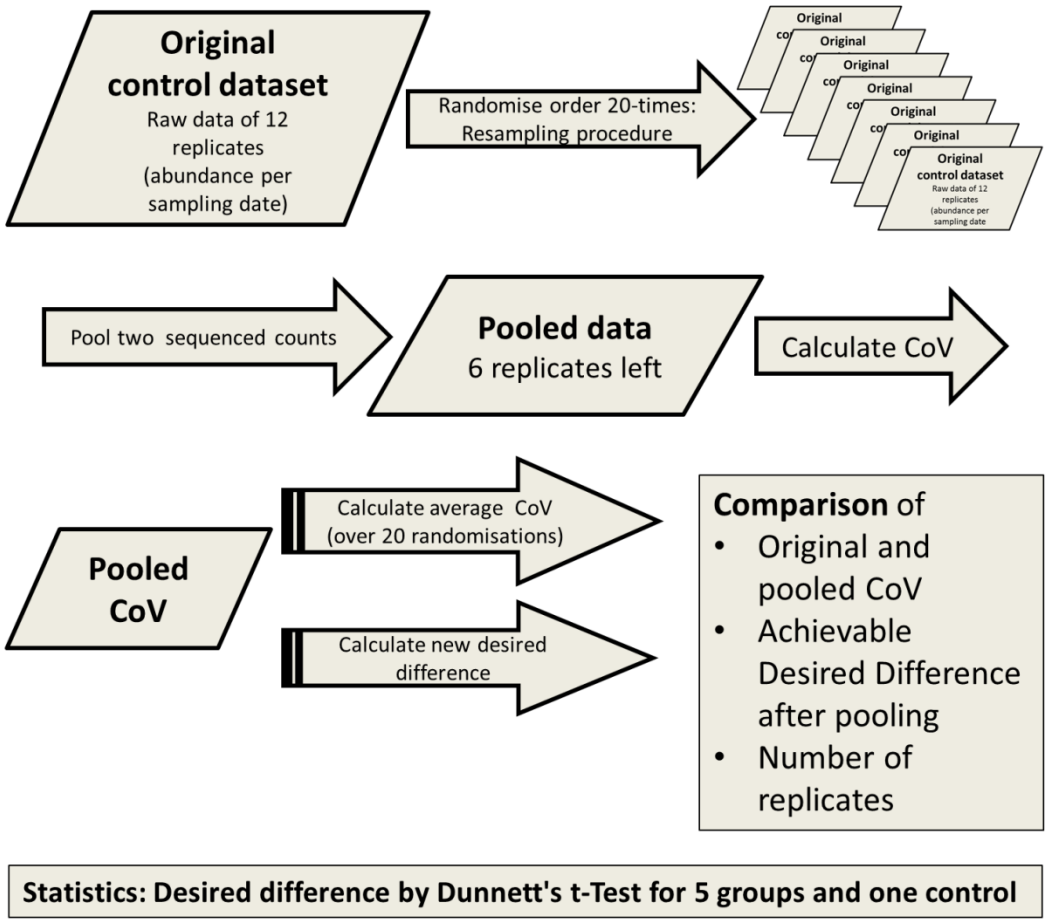


Figure V-27: Flow-chart of the data resampling procedures for the simulation of the pooling of sub-samples taken from single TME real data. The examples were taken from dose-response study 2006; total, untransformed abundance of collembolans, oribatid mites, enchytraeids and nematodes were used.

data of the dose-response experiment 2006. The outcome of this 'experimental set-up simulation' exercise could be either used to estimate the achievable desired difference after pooling (for the methodology refer to section II-4.3.6) or to quantify the number of TME replicates i.e. experimental costs and efforts that could be saved or rededicated.

It will be discussed in chapter V-3.3 how the considerations that are developed in this chapter could be of general use in future TME experiments and how our approaches have been used in following studies. The flow chart of Figure V-27 shows the procedure to rearrange the data of the dose-response study. The resulting CoV have then been put into the analyses as described in the methodology section. Figure V-29 shows that the resampling procedure, which means as a final consequence the pooling of two sub-samples of each TME, would take most probably considerable effects in the resulting variation of species counts. This is exemplary shown for the total abundance of the four focused soil organism groups, but would be valid also for the counts of single species. The mean CoV was reduced by 19 % for collembolans, by 16 % for nematodes, by 32 % for oribatid mites and by 21 % for enchytraeids.

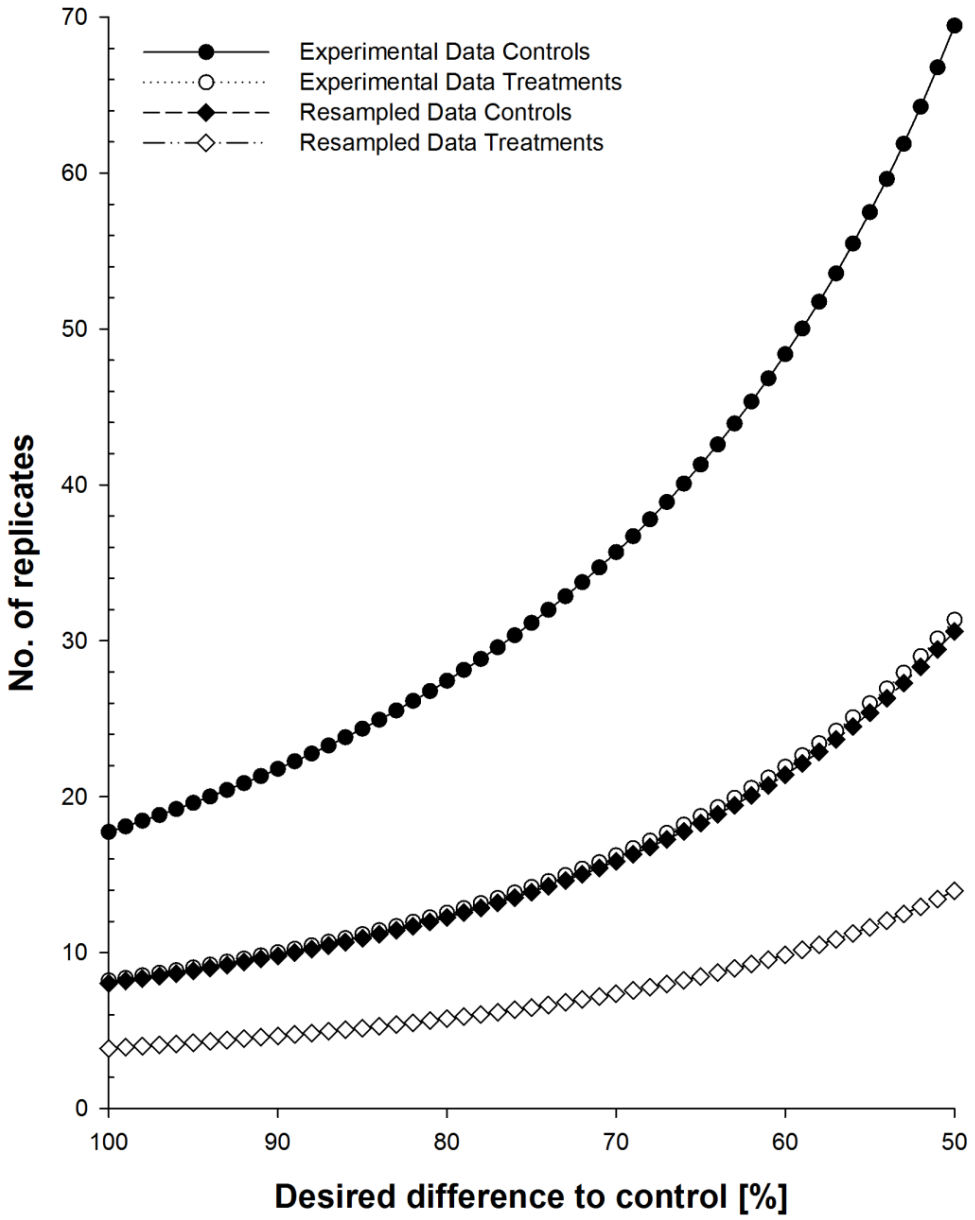


Figure V-28: The desired difference to control in the multiple comparison tests after Dunnett for 5 treatment groups and one control strongly depends on the number of replicates in the control group and the treatment groups, respectively. The figure shows exemplified how the number of necessary replicates changes after virtually pooling of two sub-samples for the group of collembolans. Please consider that here untransformed data were used.

How this finding could be used for the design of TME experiments is discussed in section V-3.3. After the pooling of sub-samples, obviously the variation in the datasets decreases. This has profound influence on the number of replicates that is necessary to reach a certain desired level of detection. Figure V-28 shows that for the detection of a 50 % difference to control approx. 70 control and 30 replicates in each treatment group would be theoretically necessary. For the resampled data, 30 replicates for controls and 14 for each treatment group are neces-

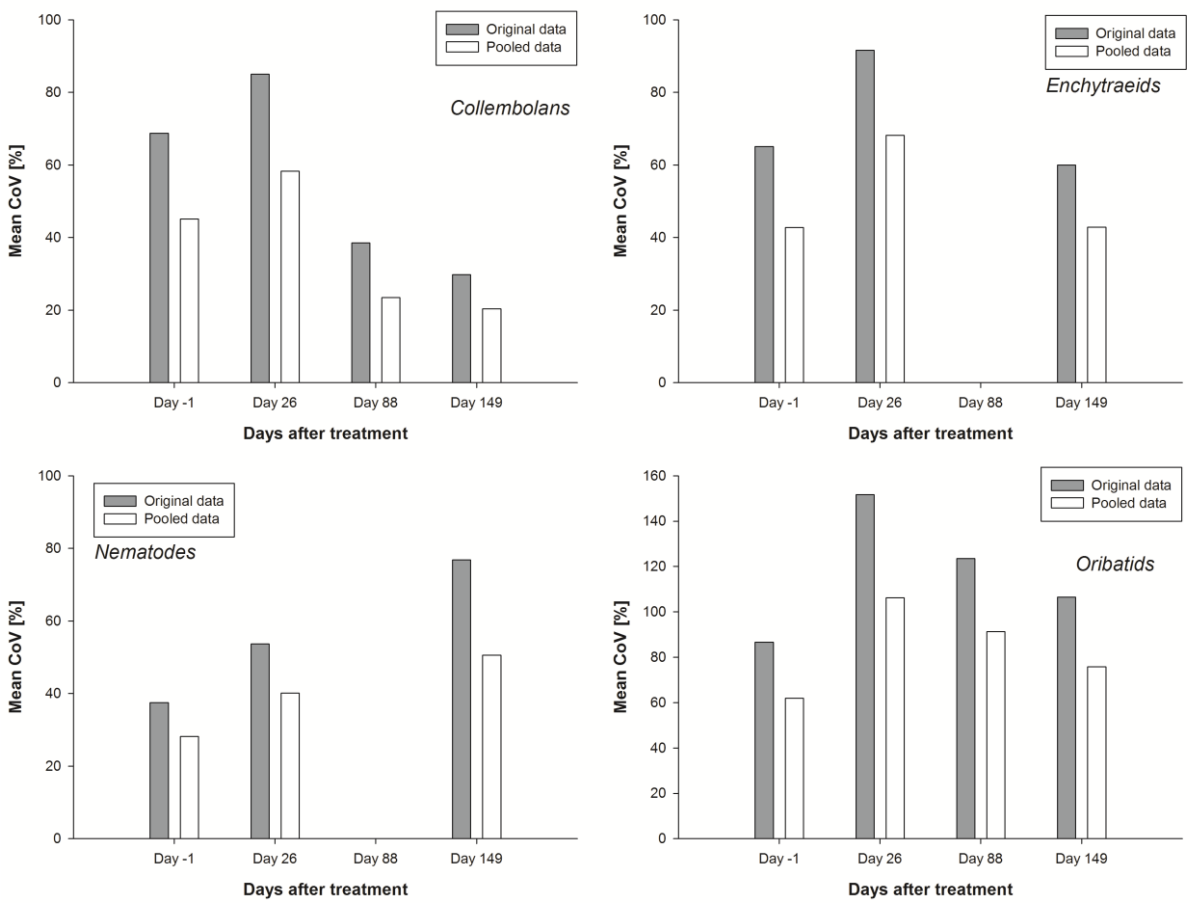


Figure V-29: Mean CoV of 4(3) sampling dates between day -1 and day 149 for the total abundance of collembolans, nematodes, enchytraeids and oribatid mites in the dose-response study 2006. The average CoV was calculated from 20 resampled datasets for the respective groups using 6 control replicates.

sary. The proportional difference between the resampled and the original data set decreases with an increasing effect level, but there is still a discrepancy of 12 to 26 control replicates needed for a 90 % desired difference.

V-3.2.5 The transformation of raw data

Usually, prior to statistical analysis, the raw data better meet the requirements of the statistical test distribution (e.g. normal distribution) after transformation, that is normality and homoscedasticity of numbers. A logarithmic transformation causes a smaller range of the data and gives large numbers less weight within the analyses. In the end, the variability decreases and so does the statistically detectable difference. This chapter illustrates the effects of logarithmic transformations as becoming apparent by the data at hand. It will be discussed which is the final consequence of using logarithmic or otherwise transformed data in testing differential hypotheses, and in interpreting results of those tests. Figure V-30 shows exemplarily for the *total number* of the groups of collembolans, nematodes, oribatids and enchytraeids the effect of transformations of the raw data by strictly taking the logarithm of the abundance to the

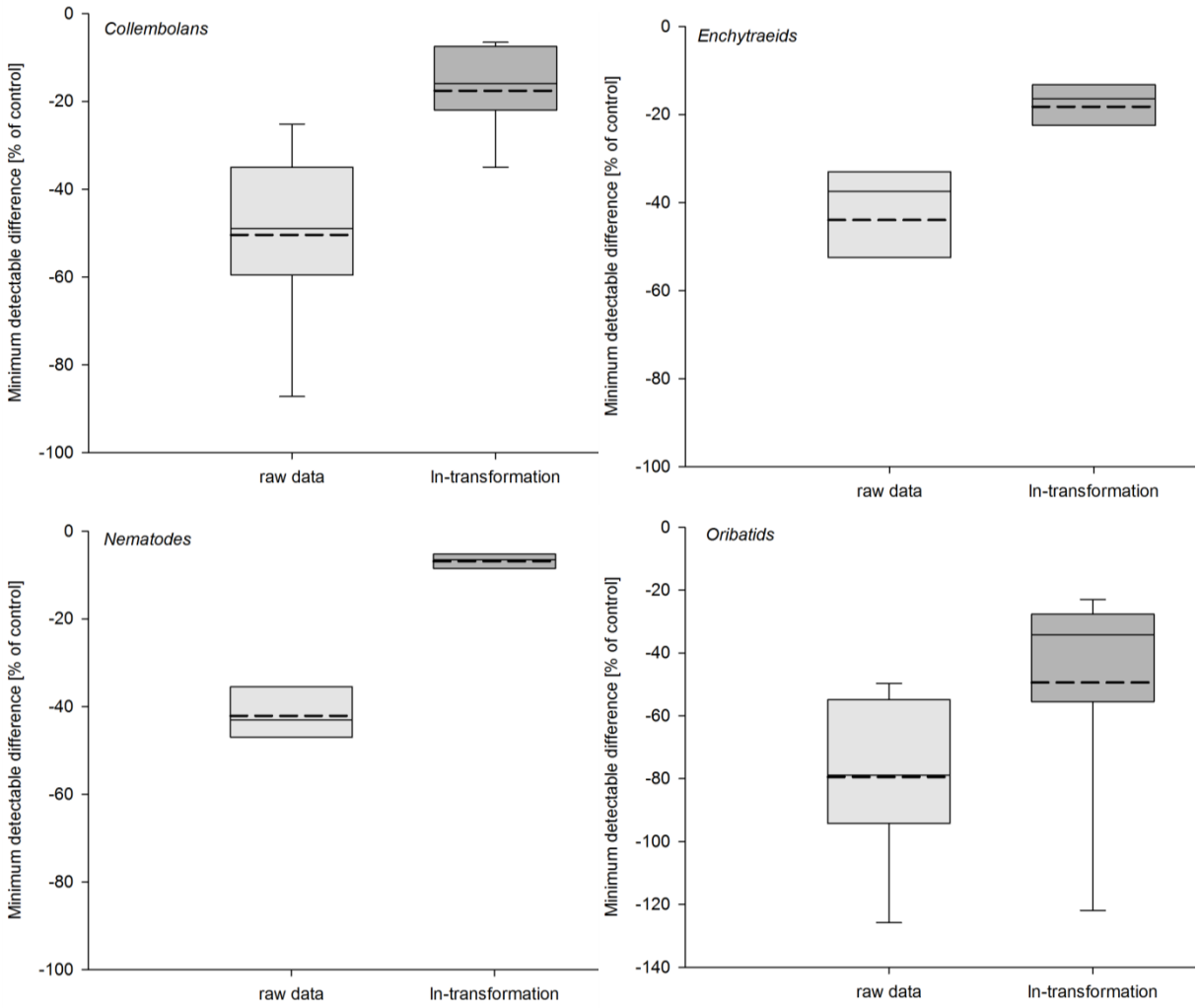


Figure V-30: Boxplots of MDDs derived from TME studies using toxic substances. Effects of data transformation exemplified on total abundances. Test statistics used: Williams multiple t-Test with $N_{control}$ = 12 or $N_{control}$ = 20; $N_{each\ treatment}$ = 6 and 12, respectively. Error rate alpha 0.05; one-sided smaller. Broken lines: arithmetic means of MDD. Straight lines: median MDD. Data taken from all available effect studies, including the TME-2007-study. N for box-plots: coll & orib: 17, nema & ench: 8 (corresponding to the number of sampling dates analysed).

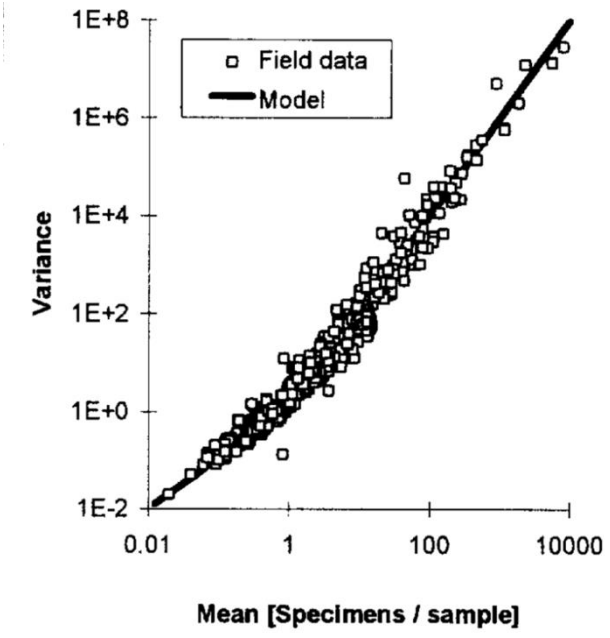
base *e*, which is Euler's number $x = \ln(e^x)$ all too clearly, and by applying the modified t-Test procedure after Williams. To start with, an average detectable difference of about 50 % between treatments and controls, the MDD after transformation amounts to less than 20 % for the *collembolans* (60 % better detection). Very similar results were observed for the effects on the abundances of the other organism groups tested here, with similar influence on less and more variable groups. For *nematodes* it was about 40 % before and 7 % MDD after transformation (82 % better detection), for *enchytraeids* it was 45 and 20 % (55 % better detection), and for the group of *oribatids* 80 % before to 50 % (47 % better detection). These examinations demonstrate a great deal of an increase of detectability of effects, but on the other hand, the interpretation of the outcome of the analysis is less trivial than for untransformed data (see the concluding chapter V-3.3).

