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Assessing the risks of pesticides to soil communities using Terrestrial Model Ecosystems

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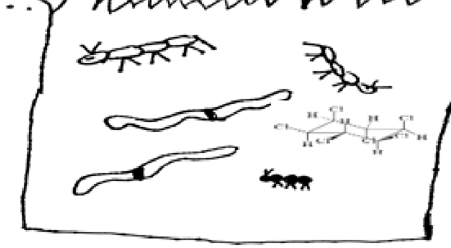
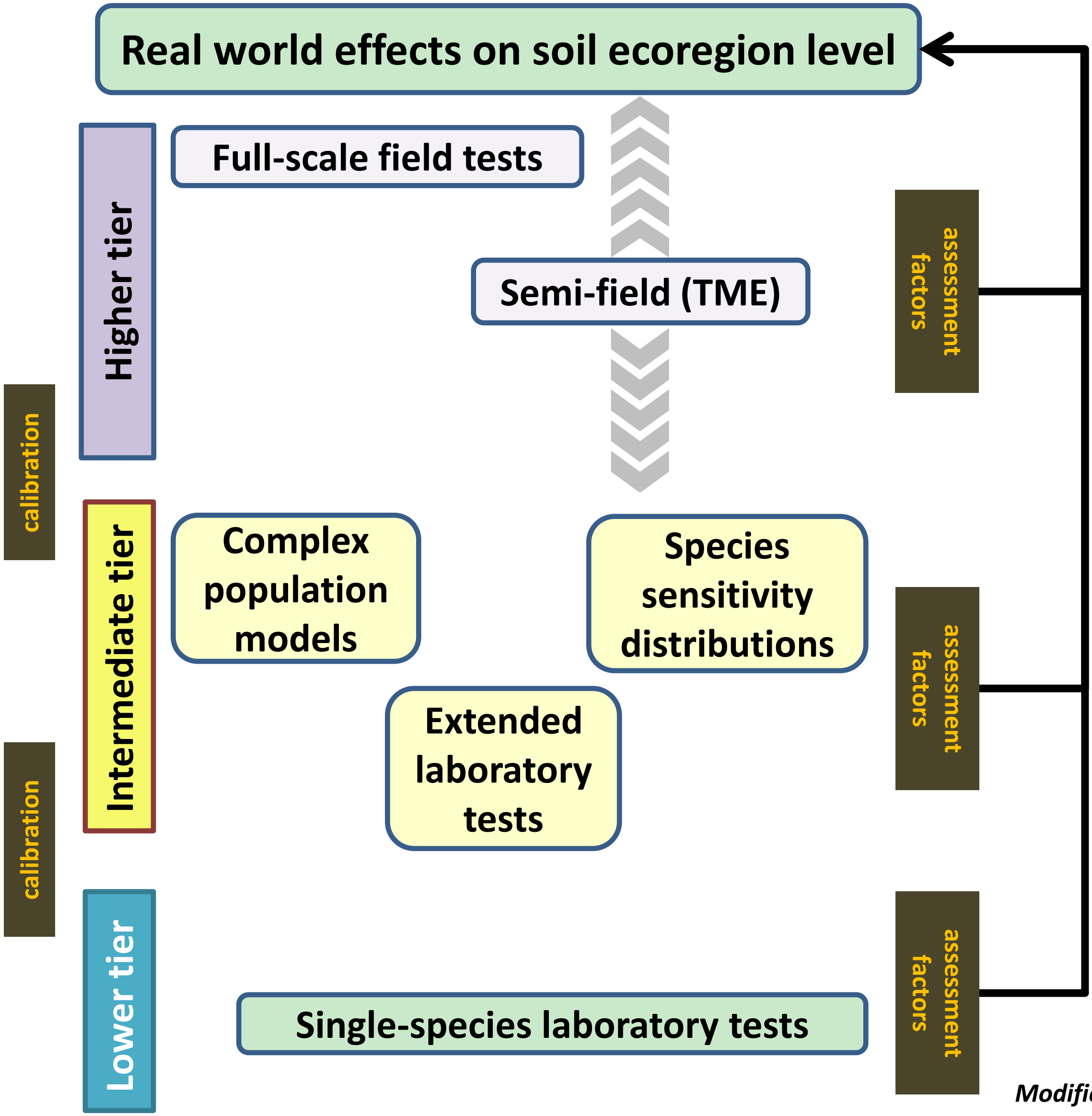
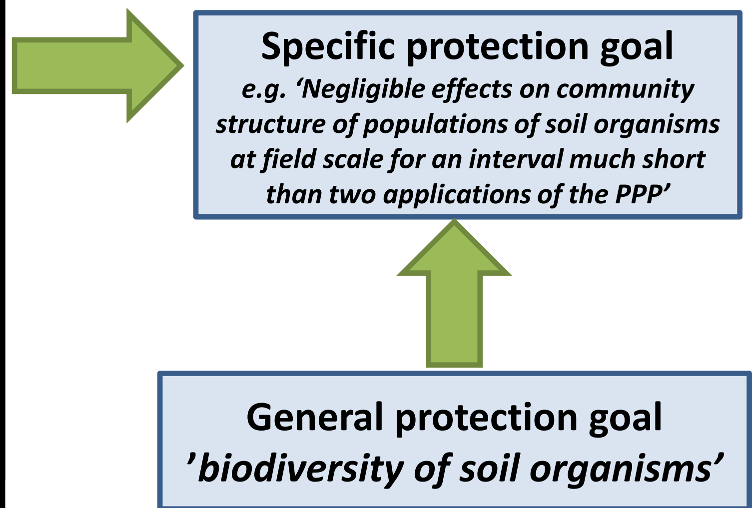


