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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)

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Abstract The effects of the fungicide carbendazim (formulation Derosal®) on enchytraeids were determined in Terrestrial Model Ecosystem (TME) tests. TMEs consisted of intact soil columns (diameter 17.5 cm; length 40 cm) taken from three grassland sites (Amsterdam (The Netherlands), Bangor (Wales, England) and Flörsheim (Germany)) or an arable site (Coimbra (Portugal)). Results for each TME site were evaluated using the multivariate Principal Response Curve (PRC) method. The resulting No-Observable Effect Concentrations (NOECs) for the community were compared with the NOECs generated by univariate statistical methods. Furthermore, the EC_{50}s (median effect concentrations) for the three taxa with the highest taxon weights determined by the PRC were compared with EC_{50}s for the other endpoints. In eight out of 16 cases the PRC revealed the lowest NOEC for the enchytraeid species community. The lowest EC_{50}s with the closest 95% confidence limits were calculated for the abundance of the three taxa with the highest taxon weights identified by the PRC. The EC_{50}s ranging from 0.19–2.79 mg carbendazim/kg soil are similar to values from laboratory toxicity studies reported in the literature. Therefore, PRC is a useful instrument to analyse microcosm and mesocosm experiments; it allows for determination of NOECs for the species community ($NOEC_{community}$), the evaluation of the taxa with the most pronounced treatment-related decrease in abundance and of the calculation of meaningful EC_{50} values for those. The resulting $NOEC_{community}$ and EC_{50} values offer a comprehensive tool for the risk assessment of chemicals at the ecosystem level.

Keywords Terrestrial Model Ecosystem · Enchytraeids · Principal Response Curve · $NOEC_{community}$ · EC_{50}

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Introduction

Risk assessment of chemicals usually is based on the results of single species toxicity tests, which are performed in the laboratory. It is, however, realised that such single species tests may not be sufficient to predict effects of chemicals on the level of ecosystem structure and functioning (Cairns 1984). In the past, such effects have been studied in the field but the results are difficult to assess and the efforts are high. Therefore, mesocosm tests have been advocated, which may close the gap between laboratory and field studies. For the soil environment, this has resulted in the development of several types of model ecosystems (Sheppard 1997; Knacker et al. 2004).

Enchytraeids (phylum Annelida, class Oligochaeta, family Enchytraeidae) are distributed all over the world and they occur in nearly all soil types and ecosystems (Didden et al. 1997). As characteristic soil-living animals with high numbers and a high biomass, they play an important role in the functioning of many terrestrial ecosystems (Didden 1993). They contribute to the decomposition of organic matter and the cycling of nutrients, and are very sensitive to anthropogenic stress factors (e.g. plant protection products (Didden and Römbke 2001)). Therefore, they fulfil important criteria to be regarded as useful indicator organisms for environmental pollution.

Effects on enchytraeids were investigated in tests using Terrestrial Model Ecosystems (TMEs). TMEs are defined as controlled, reproducible systems that attempt to simulate the processes and interactions of components in a portion of the terrestrial environment (Gillett and Witt 1980; Sheppard 1997). The tests were performed within the frame of a project that aimed at the improvement and validation of the TME test system, first described by Van Voris et al. (1985). TME tests were performed at four different European sites with different soils, but using the same equipment, test design, test chemical and the same endpoints. Carbendazim was chosen as the model chemical for the TME tests. Carbendazim is a systemic fungicide that was used at large scale in agriculture throughout Europe (Cuppen et al. 2000). The primary mode of action is binding on tubulin and therefore, the inhibition of the building of microtubulesy, resulting in a malfunction of the mitosis.

TME studies may be applied at a higher tier of risk assessment of chemicals, when results of single species laboratory tests indicate a potential concern. Data from multi-species test systems like TMEs can be evaluated on different levels. On the one hand parameters on the population level such as the total abundance of a particular taxonomical unit can be analysed by univariate statistical methods. On the other hand parameters on the community level can be evaluated by univariate as well as multivariate statistical methods. From the point of view of some authors (Landis et al. 1997) microcosm and mesocosm studies are too complex and cannot statistically be treated like single species tests. Instead, the evaluation should be made on the community level by means of multivariate statistics. The *Guidance Document on Aquatic Ecotoxicology* (EC 2002) proposes the determination of a No-Observable Effect Concentration for the species community ($NOEC_{community}$) by application of multivariate statistics.

The main objective of this paper is the statistical analysis of the effects of carbendazim on enchytraeids in TMEs by using the multivariate Principal Response Curve (PRC). The resulting community NOECs were compared with NOECs generated by univariate statistical methods. Furthermore, the EC_{50} values for the three taxa with the highest taxon weights determined by the PRC were compared with the EC_{50}s for the other endpoints. Finally, a comparison with EC_{50} values from laboratory studies described in the literature was made.

Materials and methods

Experimental set-up

The data used for this study have been published earlier (Moser et al. 2004; Moser 2004). Therefore only a brief description of the experimental design will be given.