V-3.3 Concluding 'limitations of effect detection'

One of the key questions of this thesis and in particular of the section above, i.e. which of the differences between treated TME and a control group can be detected, can be broadened to the ecological question 'which of the effects is relevant for field populations of soil organisms'. According to the findings of the TME studies on average between approx. 5-10% (group of nematodes) and 40-50 % (collembolans and enchytraeids) deviation from the control level can be detected by the TME methodology described here. Compared to results of slightly different semi-field approaches (e.g. of WEYERS & SCHUPHAN 1998), where the authors believed in detecting 10-20 % effect levels our studies allow for a precise estimate of the normal operating range of TME. The issue of the limits and prerequisites of effect detection was discussed recently between scientists, regulators and distributors of plant protection products (i.a. SCHÄFFER *et al.* 2010). It was stated at the before mentioned PERAS workshop that the variability of TME results is not to be expected exceeding the variation of field data of soil organisms. The experiences with the 'Aachen-TME' had been pivotal for the recommendations of the expert groups and included the statement that assuming a power level of 80 % a 50 % deviation from control level should be detectable. This is a quite conservative consideration and is highly dependent on the focused group of organisms and the statistical methodology applied. It has to be stated clearly that most of the results shown above base on total abundances on a taxonomical level of orders or families. The detection limits for single populations is usually lower for most of the species, except of the most dominant or equally distributed ones. Power analyses are considered very useful prior to an experiment. Figure IV-24 shows the distribution of MDD between control and treatment to find a NOEC for effects on the total abundance that could be achieved in our TME studies of the years 2005-2007 (study data unpublished and confidential). The series of experiments poses a continuum of methodological improvements. The variability of the effect detection limits reflects directly the variation within the datasets of different sampling dates due to seasonal fluctuations of the total abundance. For the groups of collembolans, nematodes and enchytraeids a good detection of 5-20% median deviation from the control can be achieved in the test design, which consists of 12-20 control replicates, and 6-10 treatment replicates; the detection for the group of oribatids is medium to low. This is in good agreement with the findings of earlier TME experiments. WEYERS & SCHUPHAN (1998) found effect detection limits of 10-20% in TME with artificial communities of soil organisms. For the detection of effects by dose-response measures as an effective concentration for the 'inhibition' of the abundance no power analysis can be done. In this experiment, the total number of significant effects, either as NOEC or as

EC_x was very similar. The conclusion could be that none of the two approaches is superior to the other. However, very clearly, it must be claimed that no compromise study design can be achieved that delivers great possibilities to determine significant dose-response relationships and effect thresholds at the same time. The experimenters have to decide in advance on one of the opposed designs. The risk regulators have to communicate transparently on which of the possible effect measures they intend to set the acceptable concentration. It would be possible to set the negligible effect to 10 % of an EC_{10} and to derive the NOEAEC on the base of either the NOEC or the EC_{10}, whichever is the most sensitive measure. This would be a practical approach to consider the precautionary principle. The thesis at hand aimed to analyse the data of TME studies by recent state-of-the-art methods as the Principle Response Curve analysis (method described in chapter II-4.3.7) and discussed the snares of statistical standard approaches as the NOEC or the ECx-approaches. The recent discussion on the use of either NOEC- or ECx-approaches is sketched by chapter V-3. Nevertheless, some authors also stipulate a complete shift of the paradigm that null hypothesis significance testing would enhance the ecological and ecotoxicological knowledge (e.g. GERMANO (1999). The spectrum of proposed new methods reaches from Bayesian statistical networks GERMANO (1999) to the use of confidence intervals (BRANDSTÄTTER 1999, CUMMING & FINCH 2005). The power estimates should be strictly 'a priori'. This means that it could be used for the planning but not for the interpretation of results of an experiment (HOENIG & HEISEY 2001). Since the significance level 'p value' of a test statistics is directly correlated to the observed power, a significant deviation from the null hypothesis (i.e. there is no difference between the treatments and controls) corresponds directly to high power, and vice versa.

As stated above in section V-3.2.2 there is only weak empirical evidence that increasing endpoint lead to decreasing variation of the data, and thus lower effect detection limits would be observed. The effect of increasing variation with decreasing organism numbers tracks mainly back on the occurrence of zero values, and on very low abundances as singletons and doubletons in the dataset. This is



a) Variance to mean relation in single species.

Figure V-31: Variance to mean variation in single species, taken from EKSCHMITT 1998.

specific for the ecosystem sampled (temperate meadow soil), the organism group focused and the extraction techniques applied. Higher numbers and less variation could be achieved by sampling exclusively at dates of especially favourable conditions (if those conditions could be forecasted) for at least one of the sensitive taxa. Another strategy could be an increased effort or a shifted focus of the TME sub-sampling towards homogenous counts per TME. It was demonstrated by the results of the pre-study (refer to chapter V-2.4) where up to seven sub-samples per date were taken from each of the TME that variation could decrease, as well as shown in chapter V-3.2.3 that the CoV in the pre-study had been very low. The probability of zero or low values declines, so will the variation. However, for the pre-study data applies additionally that there has been no treatment by a toxic substance. In cases of clear effects of high concentrations of e.g. lindane has been shown that that variation can increase with increasing treatment levels (Figure V-21: chapter V-3.1). A further aspect refers to the probability to capture the rare (or not dominant) specimen. With larger sample means, the hunt for the rare species will be much more successful (EKSCHMITT 1998). This directly relates to the frequency of zero samples. The recommendations for the design of future TME studies are based on at least the one basic principle: the individual experimental units should be tracked over the whole study period, because they constitute the smallest entity, as we understand a TME approach that should be replicated and assessed. For this reason, there is a trade-off between a homogenous experimental unit and a consistent experiment as a whole. Here, it cannot be concluded that it should be aimed for higher means in future experiments rather than for more homogenous treatment and control groups and experimental units. As proposed by SCHÄFFER *et al.* (2011), TME studies could be run in a before-after-control-impact (BACI) design (SMITH 2002). For the taxonomic group of enchytraeids, the clearly lower mean CoV in 2007 (approximately 15-20 % decrease) indicate that the combination of different non-controlled and unknown parameters led to a better situation in 2007 (‘by chance’ rather than by controlled variation of the parameters). The same interpretation could be valid for the variability of nematodes, in spite of the fact that samples were pooled and the double area was sampled in 2007 (factors not separable by statistical methods due to the non-factorial study design, in this respect). In cases where very pronounced effects became evident, (as occurred during the range-finding study), the variation increased with increasing dosages (Figure V-23). Beyond general recommendations for a favourable experimental design, in a follow-up TME-experiment of the year 2007 the diameter was enlarged to 465 mm, allowing for taking two subsamples that were pooled after extraction (results protected under the sponsoring company´s confidentiality laws). For all of the comparisons between experiments of different years,

it has to be considered that an unequal number of replicates have been compared. This only delivers comparable results if the e.g. CoV are distributed normally. In cases where the distribution is too much skewed (refer to Figure V-24, Figure V-25, Figure V-26 and Figure V-30), one should not compare the results quantitatively due to predominant outliers. Generally, the TME properly fit the prerequisites of a higher-tier test for ERA. As shown by Figure V-30, the quality of the data of the two effect studies (range-finding and dose-response study), allow for a good detection of effects of all of the four organism groups. The best detection levels offer the nematodes, mainly due to their very high abundances, followed by enchytraeids and collembolans. The worse detection can be expected for the oribatids, strongly depending on the date of the sampling occasion as indicated by the high intrinsic variability of the detection levels between the different studies and sampling dates. Data transformations, especially logarithmic calculations are broadly applied in ecotoxicology. It is a common technique to lower the variability (‘stabilise’ the variation) of a dataset prior to statistical analysis and to better fit the prerequisites of parametric statistical hypothesis testing. The recommendable transformation depends on the form of the underlying structure of the data. For Poisson-distributions (often recorded for field data of soil animals), a square-root transformation is suggested. For lognormal data, usually observed for ecotoxicological data a logarithmic transformation is recommended (an overview over the numerous publications gives ISO 2004). The profound effects of transformations were shown above by Figure V-30. However, after transformation, it should be stipulated essential to back-transform the means, confidence intervals and other specific values while reporting and interpreting the results of a study. Otherwise, the data would be misreported and misinterpreted. Beyond the effects of data transformations, there was also projection of species or family counts from a sub-set to the whole sample. In particular, for the group of nematodes an extrapolation from the first 100 determined individuals to the abundance of a specific sample was applied (the method is described in the chapter Communities of nematodes – dose-response study). An example from the nematode family Dolichodoridae, should elucidate the effects of such a projection. The highest concentration revealed the only LOEC/NOEC in this study for the group of nematodes. That is actually due to the transformation of abundance data per sample to abundance per square meter. The zero samples remain zero, whereas non-zero values are overestimated. This family was not observed in a single sample at day 149. That led to an inadequately low mean for this particular treatment level and a significant result of the statistical test. Statistical testing should always be done on non-projected data. In the light of these preliminary considerations, it can be stated that no significant deviations from control level occurred within the taxon of nematodes.

V-4 Representativeness of TME communities

Within the scope of environmental risk assessment, the extrapolation from simplified experimental to real world scenarios, from laboratory to field and from individual cases to generalities plays always a central role. Usually, for the derivation of safe concentrations of a pollutant safety factors are applied that are adapted to the particular test system. The predefinition of appropriate safety factors is beyond the scope of this thesis and up to the responsible regulatory bodies. The present chapter should give an estimate of the processes that take place after coring of TME soil cores in the course of an experiment. It is investigated whether TME would be representative to reflect the field situation after coring and transport to the experimental field site. The basic and obvious assumption is, that after coring the TME soil cores at the field site in Monheim (Rhineland) in 70 km linear distance to the experimental field site in Aachen, some key environmental parameters would change due to altered climatic conditions. Rainfall and humidity, mean temperature and sunshine duration are hypothesised to influence the relative densities of populations, the population dynamics and the community structure of soil microarthropods. It is first analysed if the differences of the initially (i.e. at the date of soil coring) identical soil microarthropod communities (drawn on the example of collembolans) become larger with succeeding time, using combined similarity and ordination methods (polar ordination). In a second section, the hypothesis was tested that differing weather conditions have profound influence on the composition of soil communities by canonical correspondence analysis. Time-series data on climatic variables of weather stations located near Monheim (Rhineland) and Aachen University were used as predictors of the community composition, respectively. The soil samples at the coring area and in the TME control groups of the range-finding and dose-response study were cored coincidentally; a direct comparison of the sampling is therefore valid. Immediately after coring the TME on the field site, many factors begin to act on the pristine communities of soil organisms as disturbing agents. Beside the differences in climatic conditions of the two areas, the soil communities could be affected by isolation or allochthonous immigration. Since organisms are restricted to a certain area and possibly negatively affected in their ability to avoid intra- and inter-specific competition, this could be a reason for community shifts (even though not expected, compare chapter V-2 for a discussion of stability aspects regarding the isolation of TME communities).

V-4.1 Similarity of collembolan communities between field and TME

For the TME-control collembolan communities of the dose-response study and the corresponding field-samples (and one early sampling date in March 2005 during the pre-study; the sampling schemes are described in chapter II-1.1) the Bray-Curtis similarity index was calculated to give an estimate of the date and site related ecological distance between two sets of samples (see Equation II-7). The results are given by a matrix of all possible combinations of pair-wise compared samples as 'within-group-similarities' (here e.g. TME- or field-samples of one sampling date) and 'between-group-similarities' (TME vs. field samples of a certain sampling date). In order to reduce the complexity of the dataset, the polar ordination method was applied (Figure V-32). It was focused on the first two axes that explain most of the variation of the collembolan dataset.

The polar ordination Figure V-32 clearly showed a seasonal trend in species data of field and TME samples. Green and red samples all originating of the year 2005 form a large group of relative high similarity. The same has been true for the samples of the year 2006, which could be subsumed to a 'seasonal meta-group'. Furthermore, field and TME samples of the same

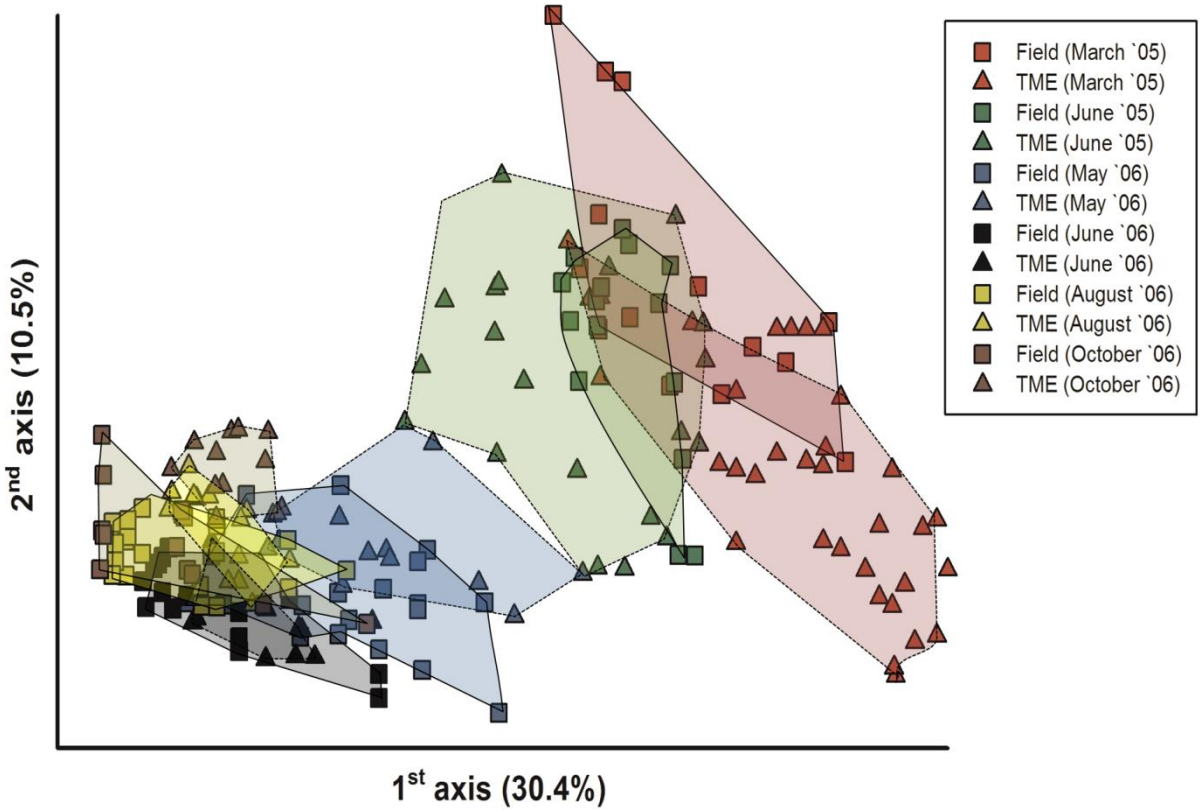


Figure V-32: Polar Ordination (Bray Curtis or Soerensen Index, Euclidean projection geometry) of six comparable sampling dates in TME and field. Data: Collembolans. Same colours indicate the same date of sampling whereas, dotted lines span the TME samples, straight lines field samples.

sampling date were on average more similar than samples of different sampling date, as indicated by the largely overlapping envelopes. It was clearly shown by this analysis that seasonal dynamics were well sustained in TME even after coring and transporting the soil cores to the experimental field site in another 'area of unspoiled nature'. This is the 'Aachener Hügelland' and the 'Köln-Bonner Rheinebene (Kölner Bucht)' (MEYNEN & SCHMITHÜSEN 1953).

It is not the same for each of the several collembolan species found both on the coring area and in TME, but the overall patterns were confounding. By analysing all combinations of samples, it was possible to test for significant differences between within similarities of TME samples of a certain date, the corresponding field sample similarities of the same date and all other possible combinations. It was hypothesised that the similarity within TME samples of a specific sampling date was the largest, followed by field samples of the same date followed by the other combinations of TME samples of one date compared to TME and field samples of other dates. The results of these comparisons are shown by Figure V-35. The within-group TME-similarities were the largest over all sampling dates, followed by mean similarities between TME and corresponding field samples of the same date. Within TME-similarity was significantly higher compared to other distributions of similarities, also for the directly corresponding field samples. The only exceptions were the similarities between TME and field samples at day -1 and day 26 of the dose-response study. There was no significant difference between TME and corresponding field samples.

While ranking the within TME similarity and TME versus field similarities with regard to the sampling dates, another striking finding is that in the ranking of of same date the next highest correlation of the community structure similarity is between TME samples of the actually regarded TME sampling and a temporally neighboured sampling in TMEs.

The within TME similarity increased with the course of the experiments. Climatic conditions, such as rainfall, temperature, sunshine duration and radiant energy could have substantial impact on the primary production and the soil moisture regime, and thus affect the communities of soil organisms. Albeit soil is a system that is highly buffered against fast alterations of environmental parameters, even slight and slow differences could have clear effects on a long term. Here, it is investigated how the differences in the community composition (as depicted above by Figure V-32 and Figure V-35) can be explained by measured environmental variables. Clear differences may be seen mainly in the sum of the radiant energy, resulting in a slightly increased mean temperature in Aachen (Figure V-33). In a *Detrended Correspondence Analysis* (DCA) the length of the gradient of the first four axes has been tested.

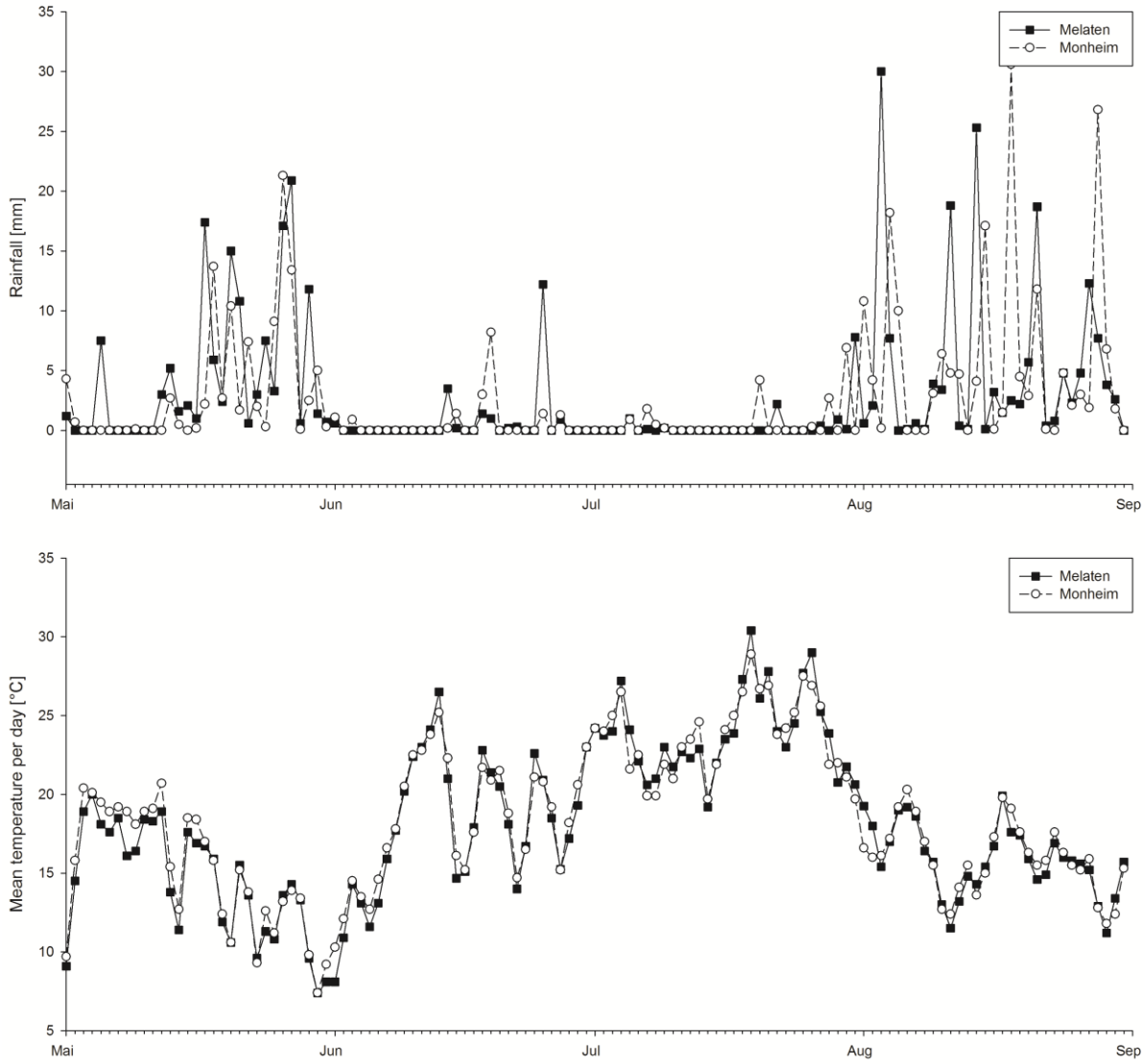


Figure V-33: Course of rainfall and daily mean temperature at TME facility site (Aachen) and field coring site (Monheim). Data from May – August 2006. Source: Weather stations 'Hörn' of the RWTH Aachen University and 'Altjudenhof' of Bayer CropScience.

It was relatively long (1st axis 4.4 and 2nd axis 3.6, respectively), so that further methods (CCA) should have been chosen that assume unimodal responses of the species rather than linear. A *Canonical Correspondence Analysis* (CCA) of 24 synchronous field and 12 TME control samples of collembolans of the first three dates of the dose-response study 2006 (day-1, day 26 and day 88) was computed. The environmental data consisted of the means of five weather parameters: daily means of a two-week period before the first sampling or of the period between two samplings of air temperature, humidity, sunshine duration, radiation energy and precipitation.

