TEXTE
193/2020

Necessary adaptations for a harmonized field-testing procedure and risk assessment of earthworms (terrestrial)

Appendix

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Necessary adaptations for a harmonized field-testing procedure and risk assessment of earthworms (terrestrial)

Appendix

by

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Abstract: Necessary adaptations for a harmonized field-testing procedure and risk assessment of earthworms (terrestrial)

The purpose of this project was to provide scientifically robust and practical information on the variability of the endpoints assessed in earthworm field studies, the statistical significance of the results and the level of the statistically detectable effects of the chemicals tested - with the aim of developing suggestions for improving the test design. Best-practice studies reveal low power to detect differences between control and test chemical treatment plots. An adapted test design should contain an option to perform regression (ECx) approaches, which have been suggested as an alternative to the currently performed threshold (NOEC) approach. A pilot field study was performed according to a newly developed combined NOEC- and ECx-test design with the test chemical carbendazim. The ECx design leads to more robust conclusions for environmental risk assessment. The calculation of effect thresholds (NOEC/LOEC) should be conducted with the most powerful multiple test procedure for given data prerequisites. If applicable to the data, the closure principle computational approach test (CPCAT) is the preferred option. The evaluation and interpretation of the data at plot (pooled samples of 1 m^2 in total used as replicates) and sub-plot level (single samples as replicates of 0.25 m^2) should be requested. According to the experiences made during the performance of the pilot study and the results of the statistical analyses, a draft OECD test guideline was developed. As of now, the discussion of the draft test guideline is ongoing.

Kurzbeschreibung: Notwendige Anpassung zur harmonisierten Freiland-Testung und Risikobewertung für Regenwürmer (Terrestrik)

Ziel dieses Projekts war es, wissenschaftlich belastbare und praktische Informationen über die Variabilität der in Feldstudien mit Regenwürmern ermittelten Endpunkte, die statistische Signifikanz der Ergebnisse und die Höhe der sicher statistisch nachweisbaren Auswirkungen der getesteten Chemikalien zu liefern, um Vorschläge für die Verbesserung des Testdesigns zu entwickeln. Best-Practice-Studien zeigen, dass die statistische Trennschärfe zur Erkennung von Unterschieden zwischen Kontroll- und mit Testchemikalien behandelten Parzellen gering ist. Ein angepasstes Testdesign sollte eine Option zur Durchführung von Regressionsansätzen (ECx) enthalten, die als Alternative zum NOEC-Ansatz vorgeschlagen wurden. Eine Pilotfeldstudie wurde nach einem neu entwickelten kombinierten NOEC- und ECx-Testdesign mit der Testchemikalie Carbendazim durchgeführt. Das ECx-Design führt zu belastbareren Aussagen für die Umweltrisikobewertung. Die Berechnung der Wirkungsschwellen (NOEC/LOEC) sollte unter den gegebenen Voraussetzungen mit dem leistungsstärksten Mehrfachtestverfahren durchgeführt werden. Wenn möglich, ist der CPCAT-Ansatz (closure principle computational approach test) die bevorzugte Option. Die Auswertung und Interpretation der Daten auf der Parzellen- (gepoolte Proben von insgesamt 1 m^2, die als Replikate verwendet wurden) sowie der Probenebene (einzelne Proben von 0,25 m^2 als Replikate) sollte gefordert werden. Basierend auf den Erfahrungen während der Durchführung der Pilotstudie und den Ergebnissen der statistischen Auswertungen wurde ein OECD-Prüfrichtlinienentwurf formuliert. Die Diskussion über den Prüfrichtlinienentwurf ist derzeit noch nicht abgeschlossen.

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AITC	Allyl isothiocyanate
a.s.	Active substance
BBA	Federal Biological Research Center for Agriculture and Forestry, Brunswig
C	**C**ontrol
CA	**C**orrespondence analysis
CCC	Chlormequat chloride
CEC	Cation exchange capacity
CIP	Chemisches Institut Pforzheim
CP	Closure principle
CPCAT	Closure principle computational approach test
CPFISH	Closure principle and Fisher-Freeman-Halton test
$\mathbf{C_{org}}$	Organic carbon
CRO	Contract research organization
CV	Coefficient of variation
DAA	Days after application
DBA	Days before application
dm	Dry matter
DNA	Deoxyribonucleic acid
DT90	90% dissipation time
DWD	Deutscher Wetterdienst, Offenbach
EC	European Community
ECT	ECT Oekotoxikologie GmbH, Flörsheim
ECx	X % effective concentration
EFSA	European Food Safety Authority
ERA	Environmental risk assessment
EU	European Union
GD	Guidance document
GLM	Generalized linear model
GPS	Global Positioning System
GSIG	Global Soil Interest Group
ISIS	Information System Chemical Safety
ISO	International Organization for Standardization
$\mathbf{K_{OC}}$	Octanol carbon partition coefficient