The TME-project was conducted at four sites throughout Europe by the following project partners: Vrije Universiteit Amsterdam (Institute of Ecological Science, The Netherlands), University of Wales (School of Agricultural and Forestry Sciences, UK), Universidade de Coimbra (Instituto Ambiente e Vida, Portugal) and ECT Oekotoxikologie GmbH (Flörsheim, Germany). In Coimbra, TME tests were performed using an arable site, whereas the respective work by the three other partners was done using grasslands (two meadows and one pasture). The TME tests started with the extraction of the TME soil cores. TMEs were taken by means of an extractor, containing a high-density polyethylene tube (diameter 17.5 cm; length 40 cm), which served as a soil core encasement. Plants were clipped just before TME extraction. TMEs were either placed in temperature-controlled carts in a climatic chamber (Amsterdam, Flörsheim, Coimbra) or in a greenhouse (Bangor). They were watered up to three times per week using artificial rainwater slightly modified according to Velthorst (1993).

The model substance Carbendazim was applied as formulation Derosal® (containing 360 g carbendazim/l) onto the TMEs after an acclimatisation period of 2–4 weeks in the climatic chamber or the greenhouse. It was applied to the soil surface of the TMEs one time using a pipette at application rates of 0 (A0), 0.36 (A1), 2.16 (A2), 13.0 (A3) and 77.8 (A4) kg carbendazim/ha. This fungicide is considered to be moderately to highly persistent in soil, i.e. its DT_{90} values determined in this study range between 12.3 and 46.1 weeks, depending on the respective soil type at the four study sites (Jones et al. 2004). For a detailed description of the set-up of the TME experiments please refer to Knacker et al. (2004).

Enchytraeid sampling and determination

Enchytraeid samples were taken one (w + 1), four (w + 4), eight (w + 8) and 16 (w + 16) weeks after application of carbendazim. At each sampling point samples were taken from six untreated TMEs and from three TMEs for each treatment level. Soil samples for the extraction of enchytraeids were taken from the top 5 cm soil using a split-corer with a diameter of 56 mm. Enchytraeids were extracted from the soil samples by wet extraction (ISO 2004) and were identified alive on the species level using appropriate keys (Nielsen and Christensen 1959, 1961, 1963; Abrahamsen 1969; Graefe 1989; Heck and Römbke 1991; Dózsa-Farkas 1992a; Rota 1994, 1995; Rota and Healy 1999; Schmelz 2003). Juveniles that could not be identified to the species level were summarised and given as genus sp. In case of the species rich genus *Fridericia* it was distinguished between small and large juveniles and they

were divided into the groups *Fridericia* sp. *small* and *Fridericia* sp. *large*, respectively.

Statistical evaluation

NOEC values were determined by univariate statistical methods for the following endpoints: enchytraeid overall abundance, species richness (the number of species), the Shannon–Wiener Diversity Index, the dominance spectrum of the genera (contribution of the respective genera to the assemblage of the enchytraeid community in % of overall abundance) and the abundance of the enchytraeid genus *Fridericia*. Other enchytraeid genera as well as single species were not evaluated by univariate statistical methods since either their abundance was too low or they did not show any treatment related effect (see Moser 2004).

NOEC values were determined from single replicate values. First, homogeneity of variances was tested applying Cochran's test. If variances were homogeneous, NOEC values were determined by analysis of variance (ANOVA) followed by a Dunnett's *t*-test (1-sided; $p \leq 0.05$). If variances were not homogeneous NOEC values were determined using a Bonferroni-*t*-test according to Holm (1-sided; $p \leq 0.05$).

The comparison of the dominance spectrum of the genera of the tested application rates with that of the control was made by a χ^2-Mehrfeldertest ($p \leq 0.05$) for the comparison of two relative frequencies. For this the mean dominance spectrum of each application rate was compared with that of the control separately.

Effects on the enchytraeid community level were analysed by a multivariate technique, the PRC. The PRC is a particular ordination method, the partial Redundancy Analysis (pRDA). The pRDA is the constrained, canonical form of the Principal Component Analysis (PCA) (Van den Brink and Ter Braak 1999). The RDA, and therefore also the PRC, is a direct gradient analysis, based on a linear distribution model. The PRC was developed and described by Van den Brink and Ter Braak (1998, 1999) and is especially suitable for the evaluation of model ecosystem experiments (e.g. aquatic mesocosms) (Cuppen et al. 2000).

PRC is a multivariate technique for the assessment of the structure of species communities, which is suitable to investigate effects of chemicals and other stressors and their changes over time. This method makes it possible to summarize effects on the community of all species and to display it in a single diagram. PRC extracts information only from this part of the variance, which is explained by the variable environmental factor (here: application rate) and the time (here: sampling date) implemented as co-variable. The focus is on the deviation of the species community in the treated model ecosystems from that in the untreated controls.

The result of the analysis is a diagram (PRC), in which the time is displayed on the *x*-axis and the canonical coefficient relative to the control (or the 'Principal Response') on the *y*-axis. The canonical coefficients of the different application rates are shown as deviations to the control at the respective sampling dates.

Together with the PRC in a second graph the taxon weight is plotted. The taxon weight can be interpreted as the weight of each single taxon for the response given in the diagram. The higher the value, the more the actual response pattern of the species is likely to follow the pattern in the PRC. In the presented work the higher a positive value of the taxon weight the higher is the contribution of the current taxon to the accomplishment of the PRC. Taxa with a high negative value show a reversed image to the PRC (e.g. in case of a negative canonical coefficient of the application rates, a positive species weight reflects a decrease of the respective species due to treatment).