Terrestrial Model Ecosystems…

- Originally developed for **risk assessment of plant protection products**
- Address the general protection goal **'no unacceptable effects on biodiversity and ecosystems'** of arable fields or grasslands (not directly off-field structures)
- Include **several species** of (relevant) soil taxa to reflect a realistic species composition, diversity and food web structure compared to lower tier studies
- Start of an experiment **with a complex community** or **after establishment of interaction patterns** typical for the respective habitat type
- **Run for several weeks up to months**, including at least one complete reproduction cycle for most taxa
- Systems reflect (semi-) **natural population dynamics** typical for the seasons around the year

Calibration of soil risk assessment by semi-field reference tiers



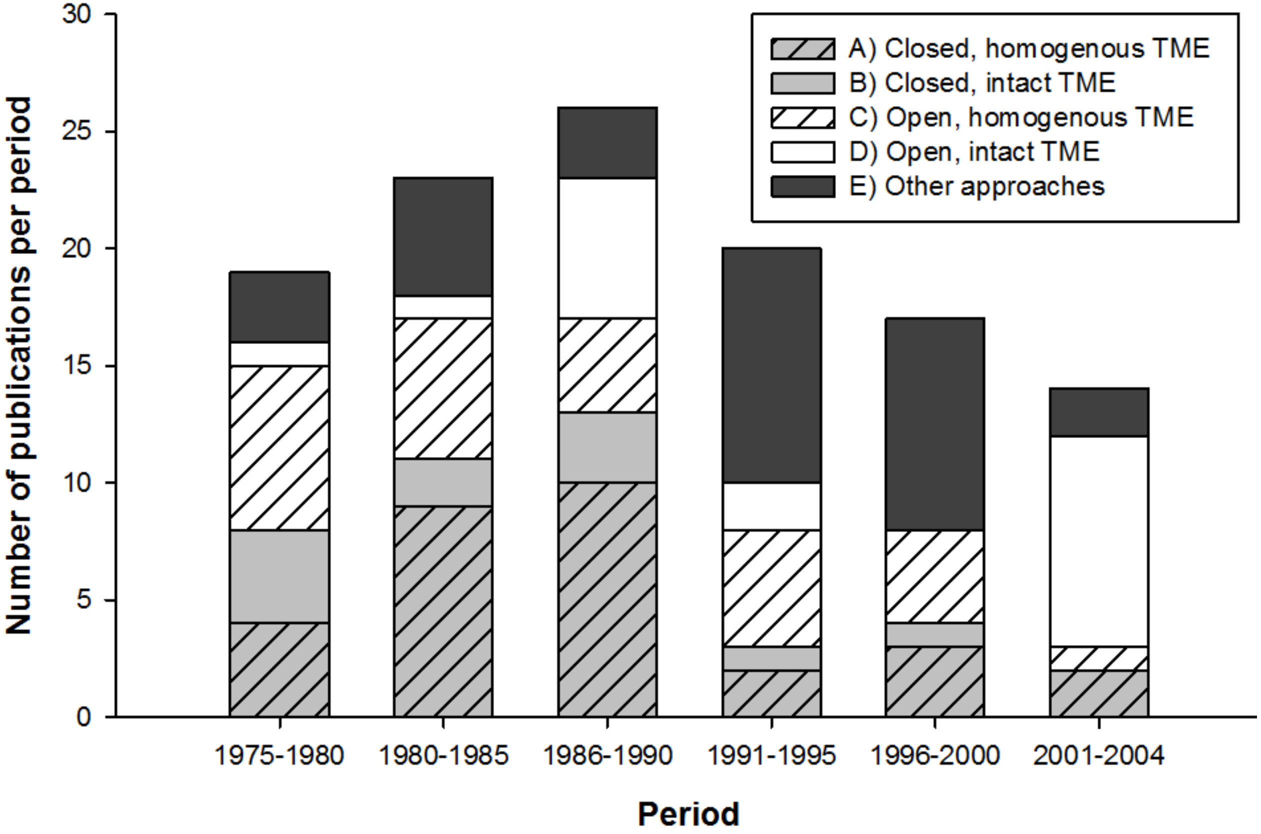


Information needed for calibration

- Variability of measurements
- Uncertainty analysis of predictions
- Representativeness of test system

Modified according to EFSA PPR Panel (2010) EFSA Journal 8(10): 1821

Availability of methods I



- **Widely agreed:** intact, open TME equipped with complex communities provide better opportunities to address biodiversity issues than assembled, homogenous systems

- Numerous different approaches from basic (ecological) to applied (ecotoxicological) research available

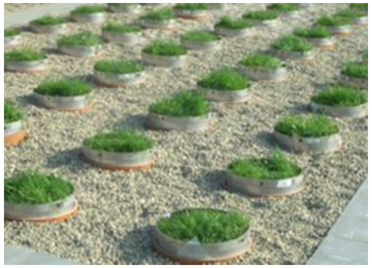
Scholz-Starke (2013) RWTH Aachen University. pp. 291.