K_{OW}	Octanol water partition coefficient
LOEC	Lowest observed effect concentration
LUFA	Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer
MDD	Minimum detectable difference
MSD	Minimum significant difference
NEC	No effect concentration
NOEC	No observed effect concentration
N_{tot}	Total nitrogen
OECD	Organization for Economic Cooperation and Development
OM	Organic matter
PPR	Panel on Plant Protection Products and their Residues
PRC	Principal response curve
QSAR	Quantitative structure–activity relationship
RDA	Redundancy analysis
RWTH	Rheinisch-Westfälische Technische Hochschule Aachen
SC	Suspensible concentrate
SETAC	Society of Environmental Toxicology and Chemistry
SSD	Species sensitivity distribution
T	Treatment
TG	Test guideline
TKTD	Toxicokinetic-toxicodynamic models
TME	Terrestrial model ecosystem
UBA	German Environment Agency
US EPA	United States Environmental Protection Agency
VR	Validation report
WHC_{max}	Maximum water holding capacity
WNT	Working Group of National Co-ordinators of the Test Guidelines Programme
WP	Work package

A Appendix

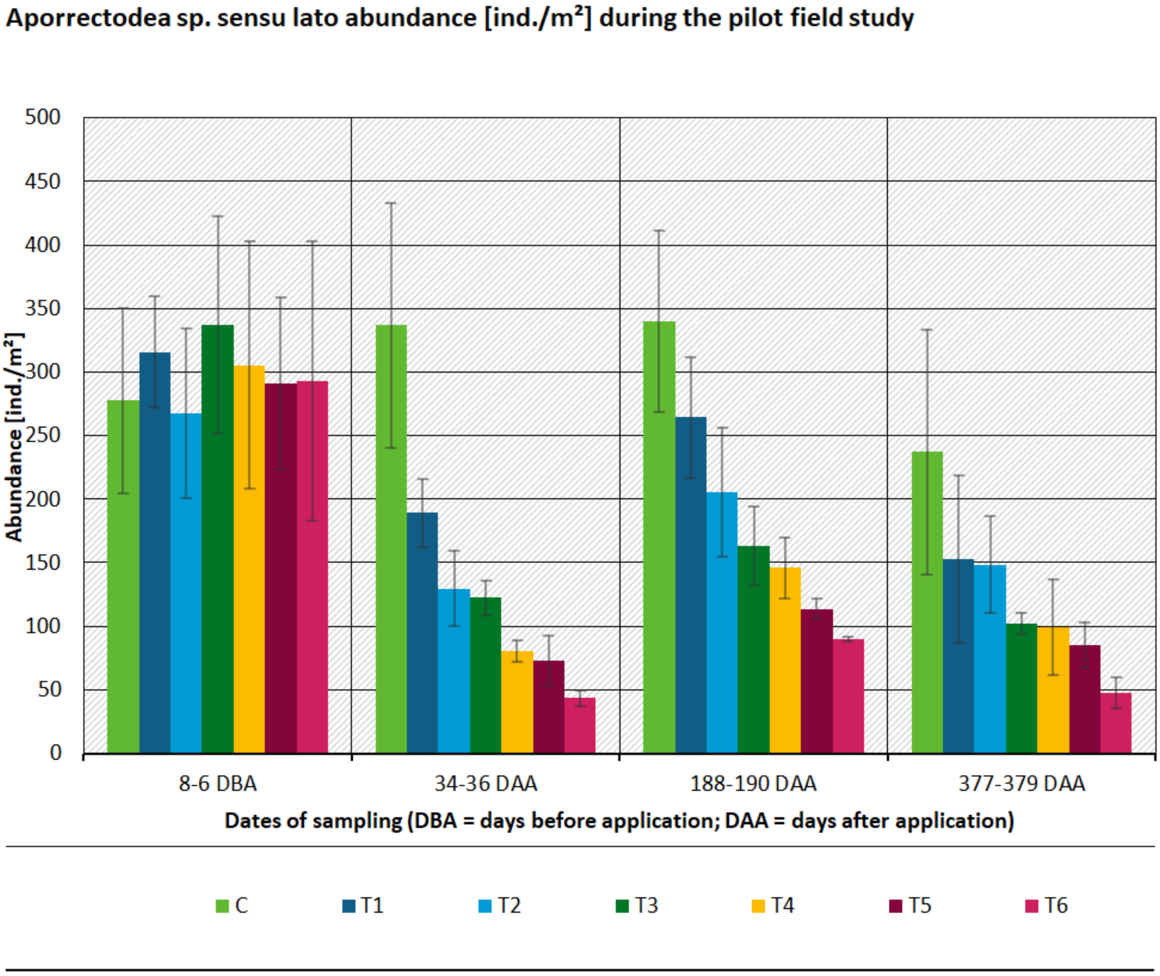
A.1 Earthworm data pilot study

Table A1-1: Application rates of the earthworm pilot field study. Concentrations are given in kg active substance (a.s. carbendazim)/hectare (ha)

Treatments	T1	T2	T3	T4	T5	T6
	0.6	1.8	3.2	5.8	10.5	31.5

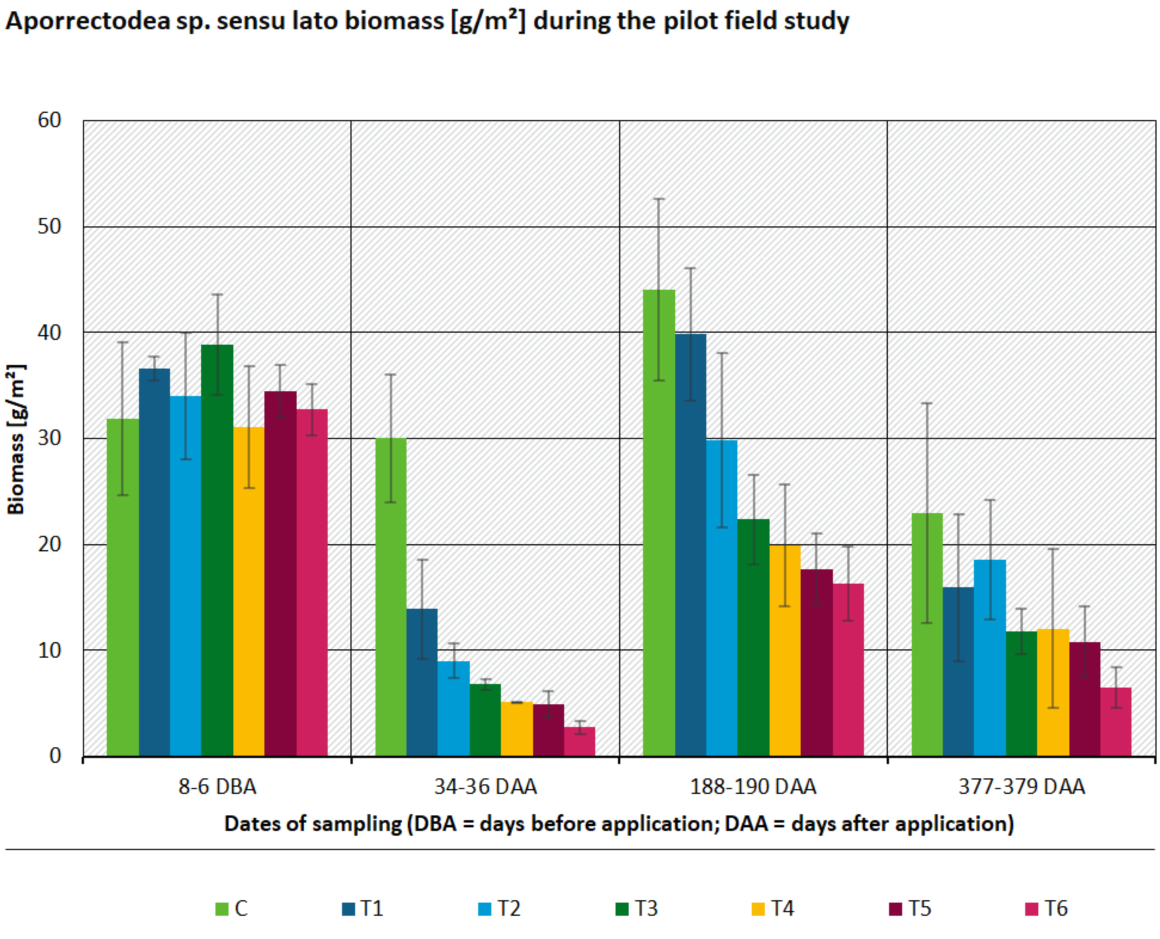
A.1.1 Abundance and biomass during the pilot field study

Figure A1-1: *Aporrectodea* sp. sensu lato abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