In addition to the diagram, a PRC-statistic is given. That contains, as a measure of the goodness of fit and of the explanatory power of the PRC, the *Eigenvalue* and the explanatory content (in %) of the first canonical axis of the PRC as well as the *F*-ratio and the *p*-value for the significance of the first canonical axis of the PRC. The *Eigenvalue* of the first canonical axis indicates which part of the variance is displayed by this axis of ordination. The explanatory content of the first canonical axis describes the percentage contribution of this axis to the sum of all axes. The *p*-value and the *F*-ratio are determined by means of a Monte Carlo permutation test and an *F*-test. Furthermore, in the PRC-statistic the proportion to the overall variance of the total dataset is listed, which on the one hand can be explained by the time ($100 \times (1 -$ sum of all unconstrained *Eigenvalues*)) and on the other hand by the influence of chemical treatment ($100 \times$ sum of all canonical *Eigenvalues*). Finally, the percentage of the variance explained by the treatment, which is captured by the first canonical axis of the PRC (and which is shown in the diagram) is specified as ($100 \times$ (*Eigenvalue* of the first canonical axis/ sum of all canonical *Eigenvalues*)). For a complete description and discussion of the PRC method see Van den Brink and Ter Braak (1998, 1999).

High abundance values are influencing the result of the PRC stronger than low abundance values. Therefore, abundances were log transformed before the analysis. Since in the abundances currently used for the calculation of the PRC many zero values were included, and the logarithm of 0 is not defined, the value of 1 was added to each abundance value before transformation. Additionally, to avoid discrepancies between zero and low abundance values, a factor *Ax* (*x* = abundance) was inserted. When using the lowest

abundance value > 0 the result should be at least 2 (Van den Brink et al. 1995). Accordingly, each abundance value was transformed by ln(2x + 1) before transformation.

For the determination of the NOEC value for the effect of carbendazim on the Enchytraeidae species community ($NOEC_{community}$) for each separate sampling date, first it was proven by Monte Carlo permutation test, if the relation between species and environment (chemical treatment) is stronger than to be expected by chance and if there is a statistically significant ($p \leq 0.05$) difference between the Enchytraeidae communities in the different treatments (including controls). If differences were found, a Williams t-test was applied to check which application rate was significantly different from the control. Since the sample scores calculated by RDA are a priori linked with, and influenced by, the application rates they are not suitable and should not be taken (Van den Brink et al. 1996). In PCA the complete unconstrained variance of the data set is incorporated and it is not a priori linked with the chemical stressor. Therefore, before applying the Williams t-test to determine the $NOEC_{community}$ sample scores were calculated by PCA.

Concentrations causing 50% reduction (EC_{50}) in enchytraeid overall abundance, the number of species, the abundance of the enchytraeid genus *Fridericia* and the abundance of the three taxa determined by the PRC (PRC-Taxon 1–3) with the highest species weight were calculated applying a non-linear, 2- or 3-parametrical logistic regression model according to Haanstra et al. (1985) using the treatment mean values.

Note: In the presented work the three taxa with the highest species weight determined by PRC are taxa with high abundance values and most decreasing in abundance due to chemical treatment. For that reason, these taxa were selected as most relevant to calculate meaningful and robust EC_{50} values. In general the highest species weight determined by PRC is not necessarily the most responding species. If a species is much more sensitive than all other species it might get a low species weight because its response deviates from the general response.

All univariate statistics were calculated with SPSS version 7.5 and ToxRat version 2.07. The multivariate analysis was made according to Van den Brink and Ter Braak (1998, 1999) by means of the program CANOCO version 4.0 (Ter Braak and Smilauer 1998).

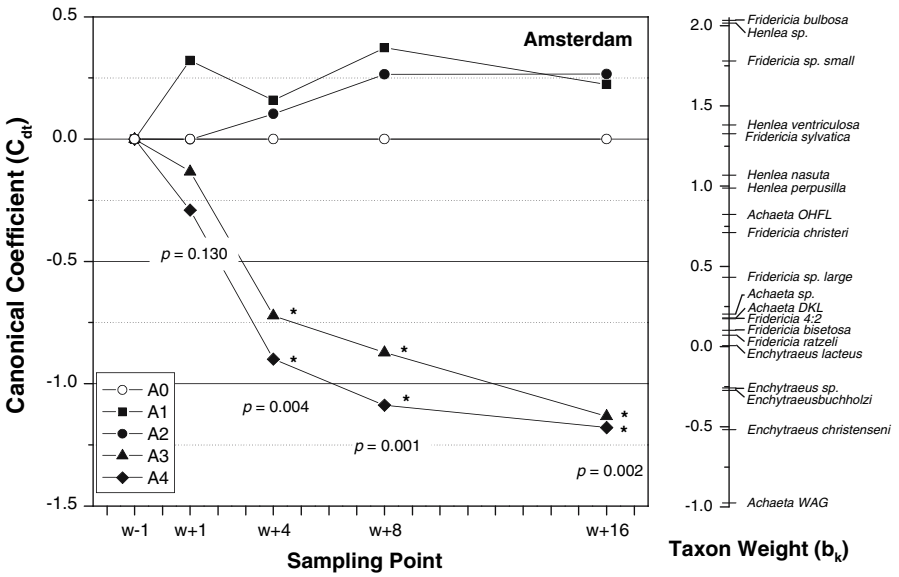
Results

The PRC calculated for the Amsterdam TME test (Fig. 1) revealed that 24.7% of the total variance of the enchytraeid data set is explained by time and 34.7% by chemical treatment. The first canonical axis of the PRC captured a statistically significant part (53.6%) of the variance explained by chemical treatment (Monte Carlo permutation test, 999 permutations, $p = 0.001$).