Availability of methods II

Wide variety of approaches have been described in the literature…

- Species composition → from assembled to natural originating from different ecosystems
- Different test substances →pesticides, veterinary pharmaceuticals, heavy metals, biocides
- Different endpoints → survival, reproduction, feeding rates, microbial, vegetation and soil animal communities
- No guidance documents but recent workshop proceedings , e.g. PERAS

(*Schäffer et al. 2011. CRC Press. pp. 106.*)



Design of case study I

Sequential sampling

Coring

Storage under controlled conditions

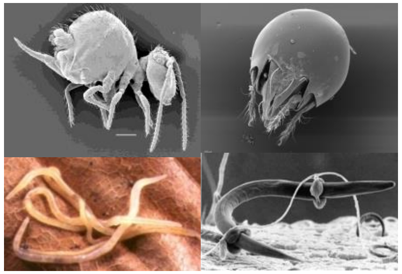
Application of a test item

Recycling

Sub-sampling

Extraction of organisms

Determination



Design of case study II

Stability over time

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- One-year ‘control’ study with full set of endpoints

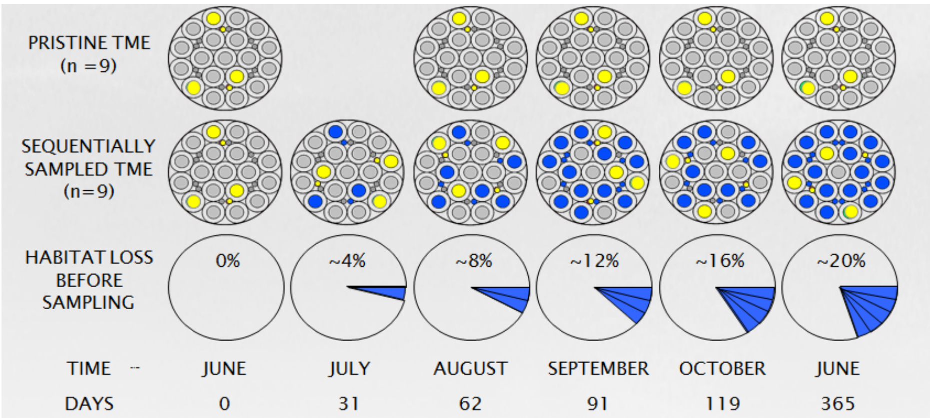
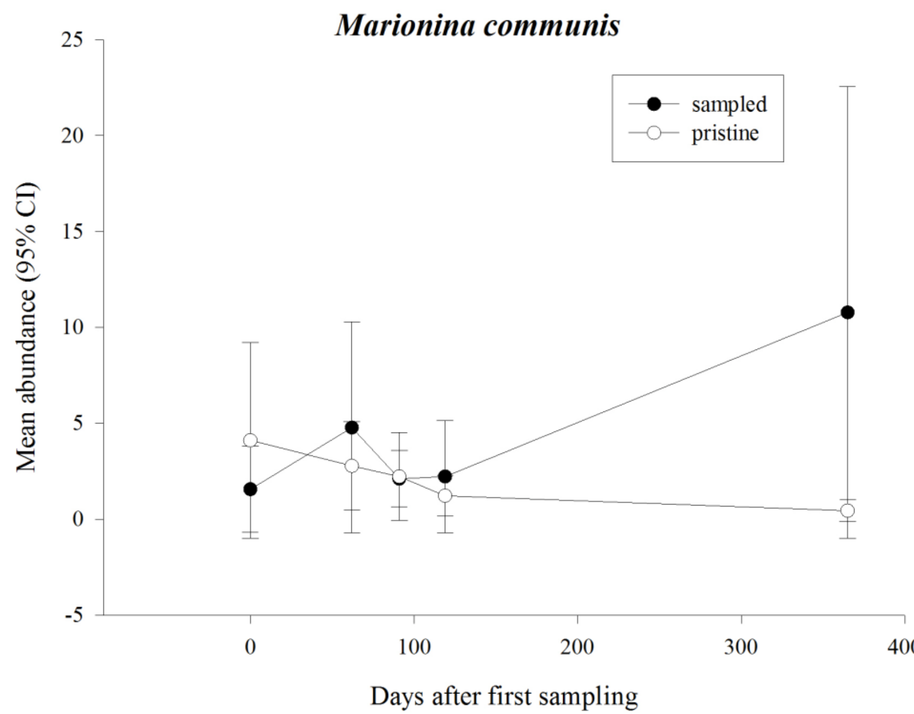


FIGURE 2: Sampling scheme, habitat loss and time scale of the presented semi-field experiment. At six sampling dates in total 54 TME were involved, 5*9 pristine and 1*9 sequentially sampled TME. Since the TME facility contains only 55 placeholders, at one date (day 31) samples were taken in the sequentially sampled treatment only.

SUItability of
Subsampling Terrestrial Model
Ecosystems
SEquentially

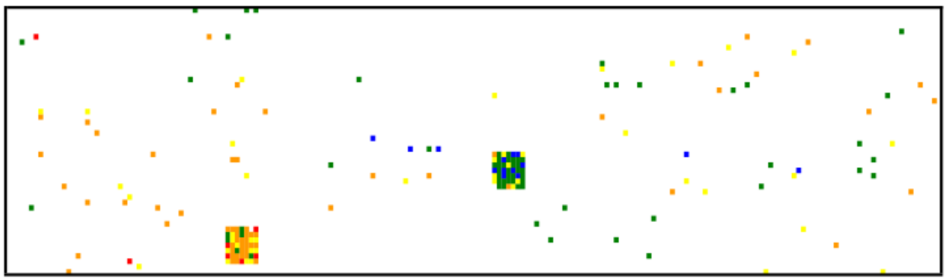


- Vast majority of endpoints did not show clear directed effects of the sampling strategy

Theißen , Scholz-Starke , Hammers-Wirtz , Kölzer , Leicher, Schäffer, Roß-Nickoll (2010) Proceedings SETAC Annual Meeting, Sevilla.