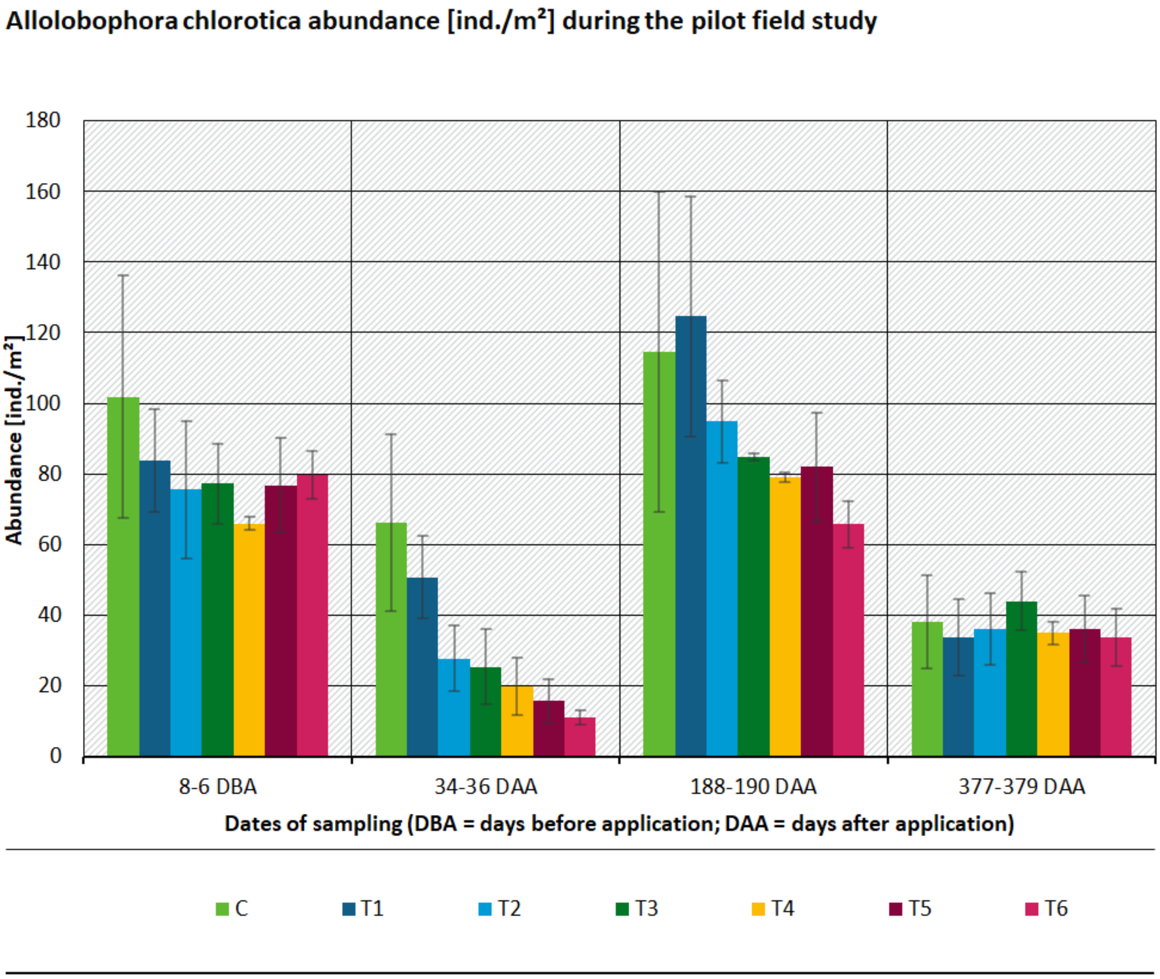
Source: ECT Oekotoxikologie GmbH

Figure A1-2: ***Aporrectodea*** **sp. sensu lato biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