At sampling points w + 4, w + 8 and w + 16 enchytraeid communities of the two highest application rates (12.96 and 77.76 kg carbendazim/ha) differed significantly from the control (Williams t-test; 2-sided, $p \leq 0.05$). The highest taxon weight was calculated for *Fridericia bulbosa* (2.03) followed by *Henlea* sp. and *Fridericia* sp. *small*. These taxa showed the strongest decrease in abundances due to carbendazim treatment. Species of the genera *Fridericia* and *Henlea* showed only positive taxon weights with values >1. All species of these genera had a treatment-related reduction in abundance. *Achaeta WAG* showed the highest negative taxon weight and therefore an increase in abundance with increasing application rate. The same was true for all *Enchytraeus* species as only negative taxon weights were determined.

The PRC for the Bangor TME test (Fig. 2) showed that 14.4% of the overall variance is explained by time and 25.9% by the chemical stressor. A significant part of 47.9% was captured by the first canonical axis of the PRC (Monte Carlo permutation test, 999 permutations, $p = 0.044$). The enchytraeid species community at the two highest application rates (12.96 and 77.76 kg a.s/ha) was significantly different from the control (Williams t-test; 2-sided, $p \leq 0.05$) at sampling point w + 4 only. However, at sampling point w + 8 deviations to the control were observed in the same order of magnitude for the application rates 77.76 and 2.16 kg a.s./ha but these were not statistically significant. *Fridericia* sp. *small* showed by far the highest species weight (3.09) and the abundance of this taxon was reduced most strongly by carbendazim treatment. For *Fridericia galba* and *Fridericia* sp. *large* the second and third highest value was determined, respectively. *Achaeta BAN* (–1.95) showed the highest and *Achaeta* sp. the second highest negative taxon weight. All other species of this genus showed also negative taxon weights only and thus, a treatment related increase in abundance.

PRC analysis of the Coimbra TME test (Fig. 3) showed that 16.9% of the total variance is explained by time and 32.1% by the chemical treatment. The first canonical axis of the PRC captured a significant part (60.0%) of the variance explained by chemical treatment (Monte Carlo permutation test, 999 permutations, $p = 0.002$). At sampling point w + 4 the enchytraeid community of the highest application rate and at sampling points w + 8 and w + 16 the enchytraeid communities of the two highest application rates differed significantly from the control (Williams t-test; 2-sided, $p \leq 0.05$). The highest species weight was calculated for *Fridericia* sp. *small* (2.47). This taxon showed the strongest decrease in abundances due to the carbendazim treatment. All species with a taxon weight > 0.5 belonged to the genus *Fridericia*. The only



PRC-Statistics

Monte Carlo permutation test on significance of the 1st canonical axis of the PRC				% of the total variance explained by		% of the variance explained by treatment captured by the 1st canonical axis of the PRC
				Time	Treatment	
Eigenvalue	0.186	*p*-value	0.001	24.7	34.7	53.6
F-Ratio	17.076	Explanatory content %	24.7			

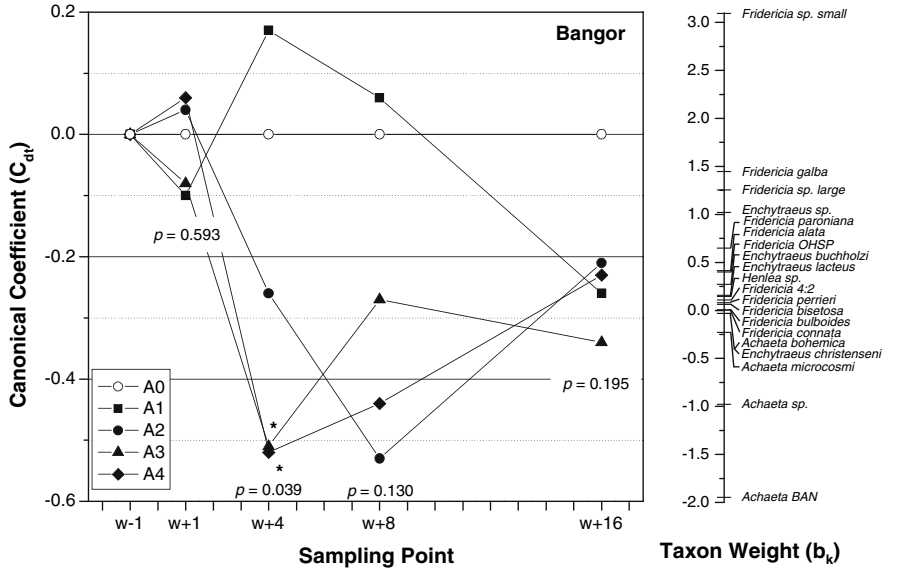
Fig. 1 Principal Response Curve (PRC) for the effects of carbendazim on the Enchytraeid species community in the Terrestrial Model Ecosystem (TME) test of Amsterdam. Presented is the canonical coefficient (Cdt) of the different application rates at the sampling points w + 1, w + 4, w + 8 and w + 16 (weeks after application) and the taxon weight (bk) for all taxa. For each sampling point the *p*-value (Monte Carlo permutation test; 999 permutations) for the comparison of all application rates (including control) is given. Significant differences of the PCA sample scores compared to the control are indicated with an asterisk (Williams *t*-test; 2-sided, $p \leq 0.05$). The PRC-Statistics show *Eigenvalue*, *F*-ratio and *p*-value of the Monte Carlo permutation test (999 permutations) on significance of the 1st canonical axis of the PRC and the explanatory content. Furthermore the part of the total variance explained by time and by treatment and the part of the variance explained by treatment that is captured by the 1st canonical axis of the PRC is given. Application rates: A0 = control, A1 = 0.36, A2 = 2.16, A3 = 12.96, A4 = 77.76 kg carbendazim/ha

taxon with a negative taxon weight was *Enchytraeus buchholzi*.