Design of case study III
Avoidance of excess variation

(1) Systematic sampling on designated coring site



(2) Description of distribution patterns

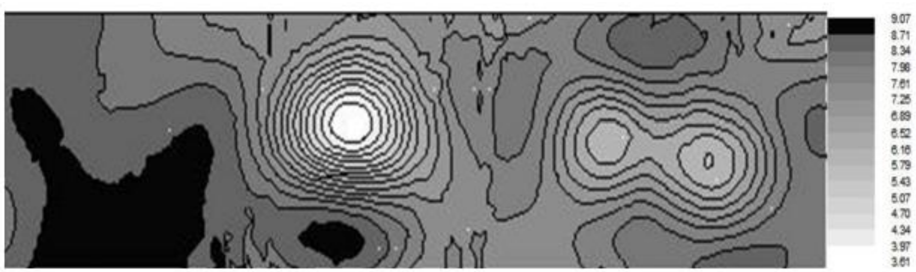
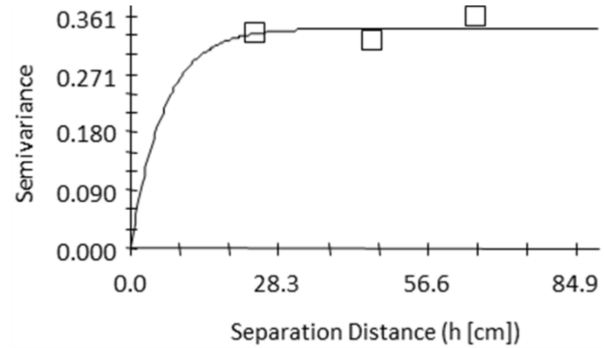


Figure V-10: Kriging diagram of *I. palustris* (abundance per sample) (data: *bsc*).

Scholz-Starke (2013) RWTH Aachen University. pp. 291.

(3) Modelling of spatial autocorrelation

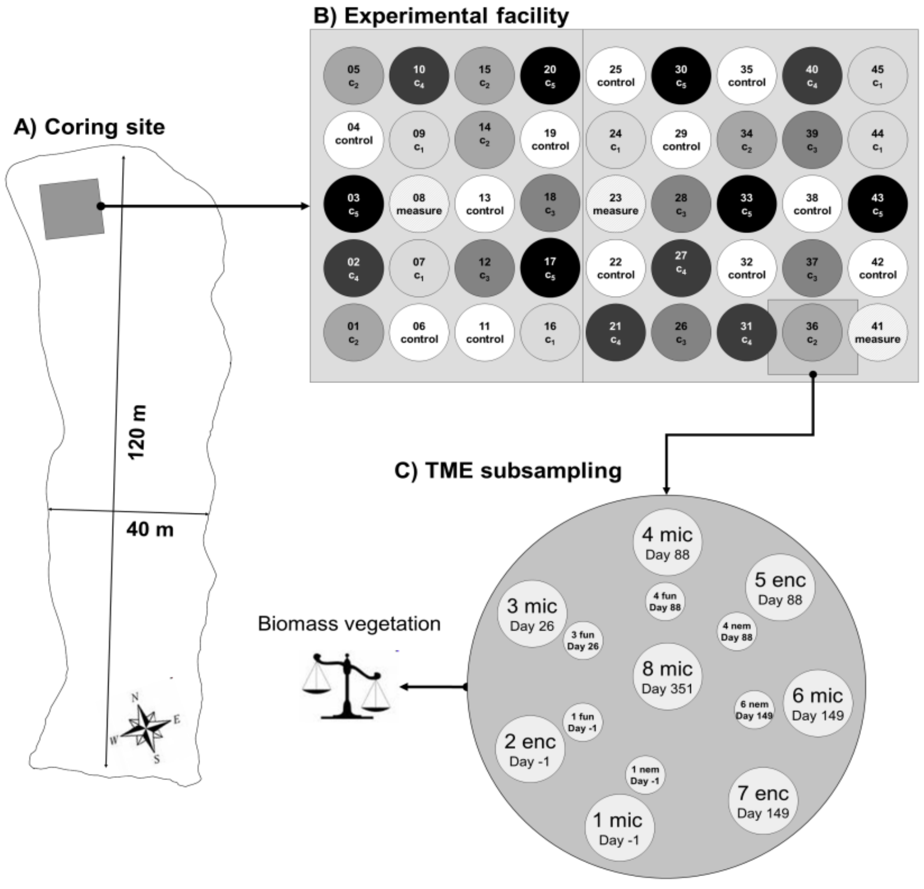


SS1*: *Exponential model
C_o = 0.0030; C_o+C = 0.3430; A_o = 6.3; r^2 = 0.041; RSS = 7.001E-04

(4) Best-fit coring strategy → e.g. not exceed 5 m distance for soil coring

Design of case study IV
Endpoints

- **Replication** $N_{\text{control replicates}} = 12$; $N_{\text{treatment replicates}} = 6$; $N_{\text{treatments}} = 5$
- **Sampling period** day -1 351 after application of lindane, 5 sampling dates
- **Endpoints**
  - Collembolans (species abundance)
  - Oribatids (species abundance)
  - Enchytraeids (species abundance)
  - Nematodes (species abundance)
  - Fungi (DGGE-patterns)
  - [species abundance Lumbricids]
  - [Plant biomass]
- **Application** of lindane/alternative insecticide
  - C_1 = 0.032 mg lindane / kg dry soil **OR** 250 g a.i. / kg dry soil
  - C_2 = 0.1 mg lindane / kg dry soil **OR** 500 g a.i. / kg dry soil
  - C_3 = 0.32 mg lindane / kg dry soil **OR** 1000 g a.i. / kg dry soil
  - C_4 = 1.0 mg lindane / kg dry soil **OR** 2000 g a.i. / kg dry soil
  - C_5 = 3.2 mg lindane / kg dry soil **OR** 4000 g a.i. / kg dry soil