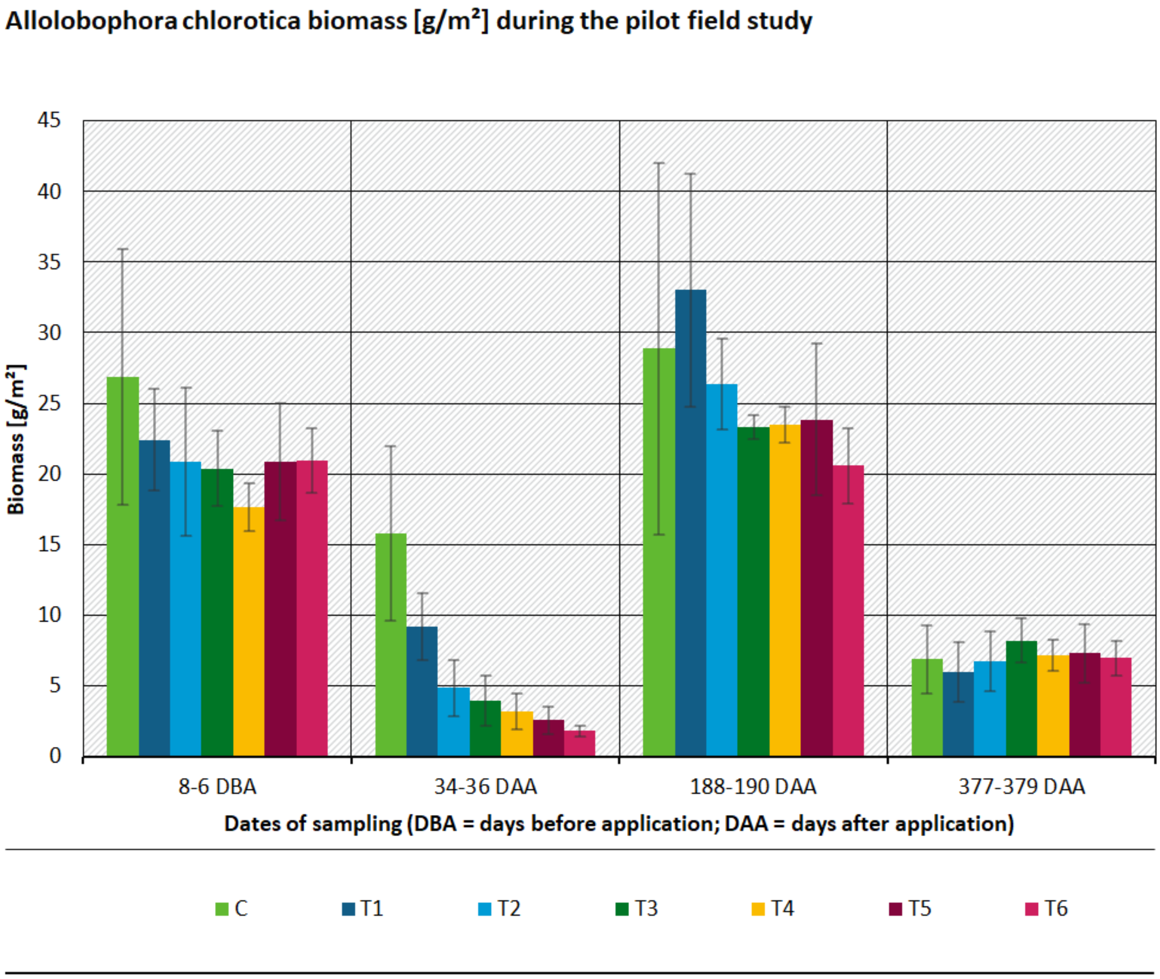
Source: ECT Oekotoxikologie GmbH

Figure A1-3: ***Allolobophora chlorotica*** **abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