The PRC calculated for the Flörsheim TME (Fig. 4) test revealed that 11.5% of the overall variance of the enchytraeid data set is explained by time and 39.7% by the treatment. A significant part of 62.2% was captured by the first canonical axis of the PRC (Monte Carlo permutation test, 999 permutations, $p = 0.001$). Concerning the enchytraeid species community statistically significant differences compared to the control were determined at sampling points w + 4 (2.16, 12.96 and 77.76 kg a.s/ha), w + 8 (0.36, 2.16, 12.96 and 77.76 kg a.s/ha) and w + 16 (12.96 and 77.76 kg a.s/ha) (Williams *t*-test; 2-sided, $p \leq 0.05$). The highest species weight was calculated for *Henlea* sp. (2.53) followed by *Fridericia* sp. *small* and *Fridericia* sp. *large*. The abundance of these taxa was reduced most strongly due to carbendazim treatment. Positive taxon weights > 1 were found only for species of the genera *Fridericia*, *Henlea* and *Marionina* as well as for *Buchholzia appendiculata*. The highest negative taxon weight was calculated for *Enchytraeus bulbosus*.

Discussion

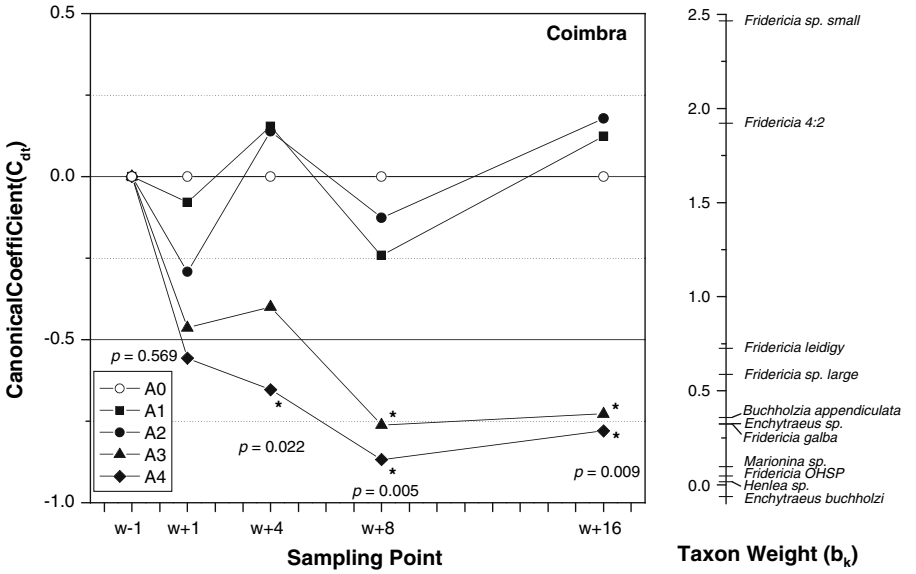
The PRC revealed differences in reacting of the different enchytraeid genera towards the carbendazim treatment. The abundance of species belonging to the genera *Fridericia* and partly *Henlea* was reduced most strongly, whereas the abundance of many species belonging to the genera *Achaeta* and *Enchytraeus* did not decrease or was partly increased. This can be attributed to the different ecological requirements and life strategies of the different genera. Species belonging to the genera *Fridericia* and *Henlea* are regarded as 'typical' epidaphic (Healy 1980; Graefe and Schmelz 1999). Most of them live in the uppermost mineral soil, i.e. close to or even in the litter layer (Dózsa-Farkas 1992b). Chemical analysis of the TMEs showed that carbendazim residues were mainly confined to the upper soil layers (Jones et al. 2004). Therefore, species of the genera *Fridericia* and *Henlea* are stronger exposed to carbendazim than those belonging to the genera *Achaeta* and *Enchytraeus*, the latter preferring much deeper soil layers (Healy 1980; Graefe and Schmelz



PRC-Statistics

Monte Carlo permutation test on significance of the 1st canonical axis of the PRC				% of the total variance explained by		% of the variance explained by treatment captured by the 1st canonical axis of the PRC
				Time	Treatment	
Eigenvalue	0.124	*p*-value	0.044	14.4	25.9	47.9
F-Ratio	8.832	Explanatory content %	14.5			

Fig. 2 Principal Response Curve (PRC) for the effects of carbendazim on the Enchytraeid species community in the Terrestrial Model Ecosystem (TME) test of Bangor. See Fig. 1 for further explanation