Table V-6: Results of the CCA of 105 samples of day -1, day 26 and day 88 of the dose-response study 2006 and the corresponding field samples. The species data were constrained to the five parameters air temperature, humidity, sunshine duration, radiation energy and precipitation. The test of significance of first canonical axis: eigenvalue =0.433; F-ratio = 12.240; P-value = 0.0005; test of significance of all canonical axes: Trace = 0.748; F-ratio = 4.702; P-value = 0.0005.

Axes	1	2	3	4	Total inertia
Eigenvalues	0.382	0.129	0.073	0.050	**3.578**
Species-environment correlations	0.851	0.687	0.570	0.472	
Cumulative percentage variance					
of species data	10.700	14.300	16.300	17.700	
of species-environment relation	58.300	78.000	89.100	96.800	
Sum of all eigenvalues					**3.578**
Sum of all canonical eigenvalues					**0.655**
Explained percent variance by env. variables	**18.3**				

The unexplained variation could be attributed to the transport, the TME-effect and immigration from outside a TME, or 'succession' during the course of the studies. The elapsing of time was not considered in the CCA. To account for the effect of the sampling date on the species composition, a PRC with the environmental variables as covariables a partial ordination approach was conducted additionally. This method could not be adapted to the unimodal response model, and was thus based on a Redundancy Analysis, as originally described by VAN DEN BRINK & TER BRAAK (1997, 1998, and 1999).

Table V-6 shows that the eigenvalues of the first axis are high, meaning that there was a strong gradient. The eigenvalues of a CCA without covariables comply with unconstraint CA analyses. The axes of the analysis are significant after Monte-Carlo permutation tests (regardless of the constraints of a multiple comparison without correction for multiple comparisons produce many falsely positive tests results). As the weighted average algorithm maximizes the correlation between species and the environment, high values of the species-environment correlations were not surprising. The descriptives of the actual weather conditions could explain 18.3 % of the total variation of the species data (Table V-6), which is highly significant after the F-test like procedure after Monte-Carlo permutation provided by the Canoco software. The CCA method has a decisive weakness: the influence of the course of time and the development of the communities in the three-month dataset could not be described.

For this reason, the PRC-method has been adapted to the question at hand: Has the treatment, i.e. the transportation, isolation and storage under altered climatic conditions an influence on the species composition, and does it change over time? These questions could be answered by a Principal Response Curve Analysis, which was introduced before as a method to depict changes of communities due to pesticide treatment (chapters III and IV).

Axis	1	2	3	4
Eigenvalues	0.115	0.04	0.01	0.169
Species-environment correlations	0.861	0.502	0.333	0
Cumulative percentage variance				
of species data	13.00	17.50	18.70	37.70
of species-environment relation	69.30	93.70	100.00	0.00
Sum of all eigenvalues	*0.888*			
Sum of all canonical eigenvalues	*0.166*			
Percentage of variance accounted for by				
Time	11.2			
Differences between replicates	72.2			
Treatment	16.6			
Percentage of treatment variance displayed in first PRC	69.3			

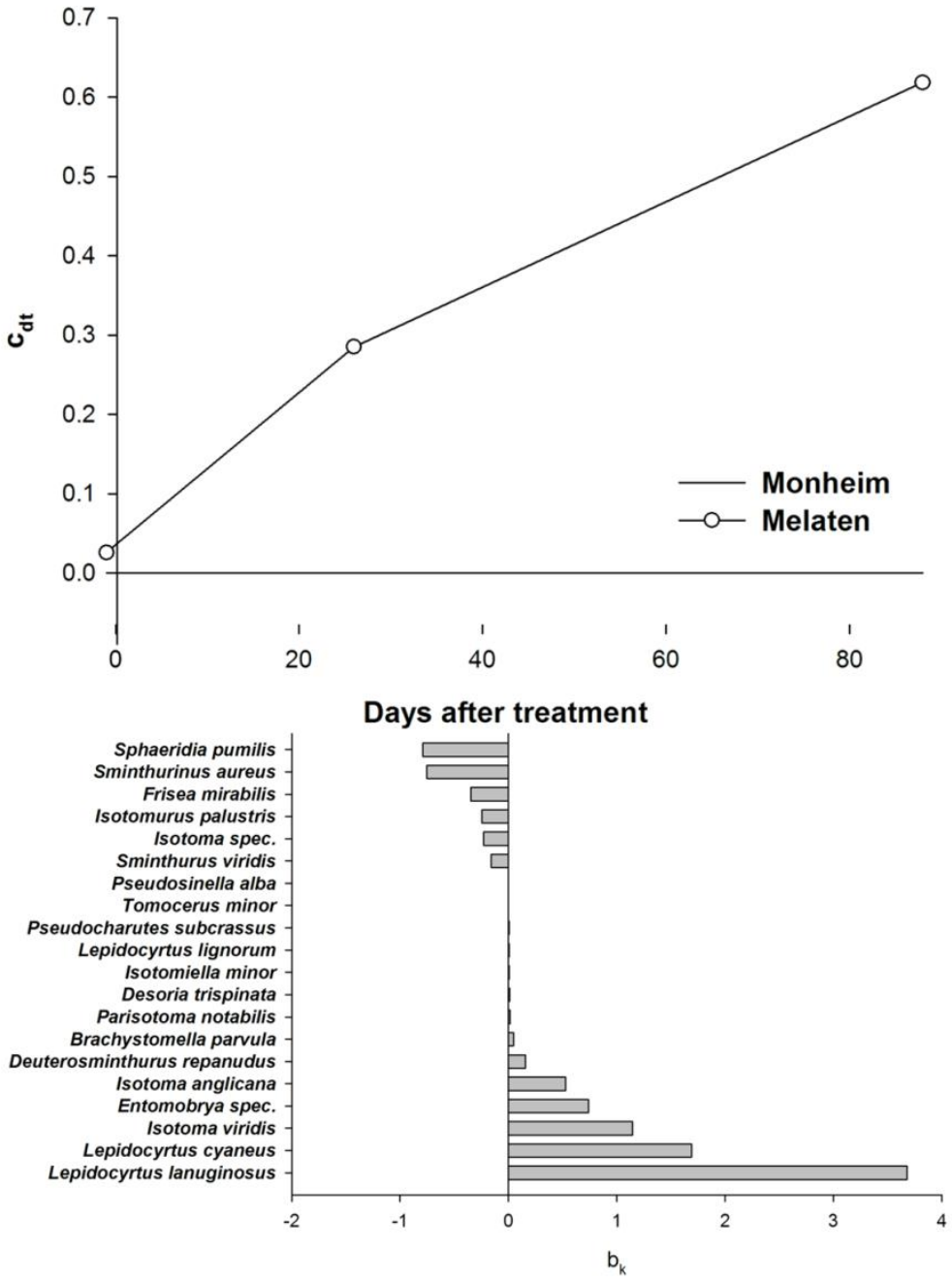


Figure V-34: PRC analysis of three sampling dates of the dose-response study 2006 (treatment 'Melaten') and the corresponding field samples (treatment 'Monheim').

Here, the pesticide treatment has been replaced by a treatment 'storing the TME at the experimental site in Aachen - *Melaten*' versus the control situation 'samples, which still remain at the coring site – *Monheim*'. The PRC (Figure V-34) shows that a significant part of the variation of species data is explained by the treatment that could be summarised as 'transport and maintenance under the conditions of 'Melaten'. The difference between Monheim and Melaten increases with time. Some species follow the overall trend (e.g. *S. pumilis* and *S. aureus*, whereas some species show the opposite behaviour (*L. cyaneus* and *L. lanuginosus*).

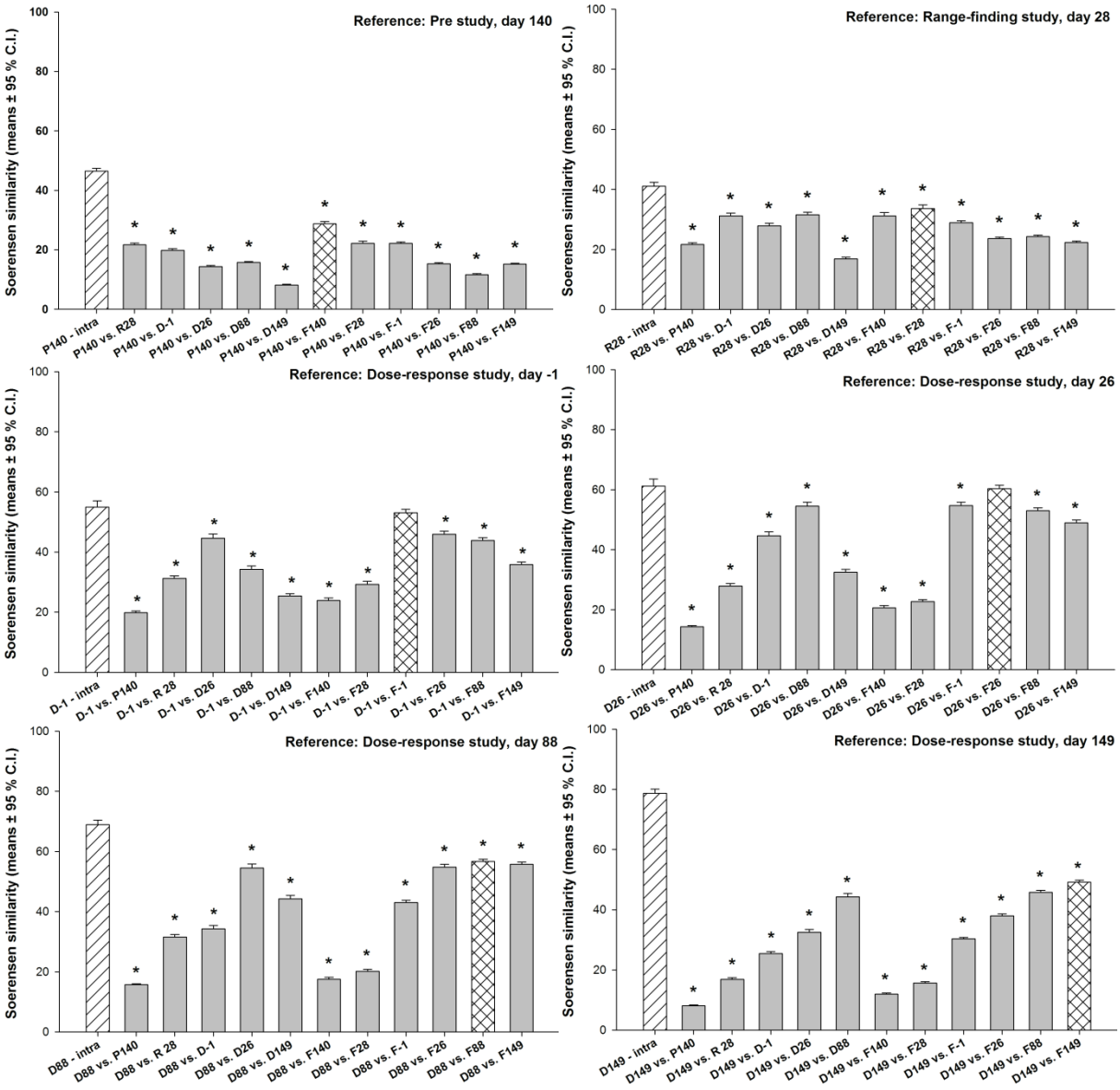


Figure V-35: Comparison between field and TME samples at corresponding sampling dates. Means of Soerensens' similarity index ± 95 % C.I. (N_{TME} between 20 and 12; N_{field} between 14 and 24 replicates). Mean and C.I. were calculated using the possible combinations without repetition of the respective replicates. Indicated by asterisks: Significant differences (Student t-Test alpha 0.05) of similarity distribution per pair-wise comparison between TME or field samples compared to the given reference within-date similarity. Labels of x-axis: P = pre-study, R = range-finding study, D = dose-response study, F = field samples combined with *number* of days after start of the experiments. Striped bars: within date similarity, hatched bars: field-TME comparison of identical dates.

V-4.2 Species inventory of TME and agricultural soils in Central Europe

A species inventory of grasslands and farmlands of Central Europe have been derived from literature and compared to the species found in TME and on the coring site (the detailed data were extracted from the literature and kindly conceded by B. Theißen, and could be partly referred to THEIßEN 2010). The compilation in Table V-7 consists of the data of 5 extensive studies and 25 sites of collembolan distribution on arable land and of 10 extensive studies and 22 sites on the distribution of collembolan species on grassland. Only species with a in the samples of more than 10 % are shown.

The presence-absence data for TME originates from 15 different sampling dates, from the TME-coring site from 8 dates. The Table V-7 was put together from a number of periodical publications and PhD Thesis's namely for arable land from WINK (1969), LÜBBEN (1991), FILSER (2001), HEISLER (1994), and HEIMANN-DETLEFSEN (1991). All of the latter sites have been located in Germany, with the one exception Poland. For grassland, the studies of WOOD 1967, SALT *et al.* 1948, CURRY (1969), WEIGMANN (1973), WINK (1969), LEUTHOLD (1961), DAVIS (1963), HERGARTEN (1985), and STERZYNSKA (1990) have been analysed. The sites were situated mainly in Germany; one was in Poland, some of them in Great Britain. Four main groups have been identified by the comparison: The typical farmland species, the typical grassland species and the ubiquists of agricultural landscapes. Together with one group that was typical for the habitat of our TME studies, the main groups have been well represented in TME.

V-4.3 Concluding 'field-extrapolation'

It could be shown by *polar ordination* and the statistical analysis of the *Bray-Curtis indices* that the similarity within a group of TME-replicate subsamples was the largest over the whole dataset that was available for the comparison between 'field' and 'TME'. Furthermore, a quite high correlation between the samples of one sampling date was observed. In addition, this was regardless if it was a field or TME sample. At the start of the experiment in March 2005, the communities changed probably due to normal seasonal fluctuations very dynamically. From the analyses in chapter V-4.1 it can be concluded that the communities of the coring site in Monheim a. Rh. and the TME, stored at the experimental field site in Aachen, represent the same type of a community.

The differences in species compositions could be partly attributed to the differing weather condition in Aachen and Monheim (around 18 % of the total variation of the dataset could be ascribed to alterations of soil-relevant and generally important weather factors, such as rainfall, sunshine duration, temperature and radiation energy. The variability that could not be explained by the weather data could be due to isolation or allochthonous immigration from the surrounding area at the experimental field site, as suspected in the introducing paragraphs

Table V-7: Presence and absence of collembolan species in agricultural landscapes as found in the literature and recorded by our own studies. References in the text.

Species	Farmland	Grassland	Grassland	Grassland
typical farmland species				
Heteromurus nitidus	X	X	X	
Isotomurus palustris	X	X	X	
Pseudosinella alba	X	X	X	
Arrhopalites caecus	X			
Bourletiella hortensis	X			
Ceratophysella succinea	X			
Folsomia candida	X			
Folsomia fimetaria	X			
Mesaphorura macrochaeta	X			
Willemia intermedia	X			
typical grassland species				
Cryptopygus bipunctatus				X
Entomobrya nivalis				X
Lepidocyrtus curvicollis				X
Metaphorura affinis				X
Protaphorura procampatus				X
Pseudisotoma sensibilis				X
Brachystomella parvula		X	X	X
Sminthurus viridis		X	X	X
openland ubiquists				
Folsomia quadrioculata/manolachei/nana	X	X		X
Friesea mirabilis/truncata	X	X	X	X
Isotoma viridis	X	X	X	X
Isotomiella minor	X		X	X
Lepidocyrtus cyaneus	X	X	X	X
Lepidocyrtus lanuginosus	X	X	X	X
Mesaphorura krausbauerii	X		X	X
Parisotoma notabilis	X	X	X	X
Sminthurinus aureus	X	X	X	X
Sphaeridia pumilis	X	X	X	X
Stenaphorurella quadrispina/denisi	X		X	X
Protaphorura armatus	X			X
Isotomodes productus	X			X
Megalothorax minimus	X			X
Ceratophysella denticulata	X			X
own studies				
Desoria tigrina		X	X	
Desoria trispinata		X	X	
Deuterosminthurus repandus		X	X	
Dicyrtomina spec.		X	X	
Lepidocyrtus lignorum		X	X	
Neanura muscorum		X		
Orchesella spec.		X	X	
Pseudosinella petterseni		X		
Tomocerus minor			X	
Tomocerus minutus		X		
Tomocerus vulgaris		X	X	
Entomobrya spec.		X	X	
Folsomides parvulus		X		
Hypogastrura spec.		X		
Isotoma anglicana		X	X	

of this chapter. The influence of time and the more general factor ‘site of storage Aachen – Melaten’ or ‘original site – Monheim’ was investigated by the PRC method. It could be depicted that the differences between the communities increase with progressing time and that a relatively small amount of variation could be attributed to the mere fact where the communities are exposed. Most of the variation has been due to differences between the replicates. It was technically not feasible to include the environment related factors as additional covariables in the analysis, because the Canoco software terminated with ‘nor-variance-left’-errors. Concluding the uni- and multivariate analysis of the differences in species composition that depend on soil coring, immigration, succession and transportation effects, it has been shown that there are no catastrophic shifts of neither the abundance of microarthropods (refer also to the chapter V-2) nor of the community composition occurred. Even the seasonal fluctuations follow still the same master clock. The analysis of the general representativeness of the approach for the agricultural area of e.g. Central Europe, as analysed by chapter V-4.2, has shown that the coring site and TME communities consist for the greater part of widespread and common species of agricultural landscapes, but also some regionally typical species occurred. Summarising the results at hand, the tested TME reflect the actualities of real world largely.

VI

Final conclusions and outlook

This thesis followed the basic principles and concepts described in chapter I-1. The guiding reasoning that were followed to describe and to assess the suitability of TME for an Environmental Risk Assessment of pesticides, or even in a broader sense the suitability of TME as replicable experimental units for eco- and ecotoxicological research was split into three main topics. The stability of an outdoor TME over a period of at least one year has been addressed just as the detectability of effects in the light of the intrinsic variability of the test systems. The representativeness of the TME communities regarding the coring site and of the coring site in relation to the agricultural landscapes of Central Europe has been examined to the issues of extrapolation from semi-field to field level. In the last chapter, it is discussed and classified, which are the persisting and lasting insights that have been gained from the development and analysis of a novel method that could be used in future environmental risk assessments of toxic substances. In the light of the new pesticide regulation in the European Union and the developments towards more community-based approaches on higher-tiers in ERA, the regulatory use of TME was focused by thesis.

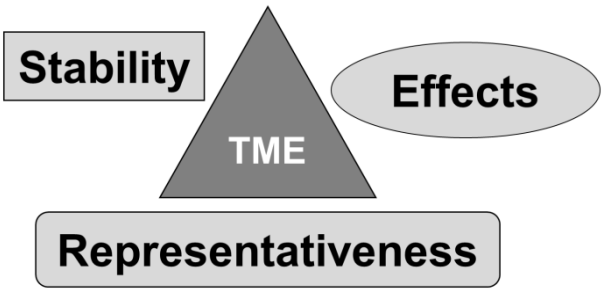


Figure VI-1: Simplified representation of the conceptual approach presented in Figure I-1.

There are some main lessons learned from the TME studies, to begin with recommendations regarding the planning of an experiment.

Semi-field approaches usually deal with high natural variability. Due to that, a careful *screening of the TME coring site* in the run-up of a study is crucial. It was demonstrated on the example of collembolans that soil organism are not equally distributed on an obviously homogenous area. It was concluded from extensive field investigations that the gradients on the coring area require a TME soil coring design on an approx. five meter square and as close as possible to each other.

Approaches using large TME in comparison with the construction used in this thesis that provide more sub-samples at each sampling date should be preferred to meliorate the prospective power of a TME experiment. The *pooling of several sub-samples* from each of the experimental units is recommended. It was shown that TME soil communities have been sufficiently stable over long periods and the sub-sampling is of minor influence, so that also disturbed system could return sound results.
The results of the analysis of the *representativeness* of the TME approach both of the coring area at landscape level and of the comparison of TME with the coring area revealed a large overlap in species composition. However, the influence of climatic conditions and the mere fact of 'transplanting' the soil communities from one site to another could cause profound differences in species composition and seasonal patterns. It is hence stipulated that TME should not be transported to climatic and physical regions with completely different prevailing circumstances. Speaking in a broader sense, it should be avoided to store the TME under laboratory conditions. As it was shown by literature, on a long-term dramatic change of the normal population dynamics such as mass reproduction of specific species could occur.

The *sufficient number of replicates* and the desired power of a test system should be estimated by sufficient pre-testing that serve as realistic estimators of the expectable variability. Furthermore, this has to be done in the light of the statistics and the relevant endpoints for decision-making. There is a marked difference in the planning of dose-response experiments to deduce an EC_x and the threshold-based studies that aim to calculate NOECs. It is not the same to apply multi- and univariate statistics. Knowledge about the communities that will be treated is crucial for designing a valuable experiment.

The underlying hypothesis that as TME 'is a useful approach for both regulators and researchers to take reasonable decisions on effects of stressors at ecosystem level' was focused by this thesis. Some aspects are particularly important for the regulatory use of TME in ERA.