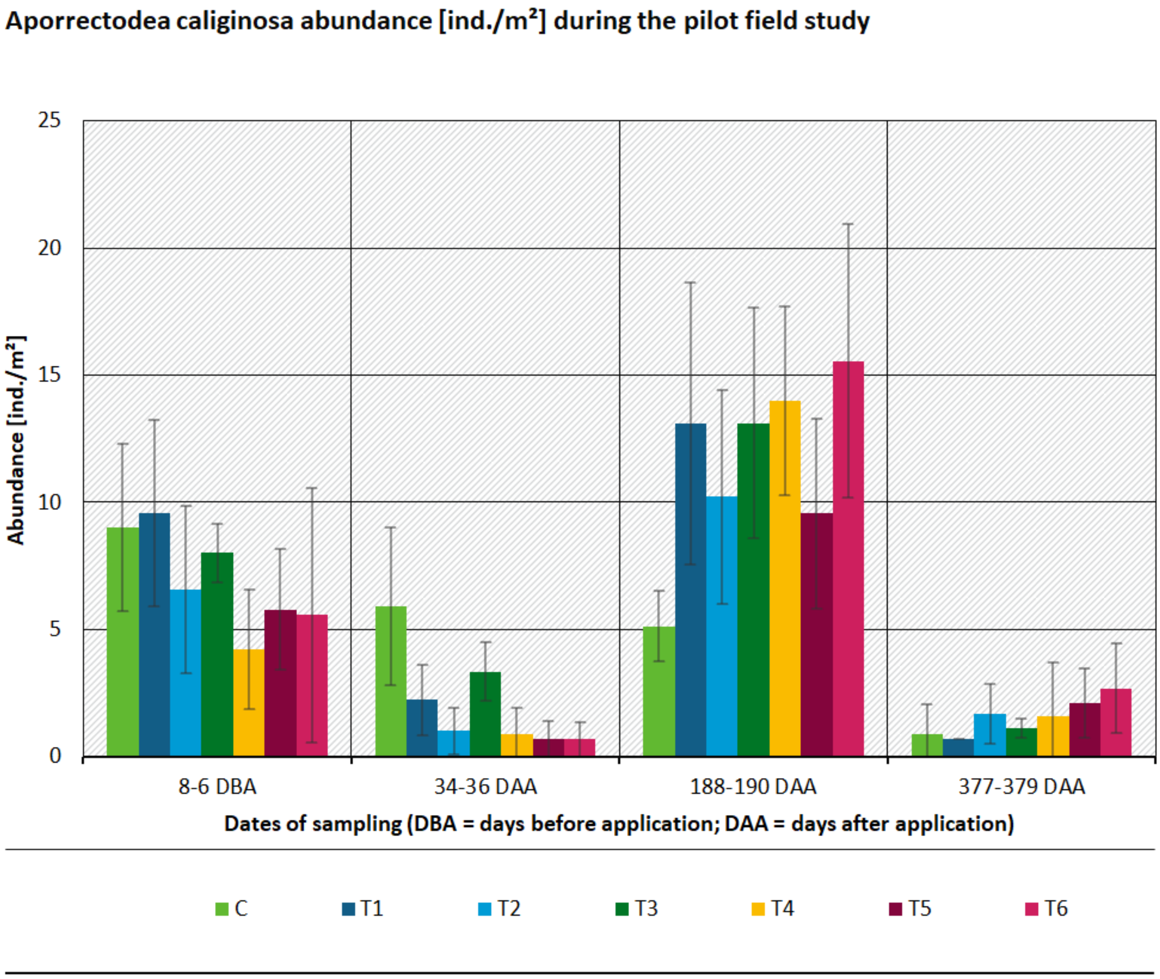
Source: ECT Oekotoxikologie GmbH

Figure A1-4: ***Allolobophora chlorotica*** **biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