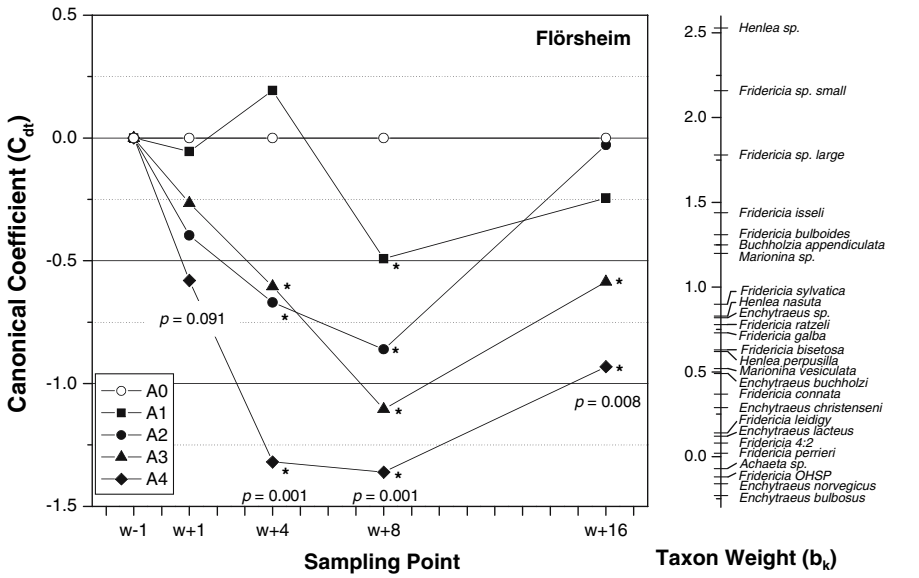


PRC-Statistics

Monte Carlo permutation test on significance of the 1st canonical axis of the PRC				% of the total variance explained by		% of the variance explained by treatmentcaptured by the 1st canonical axis of the PRC
				Time	Treatment	
Eigenvalue	0.194	*p*-value	0.002	16.9	32.1	60.4
F-Ratio	15.829	Explanatory content %	23.3			

Fig. 3 Principal Response Curve (PRC) for the effects of carbendazim on the Enchytraeid species community in the Terrestrial Model Ecosystem (TME) test of Coimbra. See Fig. 1 for further explanation

1999). Especially *Achaeta* is often considered the "typical" genus for the mineral soil layer, although this is not true for all species of this genus (Healy 1980). However, within the genus *Enchytraeus* there are also species dwelling in soil at depths down to 65 cm (Dózsa-Farkas 1992b). Furthermore, species of the latter genera usually have short life cycles (r-strategists) compared to the genera *Fridericia* and *Henlea* (K-strategists) (Graefe and Schmelz 1999). Hence,



PRC-Statistics

Monte Carlo permutation test on significance of the 1st canonical axis of the PRC				% of the total variance explained by		% of the variance explained by treatment captured by the 1st canonical axis of the PRC
				Time	Treatment	
Eigenvalue	0.247	p-value	0.001	11.5	39.7	62.2
F-Ratio	20.075	Explanatory content %	27.9			

Fig. 4 Principal Response Curve (PRC) for the effects of carbendazim on the Enchytraeid species community in the Terrestrial Model Ecosystem (TME) test of Flörsheim. See Fig. 1 for further explanation

Fridericia and *Henlea* species did not only experience higher carbendazim exposure, but may also need more time to recover from disturbances (e.g. chemical stressors) than species of the genera *Achaeta* and *Enchytraeus*.

Since the dominance spectrum of the enchytraeid communities in the soils of the different sites are different (e.g. the genus *Achaeta* was dominant in the Amsterdam and Bangor soil whereas the genus *Fridericia* was dominant in the Coimbra and Flörsheim soil) differences concerning the course of the PRC between the sites can be attributed at least partly to the described species susceptibility. However, at all sites some *Fridericia* species are always present and the EC_{50} values calculated for the taxa with the highest taxon weights identified by PRC are in a narrow range (Table 1).

The NOEC values for the enchytraeid species community determined by means of the multivariate PRC and the EC_{50} values calculated for the three taxa with the highest taxon weight identified by PRC (PRC-Taxon 1–3) were compared with the NOEC and EC_{50} values for the parameters evaluated by univariate statistical methods (Tables 2 and 3). Details of these calculations can be found in Moser (2004). This was done for each particular sampling point for each site separately.

Summarising these comparisons it can be stated that, the PRC revealed the lowest NOEC value for the enchytraeid species community in eight out of 16 cases (Table 2). Only the dominance spectrum showed the lowest NOEC values in more cases than PRC (12 out of 16). In addition, in 13 out of 16 cases the lowest EC_{50} values with the closest 95% confidence limits were calculated for the three taxa with the highest taxon weight identified by the PRC (Table 3).

According to EPPO (2003), the EC_{50} values expressed on a kg/ha basis may be converted into mg/kg soil values using a conversion factor of 1.33. This results in EC_{50} values ranging from 0.19 to 2.79 mg carbendazim/kg soil. The EC_{50} values calculated for the three taxa with the highest taxon weight are comparable to EC_{50} values and in the lower range of LC_{50} values for the effect of carbendazim on enchytraeids determined in laboratory studies (Table 4). These laboratory single-species tests used either a standardised substrate (OECD artificial soil) or a natural soil, with the chemical mixed homogeneously in with the substrate, and can be regarded a *worst-case* scenario. Therefore, and since TMEs are model ecosystems that should reflect the field situation and thus have an intrinsic heterogeneity and variability, it was expected that the effects in TMEs would be less pronounced than in the laboratory studies. That this is not the case can be attributed to the use of the PRC and the respective EC_{50} values calculated for the three taxa with the highest taxon weight determined by PRC. Furthermore, the use of the EC_{50} values calculated for the taxa with the highest taxon weights determined by PRC is recommended when using terrestrial model ecosystem data for risk assessment.