The *stability* of our TME approach over a period of one year was proven by this thesis. This period seems to be sufficient even for the testing of persistent substances and to follow the recovery of soil organism communities over long periods.

It was suggested by the comparison of two consecutive effect studies with the model compound lindane that the results of a dose-series could not directly interpolated between differ-

ent years and experiments. It was rather the case that the low doses caused transient effects on numerous endpoints, whereas the high doses had pronounced effects until the end of the study period. However, regardless of the before mentioned, we think that general response patterns caused by the intrinsic and specific sensitivities of populations and communities of soil organisms are stable and reproducible, realistically reflecting the field situation.

Unless the concrete form of a new terrestrial guidance document that is expected to be implemented not before the year 2015 (PSC 2012), the higher tier-testing procedure for the registration of plant protection products in soil will be triggered unlike the recent practice more frequently , as referred to chapter I-2.4. It could be expected that the demand for standard tests of soil organisms will increase, so will the substances of concern. A direct consequence of this enhanced amount of suspected substances would be an increasing necessity for semi-field testing. Since the protection of the biodiversity also in an agricultural context will come into effect, the functional litterbag test is yet less and less stipulated by the regulatory bodies. We then expect a *high demand for complex TME-studies* that are suitable to find a safe application rate of pesticides for the whole community of soil organisms that are directly exposed with low uncertainties. A TME approach outclasses field studies in the respect of the possibilities to design dose-response studies and to avoid excess variation and could therefore used at highest tiers of the ERA. The statistical power of field tests is widely unknown. From own experiences with field data it could be assumed to be comparably high compared to TME data. A TME semi-field approach provides less uncertainty and more confidentiality of the decisions taken by using a high number of replicates and robust statistical methods.

It is often questioned if the *data quality and the available statistical methods* from soil ecotoxicological semi-field tests is generally adequate for the detection of clear or subtle effects. Generally, it is assumed that the distribution and occurrence of soil organisms in nature is erratic and thus an investigator will face major problems representing the main effects of toxic substances to soil communities. This thesis adapted standard methodologies from aquatic semi-field tests (‘mesocosm studies’) to TME data. The mode of analysing the data was found principally adequate. It was found that in particular the results of multivariate methods that depict the changes of soil communities under stress over time have to be interpreted with caution. The use of alternatives in statistical testing, e.g. the use of confidence intervals instead of hypothesis testing, or the preference of effective concentrations [EC_x-approach] over threshold concentrations [NOEC-approach] was discussed. TME data allow for all kinds of anal-

yses. The standards have to be set by the scientific and regulatory community. However, the design of an experiment should be strictly oriented to one of the approaches. The selectivity of the statistics would profit from a focused approach rather than applying an underdone compromise.

A test system that includes species showing a range of different susceptibilities under natural conditions comprises a great range of possible responses and can help to identify a protective rate for the use of the pesticide under inspection. An undisturbed ecosystem approach offers the possibility to investigate *indirect effects* of pesticides. For example, as we have shown and discussed above, a decrease of fungal feeders from one or several of the animal groups could lead to a relative increase of the fungal biomass. The determination of a relevant endpoint in conjunction with a high degree of realism then becomes achievable. We do not keep it secret that due to very low abundances of e.g. the group of oribatids the detection of effects in our study is limited.

The use of *appropriate safety factors* for the extrapolation of results to the field and finally the deduction of regulatory acceptable application rates to protect the community structure, the diversity and the function of the soils will be subjected to further discussion within the scientific community and between the different stakeholders in the process of delivering the data base for the registration of plant protection products. For determination of appropriate safety factors of TME-studies in ERA and an extrapolation to the real field situation (not to field effect studies), the sources of variation originating by e.g. differences in the susceptibility of soil organism communities within the respective European physiogeographical authorisation zones, the differences between laboratories and the variation due to further unknown factors.

For the interpretation of a TME study, the *appropriateness* of the tested habitat in terms of specific *species sensitivity* is crucial. A common criticism towards a TME approach using undisturbed grassland soils says that our basic assumption that these diverse communities are admittedly more sensitive than likely adapted in-crop communities. However, the systems may be therefore much more sensitive and thus not suited as surrogates of the field situation. The results would overestimate the effects of plant protection products compared to in-crop habitats. It was shown here that in our TME species from all agricultural types were found and the results could be therefore extrapolated to lots of open-land habitats. The off-crop di-

versity in agricultural landscapes will be also included in future definitions of protection goals, so an approach using undisturbed grassland is assumed adequate.

A *classification of effects* leaned on the system used for aquatic mesocosm studies as performed in this thesis could help to rank the sensitivity of the endpoints investigated in a study and helps to drive application rates that caused no effect and could be therefore considered safe.

Besides, TME appear a flexible tool as they allow an adaption to the focused areas of concern in an actual ERA. *Further endpoints* could be included in a study design. In the first instance, our TME were not foreseen for excess *earthworm-testing* because of the limited volume of the soil cores. It was assumed not to provide sufficient species numbers of the larger anecic species. Recently, an adapted and enlarged TME approach in a joint project at the Institute for Environmental Research at the RWTH Aachen University attempts to include the earthworms as an additional organism group that is very much in the focus in the ERA of soils. Future TME designs could also test for other assessment areas than soil organisms. *Non-target plants* (*NTP*) could be tested as artificial but diverse assemblages. Then TME could be used to improve the database on this group of organisms, of which the risk assessment too often relies on efficiency tests of cultivated plants rather than natural plant communities. TME could be particular useful when it comes to higher-tier testing of herbicidal compounds. *Non-target arthropods* (*NTA*) other than soil organisms as nursery web spiders or ground beetles are marked by a high degree of mobility. A proper representation of this group in model ecosystem approaches seems to be restricted to field enclosures or full-scale field studies.

Our TME studies were done without verifying a successful application procedure, nor were the actual concentrations in soil measured during the experiments to *link effects and exposure* of the model substance. Assuming the behaviour of a substance is well known or could be modelled with sufficient precision, measurements are not considered necessary. On the other hand, the inclusion of fate investigations would be possible, either for the price of larger systems or less biological samples. If alternative extraction procedures would be available, it could help solving the dilemma. There would be the need of a method that firstly extracts the organisms, and then allows for the chemical analysis of the soil afterwards.

The *perspective* for the use of TME in ERA is generally promising since our and many simi-

lar semi-field approaches have been already discussed and recommended by a group of experts from academia, industry and regulatory bodies throughout Europe after the SETAC-PERAS workshop. Although the suitability was stated in principle, some research needs were posed, such as issues of extrapolation uncertainties, protection goals, basic ecological research and aspects of the experimental setup (SCHÄFFER *et al.* 2011). I think most of the research needs have been addressed and answered by the thesis at hand; others could be solved by using the methodologies presented here. It is expected that the new terrestrial guidance document will be finished in 2015. The use of TME as an important semi-field higher-tier system should be included in any case.

VII

Bibliography

Addison JA, Holmes SB (1995) Effect of two commercial formulations of *Bacillus thuringiensis subsp. Kurstaki* on the Collembolan species *Folsomia candida* in a soil microcosm study. Bulletin of Environmental Contamination and Toxicology 55: 771-778.

Anderson JM, Ineson P (1982) A soil microcosm system and its application to measurement of respiration and nutrient leaching. Soil Biology and Biochemistry 14: 415-416.

Anderson JPE, Domsch KH (1975) Measurement of bacterial and fungal contribution to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21: 314-322.

André HM, Ducarme X, Lebrun P (2002) Soil biodiversity: myth, reality or conning? Oikos 96: 3-24.

André HM, Noti M-I, Lebrun P (1994) The soil fauna: the other last biotic frontier. Biodiversity and Conservation 3 45-56.

Angle JS, Levin MA, Gagliardi JV, McIntosh MS (1995) Validation of microcosms for examining the survival of *Pseudomonas aureofaciens* (lacZY) in soil. Applied and Environmental Microbiology 61: 2835-2839.

Apitz SE, Elliott M, Fountain M, Galloway TS (2006) European Environmental Management: Moving to an Ecosystem Approach. Integrated Environmental Assessment and Management 2: 80-85.

Arthur MF, Zwick TC, Tolle DA, Van Voris P (1984) Effects of fly-ash on microbial CO2 evolution from an agricultural soil. Water, Air and Soil Pollution 22: 209-216.

ASTM - American Society for Testing and Materials (2004) Standard Guide for Conducting a Terrestrial Soil-Core Microcosm Test (E1197-87). Annual Book of ASTM Standards - Section 11: Water and Environmental Technology - Biological Effects and Environmental Fate; Biotechnology; Pesticides. pp. 13.

Barrett KL, Grandy N, Harrison EG (eds.) (1994) Guidance document on regulatory testing procedures for pesticides with non-target Arthropods. Workshop ESCORT Wageningen 28-30 March 1994, SETAC Press. pp. 51.

Becker H (1991) Bodenorganismen - Prüfungskategorien der Forschung. Umweltwissenschaften und Schadstoff-Forschung 3: 19-24.

Begon ME, Harper JL, Townsend CR (1998) Ökologie. Spektrum Akademischer Verlag, Heidelberg. pp. 750.

Bengtsson G, Annadoter H (1989) Nitrate reduction in a groundwater microcosm determined by gas chromatography-mass spectrometry. Applied and Environmental Microbiology 55: 2861-2870.

Bengtsson J (1998) Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function. Applied Soil Ecology 10: 191-199.

Bengtsson J (2002) Disturbance and resilience in soil animal communities. European Journal of Soil Biology 38: 119-125.

Bengtsson J, Setälä H, Zheng DW (1996) Food webs andf nutrient cycling in soils: Interactions and positive feedbacks. In: Polis GA, Winemiller KO (1996) Food webs: Integration of patterns & Dynamics. Chapman & Hall. pp. 30-38.

Berg MP, Bengtsson J (2007) Temporal and spatial variability in soil food web structure. Oikos 116: 1789-1804.

Beylich A, Graefe U (2009) Investigations of annelids at soil monitoring sites in Northern Germany: reference ranges and time-series data. Soil Organisms 81: 175-196.

Bieri DS, Delucchi V, Lienhard C (1978) Ein abgeänderter MacFadyen-Apparat für die dynamische Extraktion von Bodenarthropoden. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 51: 161-167.

Biggar JW, Nielson DR, Tillotson WR (1984) Movement of DBCP in laboratory soil columns and field soils to groundwater. Environmental Geology 5: 127-131.

Billings WD, Peterson KM, Luken JO, Mortensen DA (1984) Interaction of increasing atmospheric carbon dioxide and soil nitrogen on the carbon balance of tundra microcosms. Oecologia 25: 26-29.

Blair JM, Crossley DA, Rider S (1989) Effects of naphthalene on microbial activities and nitrogen pools in soil-litter microcosms. Soil Biology and Biochemistry 21: 507-510.

Block W (1966) Some characteristics of the MacFadyen high gradient extraction for soil microarthropods. Oikos 17: 9.

BMU - Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit (2001) SIDS Initial Assessment Report for SIAM 13-Dimethylformamide. Bonn-Bad Godesberg. pp. 287.

Bongers T (1988) De nematoden van Nederland: een identificatietabel voor de Nederland aangetroffen zoetwater- en bodembewonende nematoden. Koninklijke Nederlandse Natuurhistorische Vereniging No. 46. pp. 408.

Bongers T (1990) The maturity index: an ecological measure of environmental disturbance based on soil nematode species composition. Oecologia 83: 14-19.

Bongers T, Ferris H (1999) Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology and Evolution 14: 224-228.

Born H, Puschnig M, Schettler-Wiegel J (1989) Einfluß von Aldicarb und Aldicarb-Sulfon auf die Bodenmesofauna (Enchytraeidae, Acari, Collembola) in Mikrokosmen. Poster zu Verhandlungen der Gesellschaft für Ökologie, Band XVIII .

Bortz J (1985) Lehrbuch der Statistik. Springer, Heidelberg. pp. 994.

Boyle TP, Smillie GM, Anderson JC, Beeson DR (1990) A sensitivity analysis of nine diversity and seven similarity indices. Research Journal of the Water Pollution Control Federation 62: 749-762.

Brandstätter E (1999) Konfidenzintervalle als Alternative zu Signifikanztests. Methods of Psychological Research 4: 1-17.

Branham BE, Wehner DJ, Torello WA, Turgeon J (1985) A microecosystem for fertilizer and pesticide fate research. Agronomy Journal 77: 176-179.

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs 27: 325-349.

Bretfeld G (1999) Synopses on Palearctic Collembola, Volume 2: Symphypleona. Abhandlungen und Berichte des Naturkundemuseum Görlitz 71: 1-318.

Brimecombe MJ, De Leij FAAM, Lynch JM (2001) Nematode community structure as a sensitive indicator of microbial perturbations induced by a genetically modified Pseudomonas fluorescens strain. Biology and Fertility of Soils 34: 270-275.

Brock TCM, Arts GHP, Maltby L, Van den Brink PJ (2006) Aquatic Risks of Pesticides, Ecological Protection Goals, and Common Aims in European Union Legislation. Integrated Environmental Assessment and Management 2: 20-46.

Brown JKM (2004) Experimental Design Generator and Randomiser II. Cereals Research Department, John Innes Centre, Colney, Norwich, NR4 7UH, England.

Brown K, Tomlinson J, Duncan J, Hinchcliffe A, Palmquist K (2009) Critical comparison of available and potential higher tier testing approaches for the risk assessment of plant protection products, considering at least field and semi-field experimental designs, extrapolation from dose-response relationships, and increased dosages (aquatic and terrestrial). Literature reviews prepared for the European Food Safety Authority on ecotoxicology of chemicals with special focus on plant protection products. Reference: CFT/EFSA/PPR/2008/01.

Bruckner A, Kampichler C, Wight J, Bauer R, Kandeler E (1993) Using mesococms to investigate mesofaunal-microbial interactions in soil: Re-immigration of fauna to defaunated monoliths. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 69: 151-154.

Bruckner A, Wright J, Kampichler C, Bauer R, Kandeler E (1995) A method to prepare mesocosms to investigate mesofaunal-microbial interactions in soil. Biology and Fertility of Soils 19: 257-262.

Bulawa B (2004) Der mikrobielle Umsatz von Ernterückständen in einem landwirtschaftlich genutzten Boden und dessen Beeinflussung durch ausgewählte Xenobiotica. Dissertationschrift, RWTH Aachen. pp. 186.

Buldgen P, Remacle J (1988) Influence on environmental factors upon the leaching of cations from undisturbed microcosms of beech and spruce litters. Soil Biology and Biochemistry 13: 143-147.

Bundesverband Boden (ed.) (2005) Biologische Charakterisierung von Böden - Ansatz zur Bewertung des Bodens als Lebensraum für Bodenorganismen im Rahmen von Planungsprozessen. Anhang 3: Informationen zur Eignung verschiedener Bodenorganismengruppen zur Bewertung von Böden im Rahmen von Planungsverfahren. Erich Schmidt Verlag, Berlin.

Burda H, Hilken G, Zrzavý J (2008) Systematische Zoologie. UTB, Stuttgart. 1st edition. pp. 338.

Burrows LA, Edwards CA (2004) The Use of Integrated Soil Microcosms to Assess the Impact of Carbendazim on Soil Ecosystems. Ecotoxicology 13: 143-161.

Cairns J (1984) Are single species toxicity tests alone adequate for estimating environmental hazard? Environmental Monitoring and Assessment 4: 259-273.

Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (eds.) (2000) Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. ESCORT 2 workshop held at Wageningen International Conference Centre Wageningen, The Netherlands. pp. 51.

Carapelli A, Frati F, Fanciulli PP, Dallai R (2001) Taxonomic revision of fourteen southwestern European species of *Isotomurus* (Collembola, Isotomidae), with description of four new species and the designation of the neotype for *I. palustris*. Zoologica Scripta 30: 115-143.

Chalupsky J, Leps J (1985) The spatial pattern of Enchytraeidae (Oligochaeta). Oecologia 68: 153-157.

Checkai RT, Wentsel RS, Philips CT, Yon RL (1993) Controlled environment soil-core microcosm unit for investigating fate, migration and transformation of chemicals in soils. Journal of Soil Contamination 2: 229-243.

Christensen B (1959) Asexual reproduction in the Enchytraeidae (Olig.). Nature 184: 1159-1160.

Christian E, Zicsi A (2005) Ein synoptischer Bestimmungsschlüssel der Regenwürmer Österreichs. Die Bodenkultur 50: 121-131.

Cianciolo JM, Norton RA (2006) The ecological distribution of reproductive mode in oribatid mites, as related to biological complexity. Experimental andApplied Acarology 40: 1-25.

Cobb NA (1918) Estimating the nema population of the soil. United States Department of Agriculture, Agricultural Technical Circular 1. pp. 48.

Cole LK, Metcalf RL (1980) Environmental Destinies of Insecticides, andFungicides in the Plants, Animals, Soil, Air, and Water of Homologous Microcosms. In: Giesy JP (ed.): Microcosms in Ecological Research. Technical Information Center, US Department of Energy. Springfield, USA 971-1007.

Cole LK, Metcalf RL, Sanborn JR (1976B) Environmental fate of insecticides in terrestrial model ecosystems. International Journal of Environmental Studies 10: 7-14.

Cole LK, Sanborn JR, Metcalf RL (1976A) Inhibition of corn growth by aldrin and the insecticide´s fate in the soil, air, crop, and wildlife system of a terrestrial model ecosystem. Environmental Entomology 5: 583-589.

Coleman DC (2008) From peds to paradoxes: Linkages between soil biota and their influences on ecological processes. Soil Biology & Biochemistry 40: 271-289.

Council of the European Union (1991) Council Directive 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC). Official Journal of the European Communities L230: 1-210.

Council of the European Union (1992) Richtlinie 92/43 EWG des Rates der Europäischen Gemeinschaft zur Erhaltung der natürlichen Lebensräume sowie der wild lebenden Tiere und Pflanzen (Flora-Fauna-Habitat-Richtlinie - FFH-RL). Official Journal of the European Communities L206: 11-88.

Crane M, Newman MC (2000) What level of effect is a no observed effect? Environmental Toxicology and Chemistry 19: 516-51.

Cronan CS (1980) Controls on leaching from coniferous forest floor microcosms. Plant and Soil 56: 301-322.

Cumming G, Finch S (2005) Inference by Eye - Confidence Intervals and How to Read Pictures of Data. American Psychologist 60: 170-180.

Curry JP (1969) The qualitative and quantitative composition of the fauna of an old grassland site at Cellbridge, Co. Kildare. Soil Biology and Biochemistry 1: 219-227.

Daehler CC, Strong DR (1996) Can You Bottle Nature? The Roles of Microcosms in Ecological Research. Ecology 77: 663-664.

Davis BNK (1963) A study of microarthropod communities in mineral soils near Corby, Northants. Journal of Animal Ecology 32: 49-71.

De Catanzaro JB, Hutchinson TC (1985) Leaching and distribution of nitrogen and nickel in nickel-perturbed jack pine microcosm. Water, Air and Soil Pollution 26: 281-292.

De Ruiter PC, Neutel AM, Moore JC (1998) Biodiversity in soil ecosystems: the role of energy flow and community stability. Applied Soil Ecology 10: 217-228.

Debinski DM, Holt RD (2000) A survey and overview of habitat fragmentation experiments. Conservation Biology 14: 342-355.

Deventer K, Zipperle J, Kostka-Rick R (2004) Ökotoxikologische Charakterisierung von Abfall-Literaturstudie im Auftrag der Landesanstalt für Umweltschutz Baden-Württemberg. Landesanstalt für Umweltschutz Baden-Württemberg, Karlsruhe. pp. 108.

Didden W (1993) Ecology of terrestrial Enchytraeidae. Pedobiologia 37: 2-29.

Didden W, Römbke J (2001) Enchytraeids as Indicator Organisms for chemical Stress in Terrestrial Ecosystems. Ecotoxicology and Environmental Safety 50: 25-43.

Dierschke H (1994) Pflanzensoziologie. Ulmer Verlag, Stuttgart.

Djajakirana G, Joergensen RG, Meyer B (1996) Ergosterol and microbial biomass relationship in soil. Biology and Fertility of Soils 22: 299-304.

Dougherty JM, Lanza GR (1989) Anaerobic subsurface soil microcosms: methods to monitor effects of organic pollutants on indigenous microbial activity. Toxicity Assessment 4: 85-104.

Ducarmé X, André HM, Lebrun P (1998) Extracting endogeous microarthropods: A new flotation method using 1,2-dibromoethane. European Journal of Soil Biology 34: 143-150.

Dunger W, Fiedler HJ (1997) Methoden der Bodenbiologie. S. Fischer, Jena. pp. 421.

Dutta M, Schafer KS (2003) Lindane - Answers to common questions. International POPs Elimination Network. Pesticide Working Group.International POPs Elimination Network. Pesticide Working Group. pp.13.