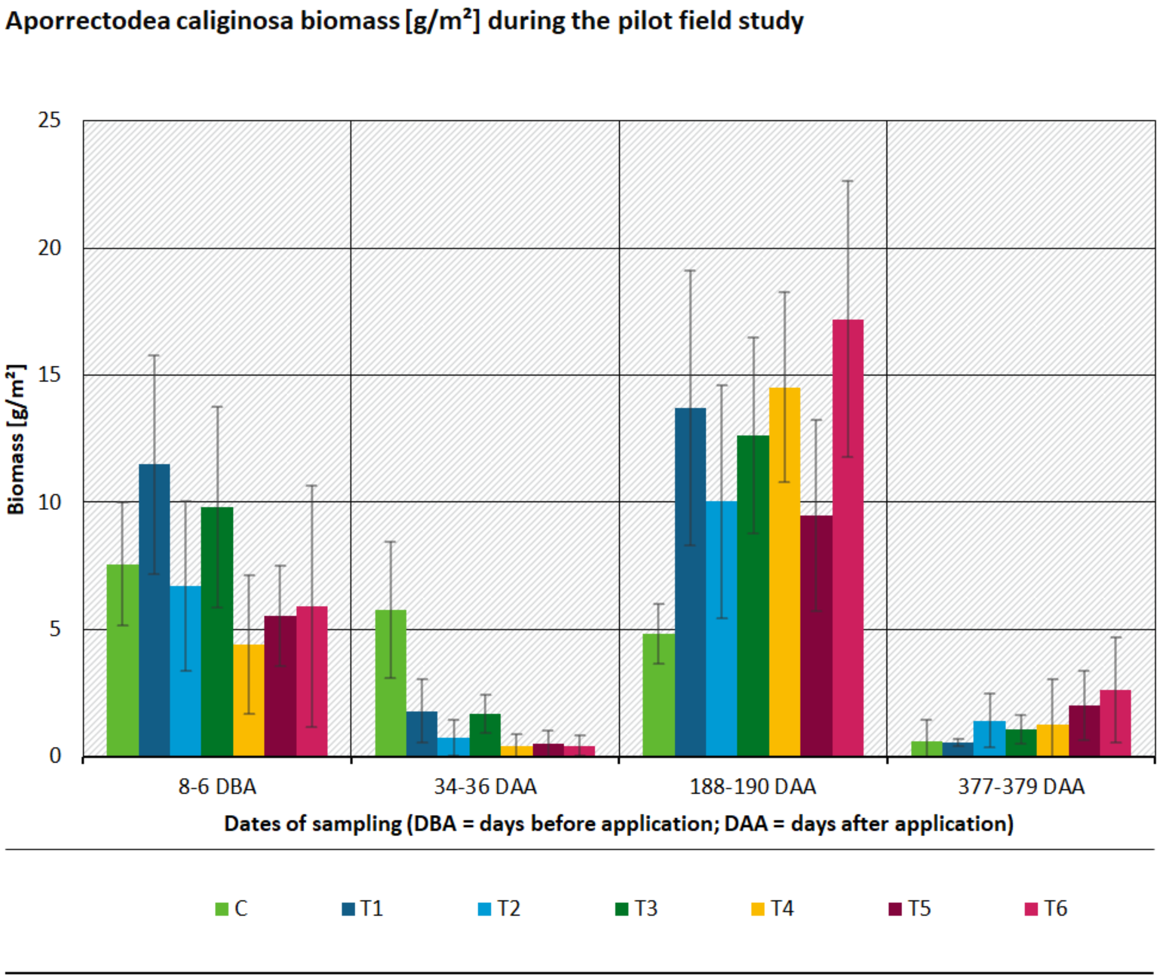
Source: ECT Oekotoxikologie GmbH

Figure A1-5: ***Aporrectodea caliginosa*** **abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