Based on the assumption that the heterogeneity of ecosystems is too complex to be captured adequately with

Table 1 The three taxa with the highest positive taxon weights identified by PRC (PRC-Taxon 1–3) for the TME-test in Amsterdam, Bangor, Coimbra and Flörsheim

Parameter	Amsterdam	Bangor	Coimbra	Flörsheim
PRC-Taxon 1	*Fridericia bulbosa*	*Fridericia* sp. *small*	*Henlea* sp.	*Fridericia* sp. *small*
PRC-Taxon 2	*Henlea* sp.	*Fridericia galba*	*Fridericia* sp. *small*	*Fridericia* 4:2
PRC-Taxon 3	*Fridericia* sp. *small*	*Fridericia* sp. *large*	*Fridericia* sp. *large*	*Fridericia leidigy*

Table 2 No-Observable Effect Concentrations for the effects of carbendazim (kg/ha) on the overall abundance, the number of enchytraeid species, the Shannon–Wiener Diversity index, the dominance spectrum, the abundance of the enchytraeid genus *Fridericia* and the enchytraeid species community

	Parameter	Amsterdam	Bangor	Coimbra	Flörsheim
w + 1	Overall abundance	≥77.76	≥77.76	≥77.76	≥77.76
w + 4		≥77.76	≥77.76	**2.16**	**0.36**
w + 8		≥77.76	≥77.76	**2.16**	**<0.36**
w + 16		≥77.76	≥77.76	≥77.76	**2.16**
w + 1	Number of species	**12.96**	≥77.76	12.96	**12.96**
w + 4		≥77.76	≥77.76	≥12.96	0.36
w + 8		≥77.76	12.96	12.96	2.16
w + 16		12.96	≥77.76	≥77.76	**12.96**
w + 1	Shannon–Wiener index	≥77.76	≥77.76	≥77.76	12.96
w + 4		**2.16**	2.16	≥12.96	12.96
w + 8		≥77.76	≥77.76	12.96	2.16
w + 16		12.96	≥77.76	≥77.76	12.96
w + 1	Dominance spectrum	≥77.76	≥77.76	**<0.36**	≥77.76
w + 4		**2.16**	**0.36**	**2.16**	2.16
w + 8		**<0.36**	**<0.36**	**2.16**	**<0.36**
w + 16		**2.16**	**<0.36**	**2.16**	**2.16**
w + 1	Genus *Fridericia*	≥77.76	≥77.76	12.96	≥77.76
w + 4		≥77.76	2.16	**2.16**	12.96
w + 8		12.96	12.96	**2.16**	<0.36
w + 16		**2.16**	2.16	≥77.76	2.16
w + 1	Enchytraeid species community	≥ 77.76	≥77.76	≥77.76	**12.96**
w + 4		**2.16**	2.16	12.96	**0.36**
w + 8		2.16	≥77.76	**2.16**	**<0.36**
w + 16		**2.16**	≥77.76	**2.16**	**2.16**

Given are the values for the TME-tests in Amsterdam, Bangor, Coimbra and Flörsheim for the sampling points w + 1, w + 4, w + 8 and w + 16. Bold indicates the lowest NOEC value for a sampling point (e.g. w + 1), at individual sites (e.g. Amsterdam) considering all endpoints measured; if the lowest value was determined for more than one parameter, all values were marked

univariate statistical methods, Landis et al. (1997) recommended that multivariate techniques are better suited for evaluating results from such test systems than univariate methods.

For the evaluation of data from mesocosm experiments in particular, Van den Brink and Ter Braak (1999) developed the 'PRC'. Currently, this method is mainly applied in aquatic ecotoxicology (Van den Brink and Ter Braak 1999; Cuppen et al. 2000). In the frame of the SCARAB project (Frampton et al. 2000) in 1997 terrestrial data were analysed by PRC the first time. More recently Smit et al. (2002) used PRC to evaluate nematode community data from soil mesocosms treated with zinc, concluding that the NOEC for the nematode community determined by PRC was lower than the NOEC for all other endpoints investigated in that study. Koolhaas et al. (2004) applied PRC to determine effects of carbendazim on soil microarthropods in the same TMEs described in this paper and they reported that PRC revealed that certain groups were more sensitive to the treatment than others. According to Frampton et al. (2000), the advantage of multivariate compared to univariate methods is that all information (here: abundance data of all taxa, all application

Table 3 Carbendazim (kg/ha) causing 50% reduction (median effective concentrations, (EC_{50}s)) of the overall abundance, the number of enchytraeid species, the abundance of the enchytraeid genus *Fridericia* and the abundance of the three taxa with the highest positive taxon weights in the respective PRC, i.e. most decreasing in abundance due to chemical treatment (PRC-Taxon 1–3)