Eash NS, Stahl PD, Parkin TB, Karlen DL (1996) A simplified method for extraction of ergosterol from soil. Soil Science Society of America Journal 60: 468-471.

Edwards CA (1991) The assessment of populations of soil-inhabiting invertebrates. Agriculture, Ecosystems and Environment 34: 145-176.

Edwards CA, Knacker T, Pokarzhevskii A (1998) The prediction of the fate and effects of pesticides in the environment using tiered laboratory soil microcosms. The 1998 Brighton Conference - Pests and Diseases 4C-1: 267-272.

Edwards CA, Knacker T, Pokarzhevskii A, Subler S, Parmelee R (1996) The use of soil microcosms in assessing the effects of pesticides on soil ecosystems. In: Environmental Behaviour of Crop Protection Chemicals -Proceedings Series. Proceedings of an International Symposium on the use of Nuclear and Related Techniques 435-451.

EFSA - European Food Safety Authority (2010) Scientific Opinion on the development of a soil ecoregions concept using distribution data on invertebrates. The EFSA Journal 8: 1-77.

EFSA - Panel of Plant Protection Products and their Residues (PPR) (2010) Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). The EFSA Journal 8: 1-55.

Ekschmitt K (1998) Population assessments of soil fauna: General Criteria for the planning of sampling schemes. Applied Soil Ecology 9: 439-445.

Elliott ET, Coleman DC, Anderson RV, Cole CV, Hunt HW, Woods LE, Gould WD, McClellan JF (1980) Microbial trophic structure and habitable pore space in soil. In: Giesy JP (ed.): Microcosms in Ecological Research. Technical Information Center US Department of Energy. Springfield, USA. pp. 1050-1070.

Engels M, Ratte HT (1992) Randomisierte Ähnlichkeitsanalyse von Lebensgemeinschaften am Beispiel von Mesokosmos-Versuchen in der Ökotoxikologie. Verhandlungen der Gesellschaft für Ökologie 21: 303-308.

Ettema CH, Wardle DA (2002) Spatial soil ecology. Trends in Ecology & Evolution 17: 177-183.

European Commission (2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union L309: 1-50.

European Commission (2000a) Commission Decision of 20 December 2000 concerning the non-inclusion of lindane in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant-protection products containing this active substance (2000/801/EC). Official Journal of the European Communities L324: 42-43.

European Commission (2000b) IUCLID Dataset on gamma-HCH. European Chemicals Bureau. Year 2000 CD-ROM Edition. pp. 49.

European Commission (2002a) Draft Working Document. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev. 2 final. Brussels, Belgium.

European Commission (2002b) Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC. Sanco/3268/2001 rev.4 (final).

Fairbanks BC, Woods LE, Bryant RJ, Elliott ET, Cole CV, Coleman DC (1984) Limitations of ATP estimates of microbial biomass. Soil Biology and Biochemistry 16: 549-558.

FAO/WHO (1968) 1967 Evaluations of some pesticide residues in food. FAO/PL: 1967/M/11/1; WHO/Food Add./68.30. .

Fermanich KJ, Daniel TC, Lowery B (1991) Microlysimeter for soil columns for evaluating pesticide movement through the root zone. Journal of Environmental Quality 20: 189-195.

Ferris MJ, Ward DM (1997) Seasonal distribution of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. Applied Environmental Microbiology 63: 1375-1381.

Fierer N, Grandy AS, Six J, Paul EA (2009) Searching for unifying principles in soil ecology. Soil Biology & Biochemistry 41: 2249-2256.

Figge K, Klahn J, Koch J (1983) Testing of chemicals by evaluation of their distribution and degradation patterns in an environmental standard system. Regulatory Toxicology and Pharmacology 3: 199-215.

Figge K, Schöberl B (1989) LAS and the application of sewage sludge in agriculture. Tenside Surfactants Detergents 26: 122-128.

Filser J (1994) The effects of the systemic fungicide Aktuan on Collembola under field conditions. Acta Zoologica Fennica 195: 32-34.

Filser J (2001) Vorkommen, Funktion und Bedeutung von Collembolen in anthropogen genutztem Böden. Entomologen Tagung Düsseldorf.

Filzek PDB, Spurgeon DJ, Broll G, Svendsen C, Hankard PK, Parekh N, Stubberud HE, Weeks JM (2004) Metal Effects on Soil Invertebrate Feeding: Measurements Using the Bait Lamina Method. Ecotoxicology 13: 807-816.

Fjellberg A (1980) Identification Keys to Norwegian Collembola. Norsk Entomologist Forening, Norway.

Fjellberg A (1998) The Collembola of Fennoscandia and Denmark. Part I: Poduromorpha. Fauna Entomologica Scandinavica Volume 35. Brill, Leiden. pp. 184.

Fjellberg A (2007) The Collembola of Fennoscandia and Denmark. Part II: Entomobryomorpha and Symphypleona. Fauna Entomologica Scandinavica Volume 42. Brill, Leiden. pp. 266.

Folker-Hansen P, Krogh PH, Holmstrup M (1996) Effect of Dimethoate on Body Growth of Representatives of the Soil Living Mesofauna. Ecotoxicology and Environmental Safety 33: 207-216.

Förster B, Eder M, Morgan E, Knacker T (1996) A microcosm study of the effects of chemical stress, earthworms and microorganisms and their interactions upon litter decomposition. European Journal of Soil Biology 32: 25-33.

Förster B, Van Gestel CAM, Koolhaas JEE, Nentwig G, Rodrigues JML, Sousa JP, Jones SE, Knacker T (2004) Ring-testing and Field-validation of a Terrestrial Model Ecosystem (TME) - An Instrument for Testing Potentially Harmful Substances: Effects of Carbendazim on Organic Matter Breakdown and Soil Fauna Feeding Activity. Ecotoxicology 13: 129-141.

Fountain MT, Hopkin SP (2005) *Folsomia candida* (Collembola): A 'standard' Soil Arthropod. Annual Reviews in Entomology 50: 201-222.

Frampton GK, Jänsch S, Scott-Fordsmand JJ, Römbke J, Van den Brink PJ (2006) Effects of pesticides on soil invertebrates in laboratory studies: A review and analysis using Species Sensitivity Distributions. Environmental Toxicology and Chemistry 25: 2480-2489.

Frampton GK, Van den Brink PJ (2007) Collembola and macroarthropod community responses to carbamate, organophosphate and synthetic pyrethroid insecticides: Direct and indirect effects. Environmental Pollution 147: 14-25.

Frampton GK, Wratten SD (2000) Effects of benzimidazole and triazole fungicide use on epigeic species of Collembola in wheat. Ecotoxicology and Environmental Safety 46: 64-72.

Fredrickson JK, Bentjen SA, Bolton H, Li SW, Van Voris P (1989) Fate of Tn5 mutants of root growth inhibiting *Pseudomonas sp*. In intact soil core microcosms. Canadian Journal of Microbiology 33: 107-172.

Frömgen M (2008) Migrationspotential epigäischer Arthropoden in und aus Terrestrischen Modellökosystemen. Forschungspraktikum am Institut für Umweltforschung der RWTH Aachen. pp. 47.

Fromin N, Hamelin J, Tarnawski S, Roesti D, Jourdain-Miserez K, Forestier N, Teyssier-Cuvelle S, Gillet F, Aragno M, Rossi P (2002) Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. Environmental Microbiology 4: 634-643.

Gagliardi JV, Angle JS, Germida JJ, Wyndham Chanway RW, Rwatson RJ, Greer CW, McIntyre T, Yu HH, Levin MA, Russek-Cohen E, Rosolen S, Nairn J, Seib A, Martin-Heller T, Wisse G (2001) Intact soil-core microcosms compared with multi-site field releases for pre-release testing of microbes in diverse soils and climates. Canadian Journal of Microbiology 47: 237-252.

Gamma Design Software (2004) GS+: Geostatistics for the Environmental Sciences. Gamma Design Software, Plainwell, Michigan USA.

Germano JD (1999) Ecology, statistics, and the art of misdiagnosis: The need for a paradigm shift. Environmental Reviews 7: 167-190.

Gescher A (1993) Metabolism of N,N-dimethylformamide: key to the understanding of its toxicity. Chemical Research in Toxicology 6: 245-251.

Gile JD (1983) 2,4-D - Its distribution and effects in a ryegrass ecosystem. Journal of Environmental Quality 12: 406-412.

Gile JD, Collins JC, Gillett JW (1980) Fate of selected herbicides in a terrestrial laboratory microcosm. Environmental Science and Technology 14: 1124-1128.

Gile JD, Collins JC, Gillett JW (1982) Fate and impact of wood preservatives in a terrestrial microcosm. Journal of Agricultural and Food Chemistry 30: 295-301.

Gile JD, Gillett JW (1981) Fate and transport of organophosphate insecticides in a terrestrial laboratory ecosystem. Journal of Agricultural and Food Chemistry 29: 616-621.

Gillett JW, Gile JD (1976) Pesticide fate in terrestrial laboratory ecosystems. International Journal of Environmental Studies 10: 5-22.

Gillett JW, Gile JD (1983) Predator-prey (vole-cricket) interactions: the effects of wood preservatives. Environmental Toxicology and Chemistry 2: 83-93.

Gisi U, Schenker R, Schulin R, Stadelmann FX, Sticher H (1997) Bodenökologie. Thieme, Stuttgart. pp. 360.

Goddard SJ (1979) A modified heat extractor for separating arthropods from decidous woodland litter. Pedobiologia 19: 26-33.

Gomes NCM, Fagbola O, Costa R, Rumjanek NG, Buchner A, Mendona-Hagler L, Smalla K (2003) Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. Applied Environmental Microbiology 69: 3758-3766.

Gosset WS (1908) The probable error of a mean. Biometrika 6: 1-25.

Greville RW, Morgan AJ (1991) A comparison of lead, cadmium and zinc accumulation in terrestrial slugs maintained in microcosms: evidence for metal tolerance. Environmental Pollution 74: 115-127.

Grimm V, Wissel C (1997) Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. Oecologia 109: 323-334.

Haderlein A, Bonin Aly-Hassan MC, Legros R, Ramsay BA (1999) The design and use of aerated microcosms in mineralization studies. Biodegradation 10: 437-442.

Hamilton WE, Dindal DL (1989) Influence of earthworms and leaf litter on edaphic variables in sewage-sludge-treated soil microcosms. Biology and Fertility of Soils 7: 129-133.

Hamilton WE, Dindal DL, Parkinson CM, Mitchell MJ (1988) Interaction of earthworm species in sewage-sludge-amended soil microcosms: *Lumbricus terrestris* and *Eisenia foetida*. The Journal of Applied Ecoloy 25: 847-852.

Hansen RA, Coleman DC (1998) Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (Acari: Oribatida) in litterbags. Applied Soil Ecology 9: 17-23.

Hawksworth DL (1991) The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycological Research 95: 641-655.

Hawksworth DL (2004) Fungal diversity and its implications for genetic resource collections. Studies in Mycology 50: 9-18.

Hedlund K, Griffiths B, Christensen S,Scheu S, Setälä H, Tscharntke T, Verhoef H (2004) Trophic interactions in changing landscapes: responses of soil food webs. Basic and Applied Ecology 5: 495-503.

Heimann-Detlefsen D (1991) Auswirkungen eines unterschiedlich intensiven Pflanzenschutz- und Düngemitteleinsatzes auf die Collembolenfauna des Ackerbodens. Dissertation Universität Braunschweig.

Heimbach F, Ratte HT (1997) Use of a similarity index to compare the species composition of aquatic communities in order to investigate ecosystem recovery. Abstract Seventh Annual Meeting of SETAC-Europe, Amsterdam, the Netherlands.

Heise M, Schuphan I, Ebing W (1988) Einsetzbarkeit künstlicher Modellökosysteme zum Testen von Umweltchemikalien. Forschungsbericht 106 05 033/ Umweltforschungsplan des Bundesministers für Umwelt, Naturschutz und Reaktorsicherheit.

Heisler C (1994) Auswirkungen von Bodenverdichtungen auf die Bodenmesofauna: Collembola und Gamasina - ein dreijähriger Feldversuch. Pedobiologia 38: 566-576.

Herbst M, Van Esch GJ (1991) International programme on chemical safety, environmental health criteria. 124 - Lindane. World Health Organization, Geneva.

Hergarten W (1985) Die Collembolenfauna verschieden bewirtschafteter Flächen am Niederrhein. Decheniana 138: 135-148.

Hill MO (1973) Reciprocal averaging: an eigenvector method of ordination. Journal of Ecology 61: 237-249.

Hoenig JM, Heisey DM (2001) The Abuse of Power: The Pervasive Fallacy of Power Calculations for Data Analysis. The American Statistician 55: 1-6.

Hommen U, Veith D, Dülmer U (1994) A computer program to evaluate plankton data of freshwater field tests. In: Hill IR, Heimbach F, Leeuwangh P, Matthiessen P (eds.): Freshwater Field Tests for Hazard Assessment of Chemicals, Lewis Publishers, Boca Raton, FL 503-513.

Hopkin SP (1997) Biology of Springtails (Insecta: Collembola). Oxford University Press Inc., New York. pp. 330.

Hopkin SP (2006) Collembola of Britain and Ireland. Map of *Isotomurus palustris*. http://www.stevehopkin.co.uk/collembolamaps/Entomobryomorpha/316IRpal/ (last accessed 12/2011).

Hubert L, Tuckowa S (2003) The oribatid communities (Acari: Oribatida) on different stands of two meadows. Ekologia (Bratislava) 22: 443-456.

Huhtaa V, Persson T, Setälä H (1998A) Functional implications of soil fauna diversity in boreal forests. Applied Soil Ecology 10: 277-288.

Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54: 187-211.

Isnard P, Flammarion P, Roman G, Babut M, Bastien Ph, Bintein S, Essermeant L, Ferard JF, Gallotti-Schmitt S, Saouter E, Saroli M, Thiebaud H, Tomassone R, Vindimian E (2001) Statistical analysis of regulatory ecotoxicity tests. Chemosphere 45: 659-669.

ISO - International Organization for Standardization (2001) Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. Soil quality - ISO/DIS 11267.

ISO - International Organization for Standardization (2004) Water quality - Guidance on statistical interpretation of ecotoxicity data. ISO TC 147/SC 5. ISO/TS 20281. pp. 242.

ISO - International Organization for Standardization (2003a) Hand-sorting and formaline extraction of earthworms. Soil quality-Sampling of soil invertebrates, ISO/DIS 23611-1.

ISO - International Organization for Standardization (2003b) Sampling and extraction of microarthropods (Collembola and Acarina). Soil quality-Sampling of soil invertebrates, ISO/DIS 23611-2 .

ISO - International Organization for Standardization (2003c) Sampling and soil extraction of enchytraeids. Soil quality-Sampling of soil invertebrates, ISO/DIS 23611-3 .

ISO - International Organization for Standardization (2003d) Sampling, extraction and identification of free-living stages of terrestrial nematodes. Soil quality-Sampling of soil invertebrates, ISO/DIS 23611-4.

Ives AR, Carpenter SR (2007) Stability and Diversity of Ecosystems. Science 317: 58-62.

Jackson DR, Ausmus BS, Levin M (1979) Effects of arsenic on nutrient dynamics of grassland microcosms and field-plots. Water, Air and Soil Pollution 11: 13-21.

Jackson DR, Levin M (1979) Transport of arsenic in grassland microcosms and field plots. Water, Air and Soil Pollution 11: 3-12.

Jackson DR, Selvidge DJ, Ausmus BS (1978B) Behaviour of heavy metals in forest microcosms: II Behaviour on nutrient cycle processes. Water, Air and Soil Pollution 10: 13-18.

Jackson DR, Selvidge DJ, Ausmus DS (1978A) Behaviour of heavy metals in forest microcosms: I Transport and distribution among components. Water, Air and Soil Pollution 10: 1-10.

Jackson DR, Washburne CD, Ausmus BS (1977) Loss of Ca and NO3-N from terrestrial microcosms as indicator of soil pollution. Water, Air and Soil Pollution 8: 279-284.

Jager T (2011) Some Good Reasons to Ban Ecx and Related Concepts in Ecotoxicology. Environmental Science & Technology 45: 8180-8181.

Jänsch S, Amorim MJ, Römbke J (2005) Identification of the ecological requirements of important terrestrial ecotoxicological test species. Environmental Reviews 13: 51-83.

Jänsch S, Frampton GK, Römbke J, Van Den Brink PJ, Scott-Fordsmand JJ (2006) Effects Of Pesticides On Soil Invertebrates In Model Ecosystem And Field Studies: A Review And Comparison With Laboratory Toxicity Data. Environmental Toxicology and Chemistry 25: 2490-2501.

Jones SE, Wiliams DJ, Holliman PJ, Taylor N, Baumann J, Förster B,Van Gestel CAM, Rodrigues JML (2004) Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - an instrument for testing potentially harmful substances: fate of the model chemical carbendazim. Ecotoxicology 13: 29-42.

Jones TM, Lewis HT Jr., Getsinger JG (1966) Formamide as a Component of Liquid Fertilizers. Journal of Agricultural and Food Chemistry 14: 20-23.

Joosse ENG (1970) The formation and biological significance of aggregations in the distribution of Collembola. Netherlands Journal of Zoology 20: 299-314.

Kampichler C (2001) Use of enclosed model ecosystems in soil ecology: a bias towards laboratory research. Soil Biology and Biochemistry 3: 269-275.

Kampichler C, Bruckner A, Baumgarten A, Berthold A, Zechmeister-Boltenstern S (1999) Field mesocosms for assessing biotic processes in soils: How to avoid side effects. European Journal of Soil Biology 35: 135-143.

Kampichler C, Bruckner A, Kandeler E, Bauer R, Wright J (1995) A mesocosm study design using undisturbed soil monoliths. Acta Zoologica Fennica 196: 71-72.

Kandeler E, Kampichler C, Joergensen RG, Mölter K (1999) Effects of mesofauna in a spruce forest on soil microbial communities and N cycling in field mesocosms. Soil Biology and Biochemistry 13: 1783-1792.

Kempson D, Lloyd M, Ghelardi R (1963) A new extractor for woodland litter. Pedobiologia 3: 1-21.

Kennedy N, Clipson N (2003) Fingerprinting the fungal community. Mycologist 17: 158-164.

Kloskowsky R, Scheunert I, Klein W, Korte F (1981) Laboratory screening of distribution, conversion and mineralization of chemicals in the soil-plant system and comparison to outdoor experimental data. Chemosphere 10: 1089-1100.

Knacker T, Marcinkowski A, Förster B, Arthur MF, Tolle DA (1989) Refinement of terrestrial microcosms for evaluating fate and effects of chemicals. Brighton Crop Protection Conference - Weeds 259-265.

Knacker T, Van Gestel CAM, Jones SE, Soares AMVM, Schallnaß HJ, Förster B, Edwards CA (2004) Ring-Testing and Field-Validation of a Terrestrial Model Ecosystem (TME) - An Instrument for Testing Potentially Harmful Substances: Conceptual Approach and Study Design. Ecotoxicology 13: 9-27.

Köhler W, Schachtel G, Voleske P (2002) Biostatistik: Einführung in die Biometrie für Biologen und Agrarwissenschaftler. Springer Verlag. pp. 302.

Koolhaas JEE, Van Gestel CAM, Römbke J, Soares AMVM, Jones SE (2004) Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - an instrument for testing potentially harmful substances: effects of carbendazim on soil microarthropod communities. Ecotoxicology 13: 75-88.

Kratz W (1998) The Bait-Lamina Test - General Aspects, Applications and Perspectives. Environmental Science and Pollution Research 5: 94-96.

Krell FT, Chung AYC, DeBoise E, Eggleton P, Giusti A, Inward K, Krell-Westerwalbesloh S (2005) Quantitative extraction of macro-invertebrates from temperate and tropical leaf litter and soil: efficiency and time-dependent taxonomic biases of the Winkler extraction. Pedobiologia 49: 175-186.

Krieg W, Becker R, Schick H (2007) In-door terrestrial model ecosystem - Experiences with TME filled with mixed field soil. Abstract Book of the SETAC Europe 17th Annual Meeting 258-259.

Kryazhimskii FV, Bolshakov VN (2008) Functional-Ecological Role of Biological Diversity in Populations and Communities. Russian Journal of Ecology 39: 383-389.

Laakso J, Salminen J, Setälä H (1995) Effects of abiotic conditions and microarthropod predation on the structure and function of soil animal communities. Acta Zoologica Fennica 196: 162-167.

Larink O, Kratz W (1994) Köderstreifen-Workshop in Braunschweig - ein Resümee. Braunschweiger Naturkundliche Schriften 4: 647-651.

Larkin RP, Kelly JM (1988) A short-term microcosm evaluation of CO2 evolution from litter and soil as influenced by SO2 and SO4 additions. Water, Air and Soil Pollution 37: 273-280.

Laskowski R (1995) Some Good Reasons to Ban the Use of NOEC, LOEC and Related Concepts in Ecotoxicology. Oikos 73: 140-144.