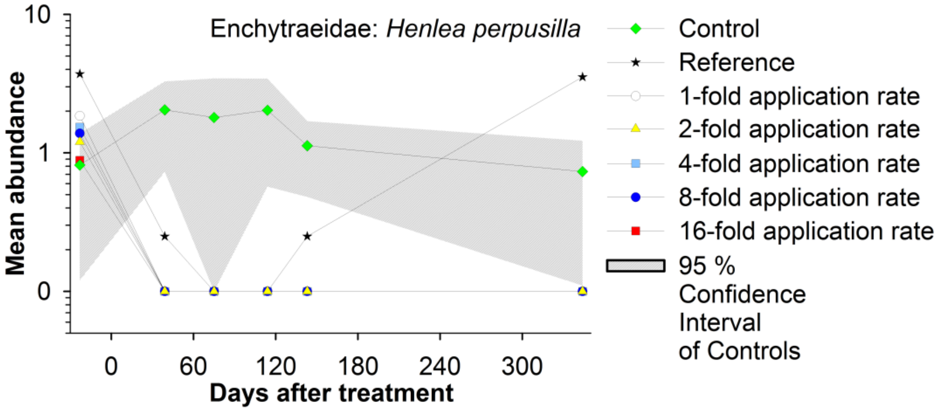


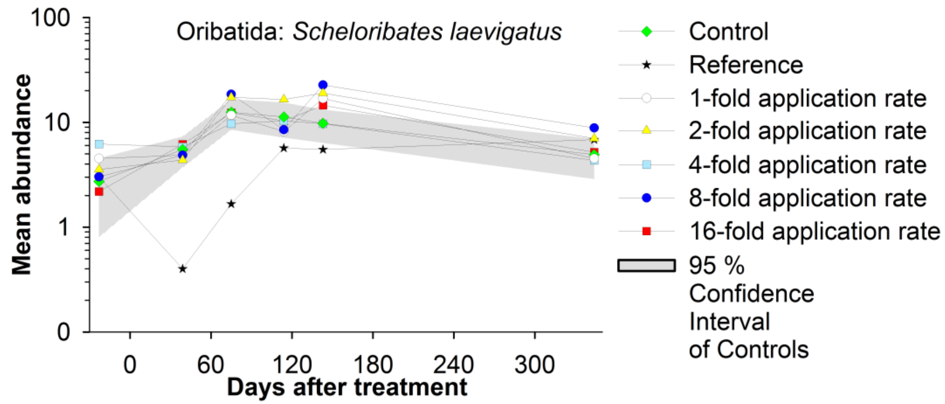
Scholz-Starke (2013) RWTH Aachen University. pp. 291.

Effects on population level

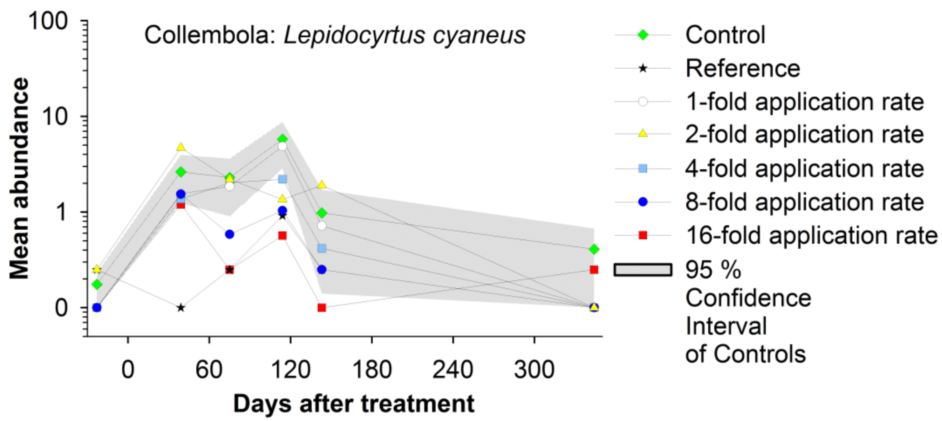
– Very sensitive → all treatments at all dates after treatment were significantly different from controls

– Insensitive → no treatment related effects, reference demonstrates principle sensitivity