	Parameter	Amsterdam		Bangor		Coimbra		Flörsheim	
		EC_{50}	95% CL	EC_{50}	95% CL	EC_{50}	95% CL	EC_{50}	95% CL
w + 1	Overall abundance	44.9	0.07–25723	n.a.	–	4.24	0.01–1477	n.a.	–
w + 4		n.a.	–	n.a.	–	6.64	1.44–30.6	2.60	0.01–678
w + 8		28.4	3.75–214	67.9	#	6.76	0.10–386	0.50	0.11–2.10
w + 16		10.9	0.03–3355	13.5	0.02–9191	10.9	0.92–129	12.1	0.12–1184
w + 1	Number of species	n.a.	–	n.a.	–	31.3	0.44–2220	n.a.	–
w + 4		n.a.	–	n.a.	–	13.2	4.29–40.3	10.5	0.22–508
w + 8		47.5	0.14–16645	31.4	#	21.0	1.59–276	7.93	6.34–9.93
w + 16		36.8	9.51–142	n.a.	–	53.0	5.26–532	39.5	3.06–512
w + 1	Genus Fridericia	n.a.	–	n.a.	–	1.53	0.09–27.1	n.a.	–
w + 4		3.37	0.002–65910	1.84	0.38–8.77	5.38	4.52–6.39	**1.30**	**0.003–633**
w + 8		3.21	0.02–502	**1.05**	**0.02–727**	4.87	0.07–336	0.27	0.06–1.24
w + 16		4.39	2.24–8.60	0.67	0.12–3.83	6.15	0.34–110	**2.31**	**0.01–469**
w + 1	PRC-Taxon 1	n.a.	–	n.a.	–	1.10	1.10–1.10	**2.29**	**#**
w + 4		2.10	0.02–264	3.08	2.16–12.96	10.4	1.01–107.8	1.95	0.005–684
w + 8		3.55	0.004–3119	1.14	0.005–291	3.80	0.02–591	**0.14**	**#**
w + 16		6.98	0.02–2897	**0.27**	**0.08–0.93**	6.87	0.07–639	16.2	0.41–634
w + 1	PRC-Taxon 2	**9.55**	**0.03–2793**	**0.23**	**a.**[a]	**0.58**	**0.11–3.22**	n.a.	–
w + 4		7.27	0.31–171	**1.02**	**0.36–2.16**[a]	2.93	2.16–12.96	1.75	0.04–81.1
w + 8		8.14	0.02–3414	n.a.	–	**0.36**	**0.06–2.27**	0.22	0.05–0.99
w + 16		6.61	0.11–383	1.55	0.01–172	**4.25**	**4.25–4.25**	11.3	0.28–435
w + 1	PRC-Taxon 3	n.a.	–	n.a.	–	1.82	0.43–7.77	n.a.	–
w + 4		4.67	3.47–6.29	**1.02**	**0.36–2.16**[a]	**1.60**	**0.67–3.83**	2.46	0.01–513
w + 8		**3.18**	**0.03–324**	3.73	#	n.a.	–	0.22	0.008–5.73
w + 16		**4.38**	**1.03–18.7**	n.a.	–	n.a.	–	6.01	0.06–620

Given are the values for the TME-test in Amsterdam, Bangor, Coimbra and Flörsheim for the sampling points w + 1, w + 4, w + 8 and w + 16 (weeks after treatment). CL, confidence limits; n.a., logistic regression not applicable, since no dose-related response was observed; #, lower 95% CL $< 10^{-3}$ $x$$EC_{50}$ and upper CL $> 10^{3}$ $x$$EC_{50}$

[a] Steep dose-response curve and therefore, 95% CL are given as the concentrations below and above the EC_{50} (a., the EC_{50} was between the control and the lowest application rate and 95% CL could not be given)

Bold indicates the lowest EC_{50} value for a sampling point (e.g. w + 1), at individual sites (e.g. Amsterdam) considering all endpoints measured; if the lowest value was determined for more than one parameter, all values were marked

Table 4 Carbendazim concentrations causing 50% reduction of reproduction or survival of enchytraeids in laboratory studies and in the TME tests (median effective concentrations, EC_{50} and LC_{50}, respectively, in mg/kg soil)

Species	EC_{50}	LC_{50}	Substrate/days	References
Enchytraeus albidus	0.24–2.4	–	OECD artificial soil/42	Römbke and Moser (1999)
Enchytraeus albidus	–	7.7	OECD artificial soil/14	Federschmidt (1994)
Enchytraeus albidus	–	4.9	OECD artificial soil/28	Federschmidt (1994)
Enchytraeus albidus	–	5.5	Natural/14	Federschmidt (1994)
Enchytraeus albidus	–	2.5	Natural/28	Federschmidt (1994)
Fridericia ratzeli	–	15.2	Natural/14	Federschmidt (1994)
Fridericia ratzeli	–	3.3	Natural/28	Federschmidt (1994)
TME (natural population)	0.19–2.79	–	Natural/7–112	This paper

rates, all sampling points) of the investigated populations is used simultaneously, thus allowing for an evaluation of the data at the community level.

Compared to other multivariate methods the graphical presentation of the PRC is less abstract and therefore much easier to interprete. In a PRC, effects of a stressor in the course of time are displayed. In doing so, information on single species is retained and a distinction between decrease and increase of species abundances with time is still possible. Furthermore, the method allows for a statistical analysis of the significance of a treatment effect. Care must be taken when interpreting the results, since very sensitive species with a different response pattern may not be recognised as important species by the PRC method.

PRC is a useful instrument to analyse microcosm and mesocosm experiments. It offers a relatively simple possibility to interpret effects of a stressor on the coenosis level at a glance. This method allows the determination of a NOEC value for effects on the species community ($NOEC_{community}$). On the other hand the taxa with the most pronounced treatment-related decrease in abundance can be evaluated by the PRC and meaningful and robust EC_{50} values can be determined for these taxa even if compared with laboratory studies. The resulting NOEC and EC_{50} values offer a comprehensive risk assessment of ecosystem effects of chemicals.

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