Lawton JH (1996) The ecotron facility at silwood park: the value of big bottle experiments. Ecology 77: 665-669.

Lebrun P, Van Straalen NM (1995) Oribatid mites: prospects for their use in ecotoxicology. Experimental & Applied Acarology 19: 361-379.

Leinaas HP (1978) Seasonal variation in sampling efficiency of Collembola and Protura. Oikos 31: 307-312.

Lepš L, Šmilauer P (1999) Multivariate Analysis of Ecological Data. Faculty of Biological Sciences, University of South Bohemia, Ceské Budejovice. pp. 110.

Leuthold R (1961) Vergleichende Untersuchungen der Tierwelt verschiedener Wiesenböden im oberbayrischen Raum, unter besonderer Berücksichtigung der Collembolen. Zeitschrift für angewandte Entomologie 49: 1-50.

Lichtenstein EP (1980) Fate and Behavior of Pesticides in a Compartmentalized Microcosm. In: Giesy JP (ed.): Microcosms in Ecological Research. Technical Information Center US Department of Energy. Springfield, USA 955-970.

Lichtenstein EP, Fuhremann TW, Schulz KR (1974) Translocation and metabolism of 14C-phorate as affected by percolating water in a model soil-plant ecosystem. Journal of Agricultural and Food Chemistry 22: 991-996.

Lichtenstein EP, Liang TT (1987) Effects of simulated rain on the transport of fonofos and carbofuran from agricultural soil in a three-part environmental microcosm. Journal of Agricultural and Food Chemistry 35: 173-178.

Lichtenstein EP, Liang TT, Koeppe MK (1982) Effects of fertilizers, captafol, and atrazine on the fate and translocation of [14C]fonofos and [14C]parathion in a soil plant microcosm. Journal of Agricultural and Food Chemistry 30: 871-878.

Lichtenstein EP, Liang TT, Koeppe MK (1983) Effects of soil mixing and flooding on the fate and metabolism of [14C]fonofos and [14]parathion in open and closed agricultural microcosms. Journal of Economic Entomology 76: 233-238.

Lichtenstein EP, Millington WF, Cowley GT (1962) Effect of various insecticides on growth and respiration of plants. Agricultural and Food Chemistry 10: 251-256.

Liebhold AM, Rossi RE, Kemp WP (1993) Geostatistics and Geographic Information Systems in Applied Insect Ecology. Annual Review of Entomology 38: 303-327.

Liess M, Beketov M (2011) Traits and stress: keys to identify community effects of low levels of toxicants in test systems. Ecotoxicology 20: 1328-1340.

Lindo Z, Winchester NN (2007) Local-regional boundary shifts in oribatid mite (Acari: Oribatida) communities: species-area relationships in arboreal habitat islands of a coastal temperate rain forest, Vancouver Island, Canada. Journal of Biogeography doi:10.1111/j.1365-2699.2007.01710.x .

Lock K, De Schamphelaere KAC, Janssen CR (2002) The effect of lindane on terrestrial invertebrates. Archives of Environmental Contamination and Toxicology 42: 217-221.

Lübben B (1991) Auswirkungen von Klärschlammdüngung und Schwermetallbelastung auf die Collembolenfauna eines Ackerbodens. Dissertation Universität Braunschweig .

LUBW - Landesanstalt für Umweltschutz Baden-Württemberg (1993) Stoffbericht Hexachlorcyclohexan (HCH). Texte und Berichte zur Altlastenbearbeitung 18/95. .

Lussenhop J (1971) A simplified canister-type soil arthropod extractor. Pedobiologia 11: 40-45.

MacFadyen A (1961) Improved funnel type extractors for soil arthropods. Journal of Animal Ecology 30: 171-184.

Malanchuk JL, Joyce K (1983) Effects of 2,4-D on nitrogen fixation and carbon dioxide evolution in a soil microcosm. Water, Air and Soil Pollution 20: 181-189.

Malanchuk JL, Mueller CA, Pomerantz SV (1980) Microcosm evaluation of the agricultural potential of fly-ash-amended soils. In: Giesy JP (ed.): Microcosms in Ecological Research. Technical Information Center US Department of Energy. Springfield, USA.

Mann HB, Whitney DR (1947) On a test of whether one of two random variables is larger than the other. Annals of Mathematical Statistics 18: 50-60.

Maraun M, Heethoff M, Schneider K, Scheu S, Weigmann G, Cianciolo J, Thomas RH, Norton RA (2004) Molecular phylogeny of oribatid mites (Oribatida, Acari): evidence for multiple radiations of parthenogenetic lineages. Experimental and Applied Acarology 33: 183-201.

Maraun M, Scheu S (2000) The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. Ecography 23 374-383.

Marshall VG (1972) Comparison of two methods of estimating efficiency of funnel extractors for soil micro-arthropods. Soil Biology and Biochemistry 4: 417-426.

May LA, Smiley B, Schmidt MG (2001) Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage. Canadian Journal of Microbiology 47: 829-841.

McCune B, Mefford MJ (1998) PC-ORD: Multivariate analysis of ecological data, version 4. Gleneden Beach, OR: MjM Software Design.

Merchant VA, Crossley DA (1970) An inexpensive, high-efficiency Tullgren extractor for soil microarthropods. Journal of the Georgia Entomological Society 5: 83-87.

Metcalf RL, Sangha GK, Kapoor IP (1971) Model Ecosystem for the Evaluation of Pesticide Biodegradability and Ecological Magnification. Environmental Science and Technology 5: 709-713.

Meynen E, Schmithüsen J (1953) Handbuch der naturräumlichen Gliederung Deutschlands. Selbstverlag der Bundesanstalt für Landeskunde, Remagen. pp. 1339.

Mieth A, Emde M, Janzen M, Nissanga J (1993) Testung von Pflanzenschutzmitteln und Umweltchemikalien in Semi-Freiland-Modellökosystemen. Bericht zum Forschungs- und Entwicklungsvorhaben Nr. 12605083 im Auftrag des Umweltbundesamtes. Berlin. pp. 209.

Mitchell MJ, Parkinson CM, Hamilton WE, Dindall DL (1982) Role of the earthworm, *Eisenia foetida*, in affecting organic matter decomposition in microcosms of sludge-amended soil. The Journal of Applied Ecology 19: 805-812.

Montforts MHMM (2006) Assessment of Persistency and Bioaccumulation in Pesticide Registration Frameworks within the Organization for Economic Cooperation and Development. Integrated Environmental Assessment and Management 2: 13-21.

Moore JC, DeRuiter PC, Hunt HW, Coleman DC, Freckman DW (1996) Microcosms and soil ecology: critical linkages between field studies and modelling food webs. Ecology 77: 694-705.

Morgan E, Knacker T (1994) The role of laboratory terrestrial model ecosystems in the testing of potentially harmful substances. Ecotoxicology 3: 213-233.

Moser T (2004) Auswirkungen des Fungizids Carbendazim auf Enchytraeidae (Annelida, Oligochaeta) in terrestrischen Modellökosystemen und im Freiland. PhD-thesis at the Johann Wolfgang Goethe Universität, Frankfurt am Main. pp. 234.

Moser T, Römbke J, Schallnaß HJ, Van Gestel CAM (2007) The use of the multivariate Principal Response Curve (PRC) for community level analysis: a case study on the effects of carbendazim on enchytraeids in Terrestrial Model Ecosystems (TME). Ecotoxicology 16: 573-583.

Moser T, Schallnaß HJ, Jones SE, Van Gestel CAM, Koolhaas JEE, Rodrigues JML, Römbke J (2004a) Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - an instrument for testing potentially harmful substances: Effects of Carbendazim on Nematodes. Ecotoxicology 13: 61-74.

Moser T, Van Gestel CAM, Jones SE, Koolhaas JEE, Rodrigues JML, Römbke J (2004b) Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - an instrument for testing potentially harmful substances: effects of carbendazim on enchytraeids. Ecotoxicology 13: 89-103.

Mulder C, Boit A, Bonkowski M, De Ruiter PC, Mancinelli G, Van Der Heijden MGA, VAN Wijnen HJ, Vonk JA, Rutgers M (2011) A Belowground Perspective on Dutch Agroecosystems: How Soil Organisms Interact to Support Ecosystem Services. In: Woodward G (ed.): Advances in Ecological Research 44 - Ecosystems in a Human-modified landscape: a European perspective 277-357.

Murray AE, Hollibaugh JT, Orrego C (1996) Phylogenetic compositions of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments. Applied Environmental Microbiology 62: 2676-2680.

Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied Environmental Microbiology 59: 695-700.

Naeem S, Thompson LJ, Lawler P, Lawton JH, Woodfin RM (1995) Empirical evidence that declining species diversity may alter the performance of terrestrial ecosystems. Philosophical Transactions of the Royal Society London 347: 249-262.

Nagpal NK (1986) Effect of soil and effluent characteristics on phosphorous sorption in dosed columns. Journal of Environmental Quality 15: 73-78.

Nash RG, Beall ML (1980A) Fate of Maneb and Zineb Fungicides in Microagroecosystem Chambers. Journal of Agricultural and Food Chemistry 28: 322-328.

Nash RG, Beall ML (1980B) Distribution of silvex, 2,4-D and TCDD applied to turf in chambers and field plots. Journal of Agricultural and Food Chemistry 28: 614-623.

Nash RG, Beall ML, Harris WG (1977) Toxaphene and l,l,l-Trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) losses from Cotton in an Agroecosystem Chamber. Journal of Agricultural and Food Chemistry 25: 336-341.

Nef L (1971) Influence de l`humidité sur le géotactisme des Oribates (Acarina) dans l`extracteur de Berlese-Tullgren. Pedobiologia 11: 433-445.

Neher DA (1999) Soil community composition and ecosystem processes - Comparing agricultural ecosystems with natural ecosystems. Agroforestry Systems 45: 159-185.

Nielsen CO, Christensen B (1959) The Enchytraeidae, critical revision and taxonomy of European species. Naturhistorisk Museum, Aarhus.

Nieminen JK, Setälä H (1998) Enclosing decomposer food web: implications for community structure and function. Biology and Fertilitiy of Soils 26: 50-57.

Niijma K (1971) Seasonal changes in collembolan populations in a warm temperate forest in Japan. Pedobiologia 11: 11-26.

Norton RA (1986) A variation of the Merchant-Crossley soil microarthropod extractor. Quaestiones Entomologicae 21: 669-671.

O´Connor GA, Wieranga PJ, Cheng HH, Dotader KG (1980) Movement of 2,4,5-T through large soil columns. Soil Science 130: 157-162.

Odum EP (1984) The Mesocosm. BioScience 34: 558-562.

OECD (2000) OECD guideline No. 217 for the testing of chemicals. Soil Microorganisms: Carbon Transformation Test. Adopted 21st January 2000, OECD, Paris.

OECD (2006) Guidance document on simulated freshwater lentic field tests (outdoor microcosms and mesocosms). OECD series on testing and assessment 53, OECD, Paris.

Oros-Sichler M, Gomes NCM, Neuber G, SmallaK (2005) A new semi-nested protocol to amplify large 18S rRNA gene fragments for PCR-DGGE analysis of soil fungal communities. Journal of Microbiological Methods 65: 63-75.

Ottermanns R (2008) Mustererkennung, Dimensionsreduktion und statistische Modellierung in der Ökologie - dargestellt am Beispiel der Lebensgemeinschaften grasiger Feldraine in deutschen Agrarlandschaften. Dissertation RWTH Aachen. pp. 468.

Overdieck D (1986) Long term effects of an increased CO2 concentration on terrestrial plants in model ecosystems; morphology and reproduction of Trifolium and Lolium. International Journal of Biometeorology 30: 323-332.

Overdieck D, Reinig E (1986A) Effect of atmospheric CO2-enrichment on perennial ryegrass and white clover competing in managed model ecosystems. I. phytomass production. Oecologia Plantarum 7: 357-366.

Overdieck D, Reinig E (1986B) Effect of atmospheric CO2-enrichment on perennial ryegrass and white clover competing in managed model ecosystems. II. nutrient uptake. Oecologia Plantarum 7: 367-378.

Parker LW, Ryder-White J, Thomas S, Whitford WG (1985) Effects of oxamyl and chlordane on the activities of non-target soil organisms. Biology and Fertility of Soils 1: 141-148.

Parmelee RW, Phillips CT, Checkai RT, Bohlen PJ (1997) Determining the effects of pollutants on soil faunal communities and trophic structure using a refined microcosm system. Environmental Toxicology and Chemistry 16: 1212-1217.

Parmelee RW, Wentsel RS, Phillips CT, Simini M, Checkai RT (1993) Soil microcosm for testing the effects of chemical pollutants on soil fauna communities and trophic structure. Environmental Toxicology and Chemistry 12: 1477-1486.

Pearson K (1896) Mathematical Contributions to the Theory of Evolution - On a Form of Spurious Correlation Which May Arise When Indices Are Used in the Measurement of Organs. Proceedings of the Royal Society of London 60: 489-498.

Perry JN (1995) Spatial analysis by distance indices. The Journal of Animal Ecology 64: 303-314.

Petersen H (1978) Some properties of two high-gradient extractors for soil microarthropods, and an attempt to evaluate their extraction efficiency. Natura Jutlandica 20: 95-122.

Pimm SL (1984) Complexity and stability of ecosystems. Nature 307: 321-326.

Piwoni MD, Wilson JT, Walters DM, Wilson BH, Enfield CG (1986) Behaviour of organic pollutants during rapid infiltration of waste water into soil 1. Processes, definition and characterisation using a microcosm. Hazardous Waste & Hazardous Materials 3: 43-55.

Polis GA, Strong DR (1996) Food web complexity and community dynamics. The American Naturalist 1547: 813-846.

Potapov M (2001) Synopses on Palearctic Collembola, Volume 3: Isotomidae. Abhandlungen und Berichte des Naturkundemuseum Görlitz 73: 1-603.

PSC - Pesticide Steering Committee (2012) Survey on needs and priorities regarding Guidance Documents for PSC October 2012. Communication & Information Resource Centre Administrator of the European Union http://circa.europa.eu/Members/irc/sanco/pest/library?l=/pesticides_committee/pesticide_committee_12&vm=detailed&sb=Title, accessed November 2012).

Raghu K, Murtghy NBK, Kale SP, Kulkarni MG (1996) Model Ecosystems for Predicting the Behaviour of Pesticides in the Environment. In: Environmental Behaviour of Crop Protection Chemicals -Proceedings Series. Proceedings of an International Symposium on the use of Nuclear and Related Techniques 209-213.

Roberts DW (1986) Ordination on the basis of fuzzy set theory. Plant Ecology 66: 123-131.

Römbke J (2006) Tools and Techniques for the Assessment of Ecotoxicological Impacts of Contaminants in the Terrestrial Environment. Human and Ecological Risk Assessment 12: 1-18.

Römbke J, Beck L, Förster B, Fründ HC, Horak F, Ruf A, Rosciczweski C, Scheurig M, Woas S (1997) Boden als Lebensraum für Bodenorganismen. Bodenbiologische Standortklassifikation. Handbuch Boden. Review edited by Landesanstalt für Umweltschutz Baden-Württemberg, Karlsruhe. pp. 404.

Römbke J, Höfer H, Garcia MVB, Martius C (2006) Feeding activities of soil organisms at four different forest sites in Central Amazonia using the bait lamina method. Journal of Tropical Ecology 22: 313-320.

Römbke J, Jänsch S, Roß-Nickoll M, Toschki A, Höfer H, Horak F, Russell D, Burkhardt U, Schmitt H (2012) Erfassung und Analyse des Bodenzustands im Hinblick auf die Umsetzung und Weiterentwicklung der Nationalen Biodiversitätsstrategie. UBA-Texte, FB370872201. pp. 386.

Römbke J, Knacker T (2003) Standardisation of Terrestrial Ecotoxicological Effect Methods: An example of successful international co-operation. Journal of Soils and Sediments 3: 237-238.

Römbke J, Moltmann JF (1996) Tests at various levels of Investigation. Laboratory Level. Semi-Field Level. Field-Level. In: Römbke J (ed.): Applied Ecotoxicology. Lewis Publishers. Boca Raton 89-158.

Römbke J, Van Gestel CAM, Jones, SE, Koolhaas JE, Rodrigues JML, Moser T (2004) Ringtesting and field-validation of a Terrestrial Model Ecosystem (TME) - an instrument for testing potentially harmful substances: effects of carbendazim on earthworms. Ecotoxicology 13: 105-118.

Rossi RE, Mulla DJ, Journel AG, Franz EH (1992) Geostatistical Tools for Modeling and Interpreting Ecological Spatial Dependence. Ecological Monographs 62: 277-314.

Roß-Nickoll M, Lennartz G, Fürste A, Mause R, Ottermanns R, Schäfer S, Smolis M, Theissen B, Toschki A, Ratte HT (2004) Die Arthropodenfauna von Nichtzielflächen und die Konsequenzen für die Bewertung der Auswirkungen von Pflanzenschutzmitteln auf den terrestrischen Bereich des Naturhaushaltes. Umweltbundesamt, Berlin. pp. 148.

Rothmaler W (2002a) Exkursionsflora von Deutschland. Gefäßpflanzen - Atlasband. Spektrum Akademischer Verlag, Heidelberg .

Rothmaler W (2002b) Exkursionsflora von Deutschland. Gefäßpflanzen - Grundband. Spektrum Akademischer Verlag, Heidelberg .

Rumpler N (2007) Einsatz von Denaturierender Gradienten-Gelelektrophorese zur Charakterisierung von Bodenpilzgemeinschaften in Terrestrischen Modell-Ökosystemen. Diplomarbeit am Institut für Umweltforschung, Lehrstuhl für Umweltbiologie und -chemodynamik, RWTH Aachen University. pp. 113.

Rusek J (1998) Biodiversity of Collembola and their functional role in the ecosystem. Biodiversity and Conservation 7: 1207-1219.

Russell DJ, Blümel H (2003) Feststellung und Modellierung der kurzfristigen Jahresdynamik und kleinräumigen Variabilität von endogäischen Insekten für Monitoringerhebungen auf Boden-Dauerbeobachtungsflächen in Baden-Württemberg. I. Horizontalverteilung bodenbiologischer Indikatoren und ihre Bedeutung für Probenahmeschemen. Zwischenbericht des Programms Lebensgrundlage Umwelt und ihre Sicherung (BWPLUS), BWC20020. pp. 16.

Rutgers M, Mulder C, Schouten AJ (eds.) (2008) Soil ecosystem profiling in the Netherlands with ten references for biological soil quality. RIVM Report 607604009/2008, National Institute for Public Health and the Environment (RIVM), Bilthoven. pp. 86.

Salt GH, Raw FSJ, Brian MV (1948) The arthropod population of pasture soil. Journal of Animal Ecology 117: 139-150.

Santos MJG, Ferreira V, Soares AMVM, Loureiro S (2011) Evaluation of the combined effects of dimethoate and spirodiclofen on plants and earthworms in a designed microcosm experiment. Applied Soil Ecology 48: 294-300.

Schäffer A, van den Brink P, Heimbach F, Hoy S, de Jong F, Römbke J, Sousa JP, Ross-Nickoll M (2008) Semi-field methods are a useful tool for the environmental risk assessment of pesticides in soil. Environmental Science and Pollution Research 50: 176-177.

Schäffer A, Van den Brink PJ, Heimbach F, Hoy SP, de Jong FMW, Römbke J, Roß-Nickoll M, Sousa JP (2011) Semi-Field Methods for the Environmental Risk Assessment of Pesticides in Soil. CRC Press. pp. 106.

Scheffer M, Carpenter S, Foley JA, Folke C, Walker B (2001) Catastrophic shifts in ecosystems. Nature 413: 591-596.

Scheu S (2002) The soil food web: structure and perspectives. European Journal of Soil Biology 38: 11-20.

Scheunert I, Schroll R, Cao G (1996) A Microcosm System to Evaluate the Fate of 14-C labelled Pesticides in Soils from Different Climate Zones. In: Environmental Behaviour of Crop Protection Chemicals (IAEA-SM-343/21). International Atomic Energy Agency, Vienna, Austria 181-186.

Schlegel HG (1992) Allgemeine Mikrobiologie. Georg Thieme Verlag, Stuttgart. pp. 634.

Schmelz RM (2003) Taxonomy of *Fridericia* (Oligochaeta, Enchytraeidae). Revision of species with morphological and biochemical methods. Abhandlungen der Naturwissenschaftlichen Vereinigung Hamburg (NF) 38: 1-415.

Schneider K (2005) Feeding biology and diversity of oribatid mites (Oribatida, Acari). Thesis TU Darmstadt. pp.127.

Schneider K, Scheu S, Maraun M (2007) Microarthropod density and diversity respond little to spatial isolation. Basic and Applied Ecology 8: 26-35.