– Sensitive with recovery → initial effects

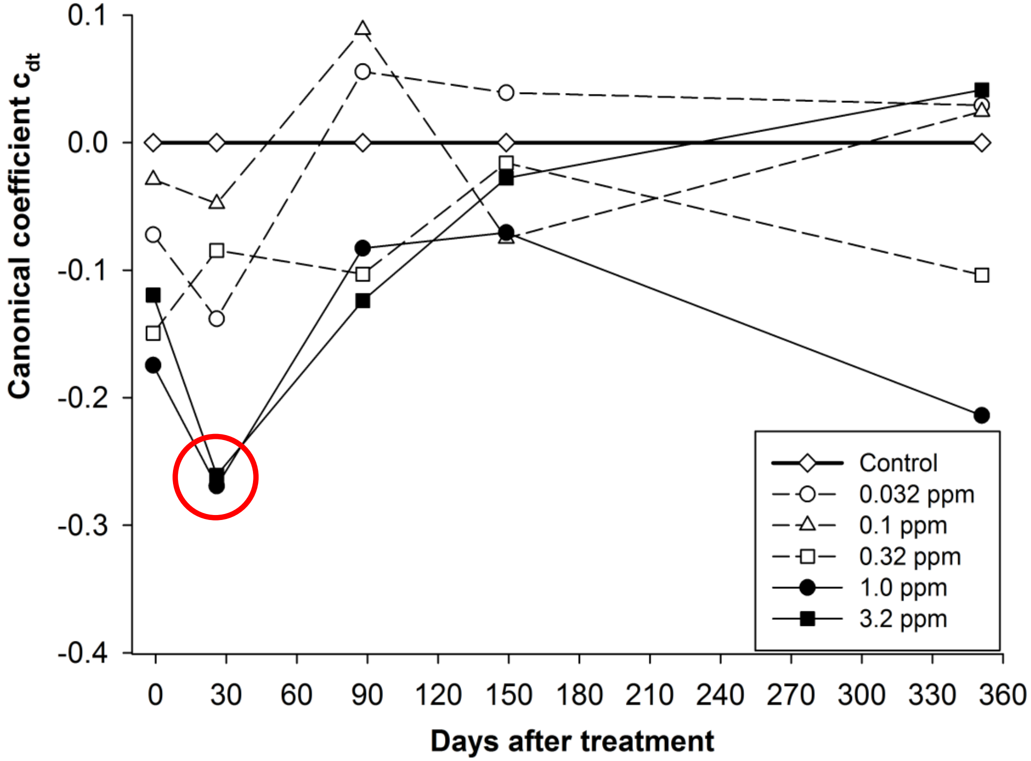


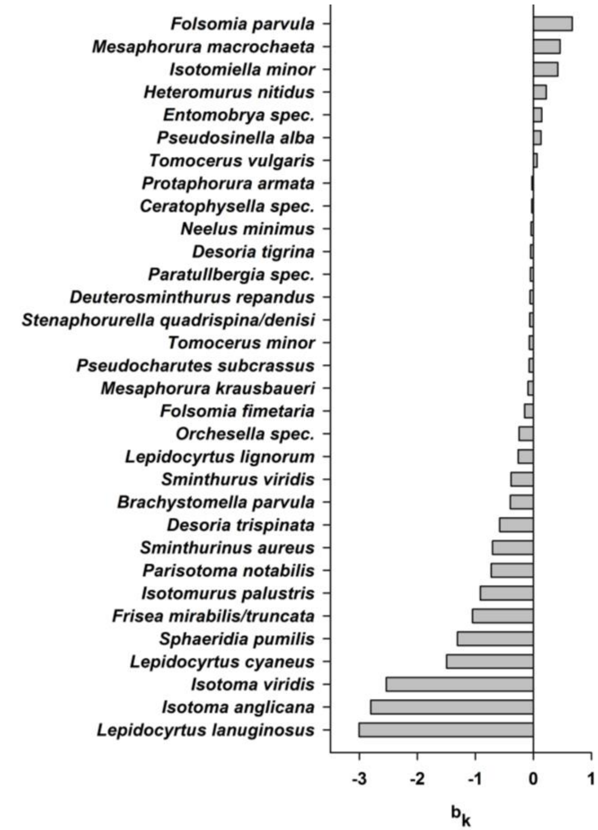
- Patterns of differentially sensitive populations, recovery could be demonstrated

Scholz-Starke et al. (2009) SETAC Annual Meeting, Gothenburg.

Effects on community level I

- Collembolans, lindane, Principal Response Curve method
- Initial effects in the highest treatments, recovery and increasing variation





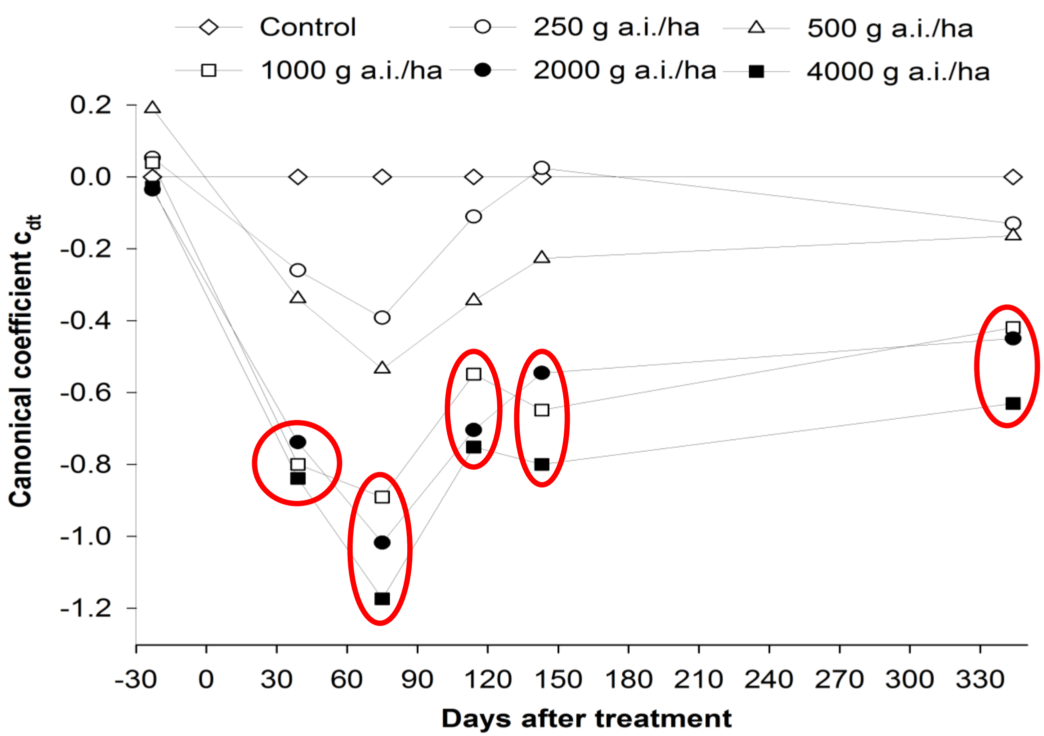
Significant treatment regime at respective sampling date and significant difference of treatment group to control

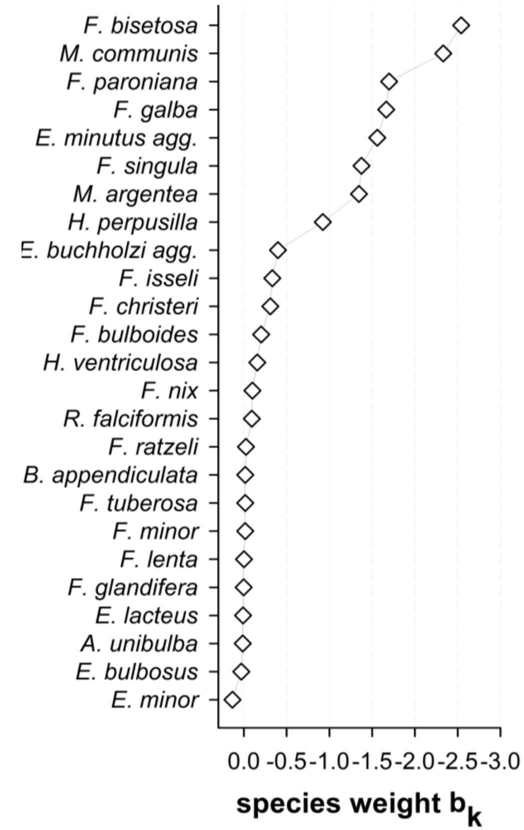
Scholz-Starke (2013) RWTH Aachen University. pp. 291.

Percentage of variance accounted for by:	
Time	40.4
Differences between replicates	51.8
Treatment	7.8
Percentage of treatment variance displayed in first PRC:	24.1

Effects on community level II

- Enchytraeids, alternative insecticide, Principal Response Curve method
- Strong effects on the community, no recovery





Significant treatment regime at respective sampling date and significant difference of treatment group to control

Percentage of variance accounted for by:	
Time	15.5
Differences between replicates	57.1
Treatment	27.4
Percentage of treatment variance displayed in first PRC:	65.6

Nikolakis et al. (2009) SETAC North America Annual Meeting, Tampa.

Use of data in ERA- Classification of effects

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

- **Recovery-based classification** of all endpoints including effects on diversity indices and single population densities allow for a comprehensive assessment

- **Specific protection goal** has to be defined: Which deviation over which period of time for which organism group and which endpoint could be acceptable for which general protection goal (e.g. agro-biodiversity)

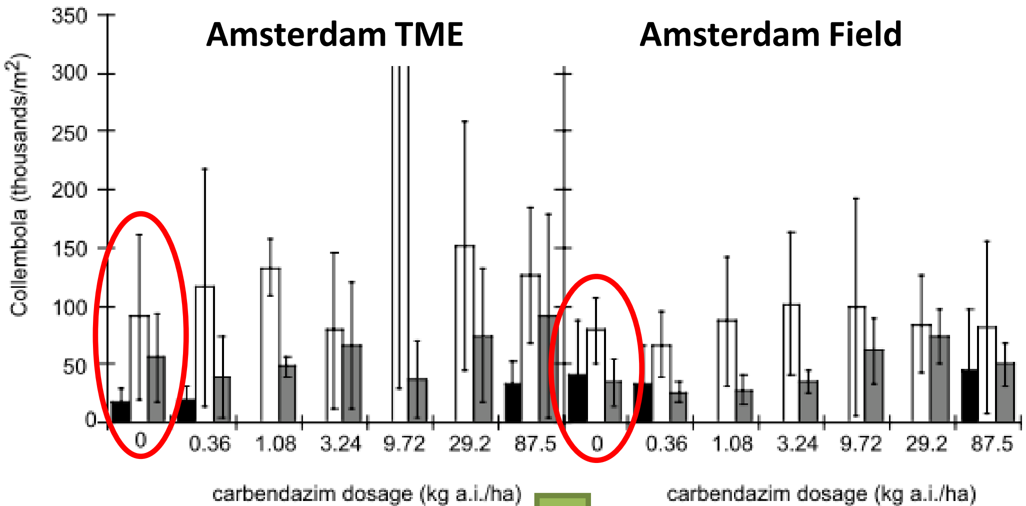
Endpoints Effects of γ-HCH (mg a.i./kg soil)	Dose-response study					Range-finding study	
	0.032	0.1	0.32	1	3.2	10	100
Principle Response	1	1	2	2	2	5	5
Shannon index	1	1	1	1	1	4	4
Evenness	1	1	1	1	1	4	4
Taxa richness	1	1	2	2	2	5	5
Total abundance	1	1	2	2	2	5	5
Brachystomella parvula	1	1	2	2	2		
Entomobrya 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

Scholz-Starke et al. (2009) SETAC Europe Annual Meeting, Gothenburg.

Calibration of higher-tier results I
Variation of TME & Field data

Collembolans

- **Field** 6 controls, 4 replicates for each treatment, 3 comparable dates
- **TME lab**: N = 6 for controls
- **CoV** estimate for untransformed data and control systems/plots



Coefficients of Variation	TME Ring test	Field test
Date 1	58%	113%
Date 3	77%	34%
Date 4	66%	50%

- Similar mean variation for TME and field testing
- More 'variable variability' for field tests
- Variation depends on distribution of organisms, which is on a scale far smaller than a typical semi-field test system