Scholz-Starke B, Beylich A, Moser T, Nikolakis A, Rumpler N, Schäffer A, Theißen B, Toschki A, Roß-Nickoll M (2012) The response of soil organism communities to the application of the insecticide lindane in terrestrial model ecosystems. Ecotoxicology DOI 10.1007/s10646-012-1030-0. Online first.

Scholz-Starke B, Nikolakis A, Leicher T, Lechelt-Kunze C, Heimbach F, Theißen B, Toschki A, Ratte HT, Schäffer A, Roß-Nickoll M (2011) Outdoor Terrestrial Model Ecosystems are suitable to detect pesticide effects on soil fauna – Design and method development. Ecotoxicology 20: 1932-1948.

Schulz HJ, Bretfeld G, Zimdars B (2003) Verzeichnis der Springschwänze (Collembola) Deutschlands. In: Klausnitzer B (editor): Entomofauna Germanica. 6. Entomologische Nachrichten und Berichte Beiheft 8: 11-25.

Schuphan I (1986a) Determination of the quantitive ecochemical and ecotoxicological behaviour of pesticides using vegetation chambers with controlled ventilation conditions. Plant Research and Development; a Biannual Collection of Recent German Contributions 23: 92-108.

Schuphan I (1986b) PCP-Verbleib mit 14C-markiertem Na-PCP in Modellansätzen unterschiedlicher Komplexizität. In: Weigmann G, Bornkamm R, Ebing W, Gruttke H, Haque A, Kielhorn U, Kratz W, Meyer G, Richtarsky G, Schuphan I: Wirkung und Verbleib von PCB-Na in einem Brachland-Ökosystem. Bundesministerium f. Forschung und Technologie, Berlin. pp. 191-221.

Schuphan I, Schärer E, Heise M, Ebing W (1987) Use of laboratory model ecosystems to evaluate quantitatively the behaviour of chemicals. In: Greenhalgh R, Roberts TR (eds.): Pesticide Science and Technology. Blackwell Science Publishers. Oxford. pp. 437-444.

Seastedt TR, Crossley DA (1978) Further investigations of microarthropod populations using the Merchant-Crossley high gradient extractor. Journal of the Georgia Entomological Society 13: 338-344.

Seastedt TR, Crossley DA (1983) Nutrients in forest litter treated with naphtalene and simulated throughfall: a field microcosm study. Soil Biology and Biochemistry 15: 159-165.

Setälä H, Haimi J, Huhta V (1988) A microcosm study on the respiration and weight loss in birch litter and raw humus as influenced by soil fauna. Biology and Fertility of Soils 5: 282-287.

Setälä H, Huhta V (1990) Evaluation of the soil fauna impact on decomposition in a simulated coniferous forest soil. Biology and Fertility of Soils 10: 163-169.

Shannon CE (1948) A Mathematical Theory of Communication. The Bell System Technical Journal 27: 379-423, 623-656.

Sheppard SC (1997) Toxicity testing using microcosms. In: Tarradellas J, Bitton G, Rossel D (eds).: Soil Ecotoxicology. Lewis Publishers. Boca Raton 345-373.

Shirazi MA, Lighthart B, Gillett J (1984) A method for scaling biological response of soil microcosms. Ecological Modelling 23: 203-206.

Siepel H (1996) Biodiversity of soil microarthropods: the filtering of species. Biodiversity and Conservation 5: 251-260.

Smit CE, Schouten AJ, Van den Brink PJ, Van Esbroek MLP, Posthuma L (2002) Effects of Zinc contamination on a natural nematode community in outdoor oil mesocosms. Archives of Environmental Contamination and Toxicology 42: 205-216.

Smith EP (1986) Randomized similarity analysis of multispecies laboratory and field studies. In: El Shaarawi AH, Kwiatkowski RE (eds.): Statistical aspects of water quality monitoring, Developments in water science 27:261-269. Elsevier Science Publishers B.V., Amsterdam, New York.

Smith EP (2002) BACI design. In: El-Shaarawi AH, Piegorsch WW (2002) Encyclopedia of Environmetrics. John Wiley & Sons, Ltd, Chichester 1: 141-148.

Smith S, Willis GH (1985) Movement of pesticides in soil columns as affected by ammonia. Environmental Toxicology and Chemistry 4: 425-434.

Snider RM, Snider RJ (1997) Efficiency of arthropod extraction from soil cores. Entomological News 108: 203-207.

Sousa JP, Rodrigues JML, Loureiro S, Soares AMVM, Jones SE, Förster B, Van Gestel CAM (2004) Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - an instrument for testing potentially harmful substances: effects of carbendazim on soil microbial parameters. Ecotoxicology 13: 43-60.

Southey JF (1986) Laboratory methods for work with plant and soil nematodes. Reference book 402, 6th edition. Her Majesty's Stationery Office, London, Ministry of Agriculture, Fisheries and Food. pp. 202.

Sovik G, Leinaas HP (2002) Variation in extraction efficiency between juvenile and adult oribatid mites: Ameronothrus lineatus (Oribatida, Acari) in a MacFadyen high-gradient canister extractor. Pedobiologia 46: 34-41.

SPSS Inc. (2005) SPSS 14.0 for Windows. SPSS Science Marketing Dept., Chicago, IL.

SRU - Sachverständigenrat für Umweltfragen (1985) Umweltprobleme der Landwirtschaft. Sondergutachten. Stuttgart: Kohlhammer.. pp. 423. (Bundestags-Drucksache 10/3613).

Stach J (1960) The Apterygotan Fauna of Poland in Relation to the World Fauna of this Group of Insects. Tribe: Orchesellini. Polska Akademia Nauk, Krakau.

Stach J (1963) The Apterygotan Fauna of Poland in Relation to the World Fauna of this Group of Insects. Tribe: Entomobryini. Polska Akademia Nauk, Krakau.

Stamou GP, Argyropoulou MD (1995) A preliminary study on the effect of Cu, Pb and Zn contamination of soils on community structure and certain life-history traits of oribatids from urban areas. Experimental & Applied Acarology 19: 381-390.

Sterzynska M (1990) Communities of Collembola in natural and transformed soils of the linden-oak hornbeam sites of the Mazovian Lowland. Fragmenta Faunistica 34: 166-260.

Storch V, Welsch U (2002) Kükenthal Leitfaden für das zoologische Praktikum. 24. Auflage. Spektrum Akademischer Verlag. Heidelberg. Berlin. pp. 496.

Strauch K (2009) Erfassung und Vergleich der epigäischen Bodenfauna in der Nachbarschaft zweier verschiedener Terrestrial Model Ecosystems (TME). Forschungspraktikum am Institut für Umweltforschung der RWTH Aachen. pp. 28.

Strenzke K (1952) Untersuchungen über die Tiergemeinschaften des Bodens: Die Oribatiden und ihre Synusien in den Böden Norddeutschlands. Zoologica Stuttgart 104: 1-173.

Suleman M, Arshad M, Jan GA (1979) Relative efficiency of mechanical and behavioral methods for the extraction of soil arthropods. Pakistan Journal of Zoology 11: 43-49.

Sulkava P, Huhta V (1998) Habitat patchiness affects decomposition and faunal diversity: a microcosm experiment on forest floor. Oecologia 116: 390-396.

Svendsen C, Weeks JM (1997) A simple low-cost field mesocosm for ecotoxicological studies on earthworms. Comparative Biochemistry and Physiology- Part C: Pharmacology, Toxicology and Endocrinology 1: 31-40.

Swedish EPA (2002) Priority list for chemicals to LRTAP and SC – Annex 1 to the Interim report. Governmental Commission, National Chemicals Inspectorate/Swedish EPA. pp. 31.

Taiz L, Zeiger E (1998) Physiologie der Pflanzen. Spektrum Akademischer Verlag, Heidelberg. pp. 773.

Takeda H (1979) On the extraction process and efficiency of MacFadyen´s high-gradient extractor. Pedobiologia 19: 106-112.

Tamura H (1976) Biases in extracting through Tullgren funnel. Revue D`Écologie et de Biologie Du Sol 17: 445-477.

Tanaka M (1970) The bio-economics on the populations of *Isotoma (Desoria) trispinata* MAC GILLIVRAY (Collembola; Isotomidae) and *Onychiurus* (Protaphorura) sp. (Collembola; Onychiuridae) in a grassfield. Publications from the Amakusa Marine Biological Laboratory 2: 51-120.

Ter Braak CJF (1986) Canonical Correspondence Analysis: A New Eigenvector Technique for Multivariate Direct Gradient Analysis. Ecology 67: 1167-1179.

Ter Braak CJF (1988) CANOCO - a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal component analysis and redundancy analysis (version 2.1). No. LWA-88-02. Technical Report. DLO-Agricultural Mathematics Group, Wageningen, The Netherlands.

Ter Braak CJF (1990) Update Notes: CANOCO version 3.10. Technical Report. DLO-Agricultural Mathematics Group, Wageningen, The Netherlands.

Ter Braak CJF, Prentice IC (1988) Partial canonical correspondence analysis. Advances in Ecological Research 18: 271-317.

Ter Braak CJF, Smilauer P (1998) Canoco Reference Manual and User's Guide to Canoco for Windows. Software for Canonical Community Ordination (version 4). Micro Computer Power. Ithaca, New York.

Teuben A (1991) Nutrient availability and interactions between soil arthropods and microorganisms during decomposition of coniferous litter: a mesocosm study. Biology and Fertility of Soils 10: 256-266.

Theißen B (2009) Die Collembolenzönosen ausgewählter Feldraine in drei unterschiedlichen Agrarlandschaften Deutschlands und die Bedeutung der Bodenfauna in der ökologischen Risikobewertung stofflicher Beeinträchtigungen aus der Agrarpaxis. Dissertation RWTH Aachen, Institut für Umweltforschung. pp. 241.

Theißen B, Scholz-Starke B, Hammers-Wirtz M, Kölzer U, Leicher T, Schäffer A, Roß-Nickoll M (2010) Does sequential sampling influence population dynamics of soil mesofauna in terrestrial model ecosystems? Poster presentation at the SETAC Europe 20th Annual Meeting 23-27 May 2010, Sevilla, Spain.

Thibaud JM, Schulz HJ, Da Gama Assalino MM (2004) Synopses on Palearctic Collembola, Volume 4: Hypogastruridae. Abhandlungen und Berichte des Naturkundemuseum Görlitz 75: 1-287.

Thomas SH (1978) Population density of nematodes under seven tillage regimes. Journal of Nematology 10: 24-27.

Tolle DA, Arthur MF, Chesson J, Van Voris P (1985) Comparison of pots versus microcosms for predicting agroecosystems effects due to waste amendment. Environmental Toxicology and Chemistry 4: 501-509.

Tolle DA, Arthur MF, Duke KM, Chesson J (1990) Ecological effects evaluation of two phosphorous-smoke producing compounds using terrestrial microcosms. In: Wang & Gorsuch (eds.): Plants for Toxicity Assessment. American Society for Testing and Materials. STP 1091 127-142.

Tolle DA, Arthur MF, Van Voris P (1983) Microcosm/field comparison of trace element uptake in crops grown on fly-ash amended soil. The Science of the total Environment 31: 243-261.

Toschki A (2008) Eignung unterschiedlicher Monitoring-Methoden als Grundlage zum Risk-Assessment für Agrarsysteme - Am Beispiel einer biozönologischen Reihenuntersuchung und einer Einzelfallstudie. Dissertation am Institut für Umweltforschung der RWTH-Aachen. pp. 162

ToxRat Solutions GmbH (2004) ToxRat Pro Version 2.09.

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11: 1111-1120.

Tuf IH, Tvardik D (2005) Heat-extractor – an indispensable tool for soil zoological studies. In: Tajovský IK, Schlaghamerský J, Pižl V (eds.) Contributions to Soil Zoology in Central Europe. ISB AS, CR, České Budějovice 191-194.

Tullgren A (1918) Ein sehr einfacher Ausleseapparat für terricole Tierfaunen. Zeitschrift für angewandte Entomologie 4: 149-150.

Turbé A, De Toni A, Benito P, Lavelle P, Lavelle P, Ruiz N, Van der Putten WH, Labouze E, Mudgal S (2010) Soil biodiversity: functions,threats and tools for policy makers. Bio Intelligence Service, IRD, and NIOO, Report for European Commission (DG Environment). pp. 250.

Ulman E (1972) Lindane, Monograph of an Insecticide. Verlag K. Schillinger, Freiberg im Breisgau.

US EPA - US Environmental Protection Agency (2006) Ecological Risk Assessment and the Ecological Technical Center. (Accessed March 2006). http://www.epa.gov/region5/superfund/ecology/index.html.

Usher MB, Booth RG, Sparkes KE (1982) A review of progress in understanding the organization of communities of soil arthropods. Pedobiologia 23: 126-144.

Van Bezooijen J (2006) Methods and Techniques for Nematology. International Report of-Nematology Departement Wageningen. Revised version of the manual. pp. 112.

Van den Brink PJ (2006) Letter to the Editor: Response to Recent Criticism on Aquatic Semi-field Experiments: Opportunities for New Developments in Ecological Risk Assessment of Pesticides. Integrated Environmental Assessment and Management 2: 202-203.

Van den Brink PJ, Ter Braak CJF (1997) Ordination of responses to toxic stress in experimental ecosystems. Toxicology & Ecotoxicology News Reviews 4: 173-177.

Van den Brink PJ, Ter Braak CJF (1998) Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis. Aquatic Ecology 32: 163-178.

Van den Brink PJ, Ter Braak CJF (1999) Principal Response Curves: Analysis of Time-Dependent Multivariate Responses of Biological Community to Stress. Environmental Toxicology and Chemistry 18: 138-148.

Van den Brink PJ, Ter Braak CJF (2011) Response to ‘‘traits and stress: keys to identify community effects of low levels of toxicants in test systems’’ by Liess and Beketov (2011). Ecotoxicology 21: 297-299.

Van den Brink PJ, Van Donk E, Gylstra R (1995) Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere 31: 3181-3200.

Van der Linden AMA, Boesten JJTI, Brock TCM, Van Eekelen GMA, De Jong FMW, Leistra M, Montforts MHMM, Pol JW (2006) Persistence of plant protection products in soil; a proposal for risk assessment. RIVM report 601506008/2006. pp. 107.

Van Gestel CAM, Koolhaas JEE, Schallnaß HJ, Rodrigues JML, Jones SE (2004) Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - an instrument for testing potentially harmful substances: effects of carbendazim on nutrient cycling. Ecotoxicology 13: 119-128.

Van Straalen NM, Leeuwangh P, Stortelder PBM (1994) Progressing Limits for Soil Ecotoxicological Risk Assessment. In: Donker MH, Eijsackers H, Heimbach F (eds.): Ecotoxicology of Soil Organisms. Lewis Publishers, Boca Raton 397-409.

Van Straalen NM, Rijninks PC (1982) The efficiency of Tullgren apparatus with respect to interpreting seasonal changes in age structure of soil arthropod populations. Pedobiologia 24: 197-209.

Van Wensem J, Adema T (1991) Effects of fluoride on soil fauna mediated litter decomposition. Environmental Pollution 72: 239-251.

Van Wensem J, Amorim M, Römbke J (2008) Soil protection in Europe. Integrated Environmental Assessment and Management 4: 519-520.

Van Wensem J, Jagers op Akkerhuis GAJM,Van Straalen NM (1991) Effect of the fungicide triphenyltin hydroxide on soil mediated litter decomposition. Pesticide Science 32: 307-316.

Van Wijngaarden RPA, Van den Brink PJ, Oude Voshaar JH, Leeuwangh P (1995) Ordination techniques for analyzing response of biological communities to toxic stress in experimental ecosystems. Ecotoxicology 4: 61-77.

Veeranagouda Y, Emmanuel Paul PV, Gorla P, Siddavattam D, Karegoudar TB (2006) Complete mineralisation of dimethylformamide by *Ochrobactrum sp*. DGVK1 isolated from the soil samples collected from the coalmine leftovers. Applied Microbiology and Biotechnology 71: 369-375.

Vink K, Van Straalen NM (1999) Effects of benomyl and diazinon on isopod-mediated leaf litter decomposition in microcosms. Pedobiologia 43: 345-359.

Von Törne E (1990) Assessing feeding activities of soil-living animals. I. Bait-lamina tests. Pedobiologia 34: 89-101.

Walter DE, Kethley J, Moore JC (1987) A heptane flotation method for recovering microarthropods from semiarid soils, with comparison to the Merchant-Crossley high-gradient extraction method and estimates of microarthropod biomass. Pedobiologia 30: 221-232.

Warwick NL (1975) The biology of free-living nematodes. Oxford University Press. pp. 219.

Weigmann G (1973) Zur Ökologie der Collembolen und Oribatiden im Grenzbereich Land-Meer. Zeitschrift für wissenschaftliche Zoologie 186: 295-391.

Weigmann G (2006) Hornmilben (Oribatida). In: Dahl F, Tierwelt Deutschlands 76. Goecke & Evers, Keltern. pp. 520.

Weigmann G, Kratz W (1981) Die deutschen Hornmilbenarten und ihre ökologische Charakteristik. Zoologische Beiträge 27: 459-489.

Weyers A, Schuphan I (1998) Variation of effect endpoint parameters in a terrestrial model ecosystem. Ecotoxicology 7: 335-341.

Weyers A, Sokull-Klüttgen B, Knacker T, Martin S, Van Gestel CAM (2004) Use of terrestrial model ecosystem data in environmental risk assessment for industrial chemicals, biocides and plant protection products in the EU. Ecotoxicology 13: 163-176.

Whittaker J (1984) Model interpretation from the additive elements of the likelihood function. Applied Statistics 33: 52-64.

Whittaker RH (1967) Gradient analysis of vegetation. Biological Reviews of the Cambridge Philosophical Society 42: 207-264.

Wiles JA, Frampton GK (1996) A field bioassay approach to assess the toxicity of insecticide residues on soil to Collembola. Pesticide Science 47: 273-285.

Wilke BM, Fleischmann S (2001) Ökotoxikologische Testbatterien. Chapter 9 in Michels J (2001) Leitfaden Biologische Verfahren zur Bodensanierung. Umweltbundesamt, Berlin.

Williams DA (1971) A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27: 103-117.

Williams DA (1972) The comparison of several dose levels with a zero dose control. Biometrics 28: 519-531.

Willmann C (1931) Moosmilben oder Oribatiden (Cryptostigmata). In: Dahl F (ed.): Die Tierwelt Deutschlands, 22 (5). Gustav Fischer, Jena. pp. 79-200.

Wilson JT, Smith GB, Cochran JW, Barker JF, Roberts PV (1987) Field evaluation of a simple microcosm simulating the behaviour of volatile organic compounds in subsurface materials. Water Resources Research 23: 1547-1553.

Wink U (1969) Die Collembolen- und Oribatidenpopulationen einiger saurer Auböden Bayerns in Abhängigkeit von der Bodenfeuchtigkeit. Zeitschrift für angewandte Entomologie 64: 121-135.

Winkelmann DA, Klaine SJ (1991) Atrazine metabolite behaviour in soil-core microcosms. Formation, disappearance, and bound residues. In: Somasundaram L, Cooats JR: Pesticide transformation products: fate and significance in the environment. ACS Symposium Series 459: 75-92.

Wood TG (1967) Acari and Collembola of moorland soils from Yorkshire, England. III. The microarthropod communities. Oikos 18: 277-292.

Wright DH, Coleman DC (1988) Soil fauna vs. Fertilization effects on plant nutrition: results of a biocide experiment. Biology and Fertility of Soils 7: 46-52.

Xiao CL, Hao JJ, Subbarao KV (1997) Spatial Patterns of Microsclerotia of Verticillium dahliae in Soil and Verticillium Wilt of Cauliflower. Phytopathology 87: 325-331.

Yeates GW (2003) Nematodes as soil indicators: functional and biodiversity aspects. Biology and Fertility of Soils 37: 199-210.

Yeates GW, Barker GW, Pottinger RPAF (1983) Effects of Oxamyl and Carbofuran on nematode populations below 10 grass cultivars. New Zealand Journal of Experimental Agriculture 11: 147-151.

Zechmeister-Boltenstern S, Baumgarten A, Bruckner A, Kampichler C, Kandeler E (1998) Impact of faunal complexity on nutrient supply in field mesocosms from a spruce forest soil. Plant and Soil 1: 45-52.

Zimdars B, Dunger W (1994) Synopses on Palearctic Collembola, Volume 1: Tullbergiinae. Abhandlungen und Berichte des Naturkundemuseum Görlitz 68: 1-71.