Koolhaas et al. (2004) Ecotoxicology 13: 75-88

Calibration of higher-tier results II

Effect detection TME vs. field experimental designs

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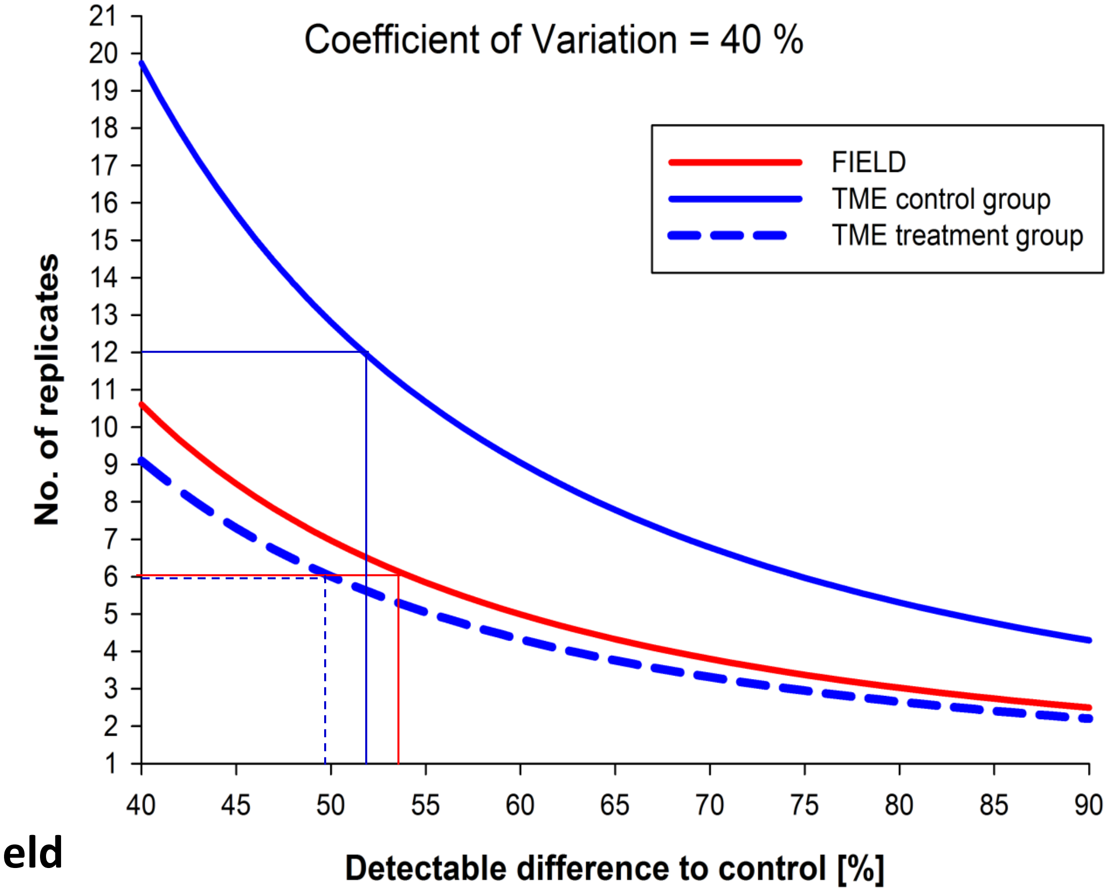
Example calculation for a Dunnett test

- **One control group vs. one** (**field**) or **five** (**TME**) treatment groups
- High error rates **α = 10%** and common power with **β = 20 %** allowed
- **Design FIELD study**
  - $N_{control}$ = 6; $Nt_{treatment}$ = 6
- Design TME study
  - $N_{control}$ = 12; $N_{treatment}$ = 6



- **No decisive difference between field and TME studies in terms of detection limits that could be ascribed to the experimental design or system characteristics**

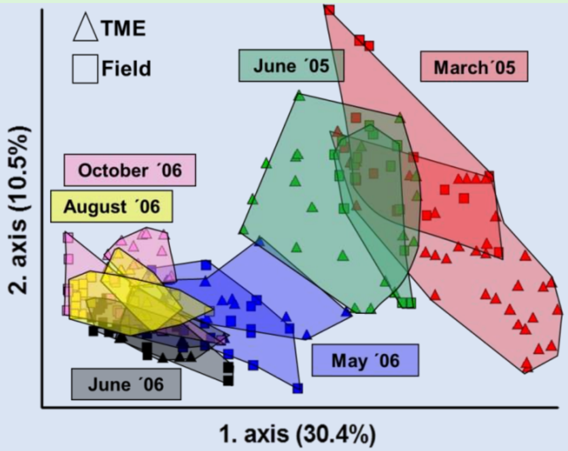
Data estimates deduced from Koolhaas et al. (2004) Ecotoxicology 13: 75-88
Design field study . Römbke et al. (2009) Soil Organisms 81: 237-264.
Design TME study Scholz-Starke (2013) RWTH Aachen University.

Terrestrial Model Ecosystems could serve as a reliable higher-tier test system

- Methodology is well developed, however standardisation is not considered necessary
- High statistical power to detect differences of treatments to control levels due to homogenous test units and high replication
- High stability over time and minor influence of subsampling
- Fit-to-purpose → Various fate and effect endpoints for many organism groups (microbes, fungi, arthropods, lumbricids) and substance classes (pharmaceuticals, metals, pesticides) applicable
- Data can be used for complex effects modelling

(Filser et al. (2014) SETAC Europe Annual Meeting, Basel.)

- Estimation of representativeness by 'general' reference communities for the type of habitat
  - Query site-related soil zoology repositories (e.g. Edaphobase.org)
  - Compare actually tested species composition with indicator assemblages



- Estimation of representativeness by 'internal' references → Coring site communities compared to TME communities

- Classification schemes of effects should incorporate specific protection goals

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Thank you for your attention

Institute for Environmental Research

Andreas Schäffer, Bernhard Theißen, Andreas Toschki, Hans-Toni Ratte, Julia Bless, Martina Roß-Nickoll

Cooperation & Funding Bayer Cropscience

Thorsten Leicher, Alexander Nikolakis, Fred Heimbach, Christa Lechelt-Kunze, Ursula Kölzer