VIII
Additions

VIII-1 Abstract

The Environmental Risk Assessment (ERA) of pesticides in soil is based on a tiered approach that uses surrogate indicators of risk spanning from single species in the laboratory at the first stage to soil organism communities in the field. This thesis aims to contribute to the usability of semi-field methods in ERA using a Terrestrial Model Ecosystem (TME) approach to bridge the gap between laboratory approaches and full-scale field studies. It was purposed to give a comprehensive evaluation of the recent knowledge on higher-tier semi-field methods in soil against the background of the requirements of the international regulatory demands and in the light of the new European pesticide regulation 1107/2009/EC. The scientific base has been laid down by a series of own TME-experiments. The subsequent analysis of the data regarding the stability, ecotoxicological sensitivity and representativeness of the systems was focused on effects on community structures of soil organisms, such as collembolans, oribatid mites, nematodes, enchytraeids and soil fungi. This thesis hypothesises that TME-experiments are well suited to answer relevant ecotoxicological questions as long as performed on a sound statistical, methodological and ecological basis.

Chapter I provides a framework of the systematic approach that is applied in this thesis. The effects to soil communities and the corresponding detection limits depend largely on the intrinsic variability and the sensitivity (combined resilience and resistance) of the systems. Further, a profound background on the legal and regulatory framework is given. Since TME are meant to provide a high degree of realism comparable to field studies, the first chapter gives an overview on the characteristics of soil ecosystems and the ecology of the soil organisms gathered in TME studies at hand.

Chapter II gives an overview of the experimental approaches, the test compound lindane (gamma-hexachlorocyclohexane) and the assessment endpoints measured in both TME and field studies. The complex community-level data of different soil organism groups requires a variety of uni- and multivariate statistical methods.

Chapter III involves the description and interpretation of effects of the persistent and toxic pesticide lindane on soil microarthropod communities that were detected and assessed in a one-year range-finding study in TME. The open, intact soil cores (diameter 300 mm, height 400 mm) included indigenous soil organisms of the undisturbed grassland of the coring area. Forty units were placed outdoors in an experimental facility at the RWTH Aachen University between spring 2005 and 2006. The TME experiment was designed to focus on structural endpoints such as population dynamics of soil organisms and their community structures in case higher-tier evaluation is triggered under the propositions of the European pesticide regulation 1107/2009/EC. The key objective was at first to evaluate the dynamics and stability of species-diverse microarthropod communities on grassland over a period of time that is relevant for assessing the recovery potential of the TME communities following toxic stress. On grassland soils, less selection pressure towards insensitive species compared to arable land was presumed. Sufficient numbers of organisms and replicates of the experimental units ensured that a statistical evaluation could be performed to estimate the sensitivity of the organisms upon application of lindane applied at high rates of 7.5 and 75 kg active ingredient (a.i.)/ha. The application rates resulted in nominal concentrations of 10 and 100 mg a.i./kg dry soil referred to the top 5 cm soil layer of 10 TME each; 20 untreated TME served as controls and were used to study the natural dynamics and the variability of populations under controlled field conditions. The results showed that TME soil cores maintained communities of soil organisms marked by typical diversity of improved grassland. Lindane applied at excessive rates caused clear dose-related and long-lasting effects on the communities of microarthropods. On the contrary, lumbricids, the total feeding activity (bait lamina) and the growth of plant biomass were not affected by both treatments. Based on the results of this study, we assumed the methodology to be suitable for use in a regulatory context of ERA.

Based on the results of the first effect study, a modified ‘dose-response study’ with the same compound lindane was designed (**Chapter IV).** Further organism groups were included, so that the effects on collembolans, oribatid mites, nematodes, soil fungi and plant biomass could be determined in forty-two TME. Lindane was applied in five concentrations between 0.032 mg a.i./kg dry soil and 3.2 mg a.i./kg dry weight soil, six-fold replicated each. Twelve TME served as untreated controls. Abundance and community structures of oribatids, collembolans, enchytraeids, nematodes and fungi were recorded. Oribatid mites’ community responded three months after treatment, although they were not significantly affected by the overall treatment regime. Collembolans in total and species-specific abundance as well as the community endpoints were adversely affected by moderate dosages of lindane. Effects were tran-

sient between three and five months after treatment with a recovery within one year. No significant effects were detected for enchytraeids, nematodes and fungi. The study design and the obtained results allow for calculations of no observed effect concentrations below the highest treatment level for populations and for soil communities as defined entities, as well as effective concentrations as indicators of dose related responses.

Chapter V leaves behind the field of solely ecotoxicological evaluation and turns towards general considerations of the suitability of TME for applied and conceptual research questions. The ecology of the coring area is described by means of floristic and faunistic surveys. The distribution of collembolans is analysed by geostatistical methods and categorized as patchy or gradiental. These findings lead to conclusions on optimized soil coring strategies, which are proposed as small-scaled as possible to avoid excess variability. The temporal stability of TME is investigated under the propositions of the criteria 'no tendency towards altered abundances', 'no tendency towards altered diversity structures' and 'similarity of communities compared to the original state'. The important issue 'for which kind of habitat is the particular TME representative' is questioned. It is answered by the statistical comparison of similarities between TME and soil organism communities in the field. It has been concluded that a high degree of similarity between field and TME samples remains manifest over time and the pattern persists the large seasonal variation of community structures. The species inventory of our TME includes many common collembolan species of typical agricultural landscapes in Central Europe. Recurring more closely to the subject of ecotoxicology, prospective power analyses led to an estimation of the limits of effect detection. For this purpose, the intrinsic variability of either grassland or TME, which was estimated from, own data were used. On average, the detectable differences of abundances of treatment groups compared to control level (MDD) was between five percent for nematodes and about fifty percent for enchytraeids, collembolans and oribatids, markedly varying between sampling dates.

This thesis gives overview of the available semi-field approaches, offers guidance how to design and interpret TME studies and demonstrates the potential of TME in ecotoxicological studies. The chosen TME approach was examined from different angles and was finally considered suitable for a variety of scientific and regulatory problems regarding stressor-induced changes of soil communities.

VIII-2 Zusammenfassung

Die Zulassung von Pflanzenschutzmitteln beruht auf Abschätzungen des Umweltrisikos für Bodenorganismen in einem gestuften Ansatz, der sich zunächst auf Effekte stützt, die in Einzelartentests unter Laborbedingungen erfasst worden sind. Ergeben sich auf den ersten Bewertungsstufen unvertretbare Risiken, so werden höherwertige Testsysteme zur Risikoabschätzung herangezogen. In der vorliegenden Arbeit wird die Eignung von Terrestrischen Modellökosystemen (TME) als höherwertige Halbfreilandtests in der Umweltrisikobewertung untersucht, um einen Beitrag zu leisten, die Lücke zwischen Labortests und Feldstudien zu schließen. Es wird eine umfassende Beschreibung und Bewertung des Wissensstandes über Halbfreilandmethoden als höherwertige Testverfahren in der Pestizidzulassung angestrebt, die vor dem Hintergrund der aktuellen regulatorischen Entwicklungen seit dem Inkrafttreten der neuen Verordnung 1107/2009/EC zum Inverkehrbringen von Pflanzenschutzmitteln von zunehmender Relevanz sind. Die wissenschaftliche Basis dazu wurde in einer Reihe von TME-Experimenten gelegt, die zum Ziel hatten, die Stabilität, die ökotoxikologische Empfindlichkeit und die Repräsentativität von TME analysieren zu können. Dazu wurde besonderes Augenmerk auf Effekte auf Ebene der Lebensgemeinschaften von Springschwänzen, Hornmilben, Nematoden, Enchytraeen und Bodenpilzen gelegt.

Die vorliegende Arbeit basiert auf der Hypothese, dass die Verwendung von TME geeignet ist ökotoxikologische Fragestellungen adäquat zu bearbeiten, solange sie auf belastbarer statistischer, methodischer und ökologischer Grundlage geplant werden.

Kapitel 1 legt den Rahmen des systematischen Ansatzes fest, der der vorliegenden Arbeit zugrunde liegt. Die detektierbaren ökotoxikologischen Effektschwellen hängen stark von der systemimmanenten Variabilität und der toxikologischen Empfindlichkeit (als Kombination von Resilienz und Resistenz) der Lebensgemeinschaften ab. Es wird ein Überblick über die regulatorischen Hintergründe von Halbfreilandstudien sowie über die Eigenschaften von Bodenökosystemen und die Ökologie von Bodenorgansimen gegeben.

Kapitel 2 beschreibt den experimentellen Ansatz, die stofflichen und toxikologischen Eigenschaften der Modellsubstanz Lindan (gamma-Hexachlorocyclohexan) und die Vorgehensweise bei der Applikation der Testsubstanz. Die methodischen Grundlagen zur Erfassung der Umweltvariablen und der funktionellen und strukturellen Endpunkte werden vorgestellt. Die Auswertung der komplexen Datensätze wird mit uni- und multivariaten statistischen Methoden ausgeführt.

In **Kapitel 3** werden die Effekte hoher Konzentrationen der persistenten und toxischen

Testsubstanz Lindan auf die Lebensgemeinschaften von Boden-Mikroarthropoden in einer TME-Studie zum „range-finding“ über den Zeitraum von einem Jahr verfolgt. Die offenen, intakten Bodenkerne (Durchmesser 300 mm, Höhe 400 mm) waren von der ursprünglichen Lebensgemeinschaft eines unbehandelten Wiesenbodens besiedelt, wie er auf der TME-Entnahmefläche vorgefunden wurde. Vierzig Versuchseinheiten wurden zwischen dem Frühjahr 2005 und 2006 unter Freilandbedingungen in einer Versuchsanlage untergebracht. Die Studie war darauf ausgerichtet, strukturelle Endpunkte im Boden zu erfassen, die unter den Bedingungen der neuen Pestizidgesetzgebung in der Europäischen Union zur Umweltrisikobewertung herangezogen werden. Das Hauptaugenmerk lag dabei zunächst auf der Bewertung der Stabilität von TME hinsichtlich der Artenvielfalt von Boden-Mikroarthropoden über einen längeren Zeitraum, der relevant für eine Abschätzung des Wiedererholungspotentials der Lebensgemeinschaften nach toxischer Belastung mit persistenten Substanzen ist. Die Behandlung erfolgte durch Aufsprühen von zwei Applikationsraten (7.5 und 75 kg Lindan/ha) auf jeweils 10 TME. Diese Menge entspricht Bodenkonzentrationen von 10 und 100 mg aktive Substanz (a.s.)/kg Trockengewicht Boden in den obersten fünf Zentimetern des Bodenschicht. Zwanzig TME dienten als unbehandelte Kontrollen und wurden auch dazu genutzt die natürlichen Populationsdynamiken und die Variabilität der Bodenorganismen zu studieren. Die Ergebnisse zeigen, dass die TME diverse und typische Lebensgemeinschaften von bewirtschafteten Wiesenböden aufrechterhalten können. Die Modellsubstanz Lindan zeigte klare dosisabhängige und langanhaltende Effekte auf die Lebensgemeinschaften der Mikroarthropoden. Im Gegensatz dazu konnten keine Effekte auf Regenwürmer, auf die Fraßaktivität (Köderstreifenmethode) und die pflanzliche Biomasse nachgewiesen werden. Aus den Ergebnissen und Erfahrungen, die in dieser Studie gewonnen wurden, konnte zunächst die grundsätzliche Eignung der Methode zur Verwendung in der Umweltrisikobewertung von Pflanzenschutzmitteln abgeleitet werden.

Auf den Erkenntnissen der ersten Effektstudie wird im **Kapitel 4** beschrieben, wie mit einem modifizierten Versuchsdesign unter Verwendung derselben Testsubstanz Lindan die Ableitung von Dosis-Wirkungsbeziehungen unternommen wurde. Es wurden weitere Organismengruppen einbezogen, so dass schließlich die Effekte auf die Abundanzen und Strukturen der Lebensgemeinschaften von Collembolen, Oribatiden, Nematoden, Enchytraeen und Bodenpilzen sowie auf die Biomasse der Vegetation in insgesamt 42 TME erfasst werden konnten. Lindan wurde in fünf Behandlungsstufen von 0.032 und 3.2 mg a.s./kg Trockengewicht Boden eingesetzt. Jede Behandlungsstufe wurde 6-fach repliziert. Zwölf TME dienten als unbehandelte Kontrollen. Die Lebensgemeinschaften der Hornmilben zeigten drei Monate nach

der Applikation der Testsubstanz Abweichungen vom Kontrollniveau, während über den gesamten Versuchszeitraum betrachtet keine signifikanten Effekte auf die Struktur nachgewiesen werden konnten. Die Collembolen zeigten sowohl auf Ebene der Abundanzen der gesamten Gruppe als auch einzelner Arten, wie auch auf Ebene der Struktur der Lebensgemeinschaften signifikante Abweichungen zu den Kontrollen. Alle Endpunkte zeigten eine Wiedererholung zum letzten Probenahmezeitpunkt ein Jahr nach der Applikation. Für die Gruppen der Bodenpilze, der Enchytraeen und Nematoden konnten keine signifikanten Effekte nachgewiesen werden. Es zeigte sich, dass das Studiendesign geeignet war, sowohl Effektschwellen (als no-observed effect concentrations „NOEC“) oder Dosis-Wirkungsbeziehungen (als effective concentrations „EC_x“) abzuleiten, wobei eine konsequente Ausrichtung auf eines der beiden Konzepte der Trennschärfe der Analysen zugutekommt.

Kapitel 5 wendet sich von der rein ökotoxikologischen Evaluation ab und den allgemeingültigen Analysen der Eignung von TME für angewandte und konzeptionelle Fragestellungen zu. Im Rahmen dieser Arbeit wurde die Ökologie der TME-Entnahmefläche in floristischen und faunistischen Erhebungen intensiv untersucht. Geklumpte, gleichmäßige oder an von Umweltgradienten abhängige Verteilungsmuster von Collembolen werden mit geostatistischen Methoden beschrieben. Die Schlussfolgerungen aus diesen Analysen werden für Empfehlungen zu optimierten Entnahmestrategien für TME-Bodenkerne genutzt. TME sollten so kleinräumig wie technisch möglich gestochen werden, um homogene Versuchseinheiten zu erhalten und übermäßig hohe Varianz zu vermeiden. Die Stabilität von TME über die Zeit ist hinsichtlich der Kriterien „Systematische Tendenz zu Änderungen der Abundanz und der Diversität“ und „Ähnlichkeit der TME-Lebensgemeinschaften zur ursprünglichen Artzusammensetzung“ untersucht worden. Die TME bleiben über einen Zeitraum von einem Jahr hinsichtlich aller Kriterien stabil. Der für die Übertragbarkeit von TME-Studien entscheidende Frage, für welche realen Habitate die Testsysteme repräsentativ sind, wird anhand des Vergleichs mit der Entnahmefläche und mit dem Arteninventar von Bodenarthropoden mitteleuropäischer Agrarlandschaften nachgegangen. Es kann der Schluss gezogen werden, dass die Ähnlichkeit zwischen dem Freiland und den TME auch über längere Zeiträume groß bleibt und nicht von saisonalen Verschiebungen überlagert wird. Die Zusammensetzung der Collembolen-Lebensgemeinschaften der TME wird von hohen Anteilen typischer Arten der Agrarlandschaften bestimmt. Prospektive Abschätzungen der statistischen Power zur Abschätzung der Detektierbarkeit von ökotoxikologischen Effekten wurden unter Verwendung der Variabilität durchgeführt, die in den eigenen Untersuchungen aufgetreten ist. Die im Mittel unterscheidbare Differenz zwischen einer Behandlungsgruppe und einer Kontrollgruppe (MDD) beträgt

zwischen fünf Prozent für Nematoden und 50 Prozent für Collembolen, Oribatiden und Enchytraeen.

Diese Arbeit gibt einen Überblick über die verfügbaren Halbfreilandsysteme in der Ökotoxikologie, stellt Orientierungshilfen für Planung und Durchführung von TME-Studien dar und zeigt das große Potential auf, das dem TME-Ansatz innewohnt. Der hier gewählte TME-Ansatz ist von unterschiedlichen Seiten beleuchtet worden und ist schließlich als geeignet für wissenschaftliche und regulatorische Fragestellungen betrachtet worden.

VIII-3 Curriculum vitae

Personal information

Name: Björn Scholz-Starke

Address: Von-Broich-Str. 23

Date and place of birth: 1977-05-16, Krefeld-Uerdingen

Marital status: single

Education and training

1983-1987	Primary school: Gemeinschaftsgrundschule Sonsbeck
1987-1996	Secondary school: Städtisches Stiftsgymnasium Xanten
08/1996-08/1997	Civil service: Initiative Integratives Leben e.V. in Sonsbeck
10/1997-05/2004	Studies of Biology at the RWTH Aachen University (focus on Ecology, Ecotoxicology, Microbiology, Animal Physiology and Environmental Health, Degree: Diploma
10-12/2004	Contractor at the Institute for Environmental Research, RWTH Aachen University
01/2005-10/2009	Scientific assistant at the Institute for Environmental Research, RWTH Aachen University
11/2009- 04/2011	Scientific assistant at the Federal Environmental Agency of Germany, Section IV 1.3 Pesticides
Since 05/2011	Scientific assistant at the Institute for Environmental Research, RWTH Aachen University

VIII-4 Referred Articles

Parts of this thesis have been published in scientific journals.

Scholz-Starke B, Beylich A, Moser T, Nikolakis A, Rumpler N, Schäffer A, Theißen B, Toschki A, Roß-Nickoll M (2012) The response of soil organism communities to the application of the insecticide lindane in terrestrial model ecosystems. Ecotoxicology DOI 10.1007/s10646-012-1030-0 (Online first).

Scholz-Starke B, Nikolakis A, Leicher T, Lechelt-Kunze C, Heimbach F, Theißen B, Toschki A, Ratte HT, Schäffer A, Roß-Nickoll M (2011) Outdoor Terrestrial Model Ecosystems are suitable to detect pesticide effects on soil fauna: design and method development. Ecotoxicology 20: 1932-1948.

VIII-5 Acknowledgements

Quite exhausted at the end of the reading of an extensive PhD thesis that comes under the name of a single author, it could be suggestive that it was done in complete independence. However, I think you already anticipated that many people must have contributed their knowledge, support, power, patience and passion.

First of all, I would like to thank my direct supervisor Andreas Schäffer to give me the opportunity to stay at your Institute for quite a time and your confidence that the work will be finished at the end. Toni Ratte is thanked for actively participating in many meetings, for observing and advising during the work. I thank both of you for finally reviewing the thesis.

Martina, I thank you for the great deal of trustfulness and the constant support over the years. In particular, I owe you the opportunity to come home after my eastern exile. You gave me the freedom to develop own ideas, ceded me far-reaching responsibilities and mentored me whenever necessary.

I would particularly like to thank the people of Bayer CropScience Fred Heimbach, Christa Lechelt-Kunze, Jürgen Keppler, Frank Saalfeld, Andreas Richter, Thorsten Leicher, Alexander Nikolakis and Ursula Kölzer. You really brought the idea of complex semi-field studies forward with your funding, discussions and the generous attitude you ceded me the data gathered in the TME studies.

Thank you David ‘the magic snap hooker’, Wege and Hans for carrying the heavy stuff during facility building and TME coring. Thanks to the people of Gut Melaten and the staff of the workshop of the Biology of the RWTH Aachen University (AVZ + Sammelbau) for providing me tools, ideas and specialized constructions that I urgently needed. Being all thumbs, I couldn’t do without your help. Many further people from the Institute has to be thanked for their field and lab work, the PhD-students Gregor, Matthias, Michael, the helping hands Philipp, Ingvar, Sabrina, Saskia, Viola and especially Jule, head of the lab raising the quality of sample preparation nearly to a GLP gold standard. Special thanks to the Diploma student Nadine ‘Rumpelchen’ and the young researchers Gus, Marko and Katrin who contributed substantially to some of the questions raised by this work. Monika from the research institute gaiac is thanked for linking the aquatic mesocosm experiences with the peculiarities of terrestrial systems. Richard, for statistical advice when it came to complicated multivariate issues. Telse, thank you for your great cooperativeness in every way and many enriching non-scientific conversations.

Mascha, I thank you for your support of all kinds in the first couple of years of my work on

this long-term project, and for opening my mind towards the habits and customs of our western neighbours.

My colleagues Bernhard and Tosch, you have been the last of my office mates that sustained in the longer term. You have been the pioneers of TME enthusiasm (together with Martina) with a high risk of infection. We discussed all kinds of scientific issues and in fact issues of life after-work, even to the point of transcendental phenomena. It was a lot of fun.

Thanks to my parents Irmgard and Herbert for never giving up asking for progress reports and for not going too much into the details if they turned out to be short. My brother Kai, thank you for providing your help from the sturdy tasks at the beginning of an experiment to the intellectual challenges of statistical data analysis.

Mela, my love, I am happy and proud that we shared the last, hardest and most intensive part of the path. I am very thankful for high-speed proofreading and important suggestions, and for doing everything that I could focus completely on my personal progress. This thesis would have never been finished without you. Emma und Nina for your empathy and constant wondering what on earth is intended by all the symbols, lines, tables and endless reasoning.

IX

Register

IX-1 Figures

IX-2 Tables

IX-3 Equations

IX-4 Tabular appendices

The tabular appendices can be accessed online at **www.bio5.rwth-aachen.de/bss**