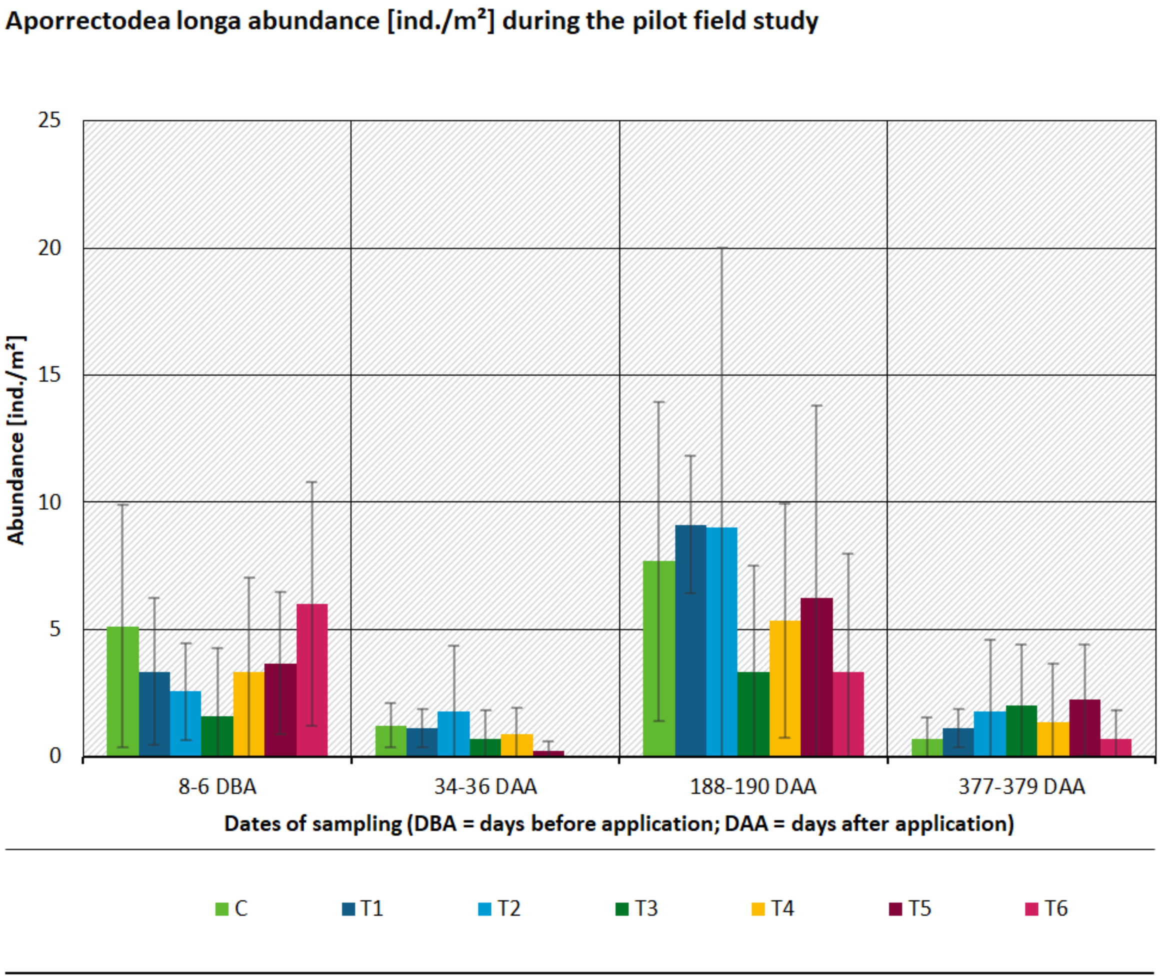
Source: ECT Oekotoxikologie GmbH

Figure A1-6: *Aporrectodea caliginosa* **biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



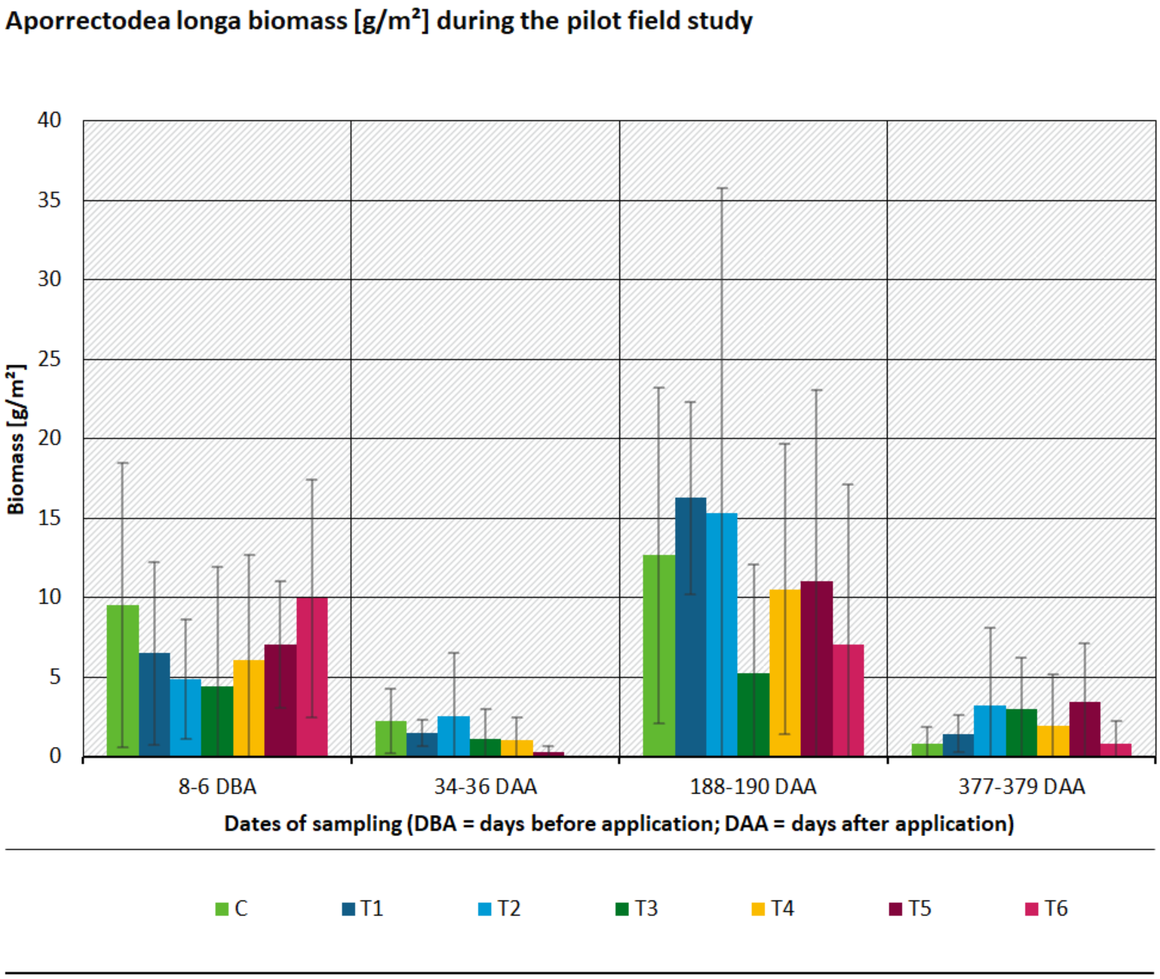
Source: ECT Oekotoxikologie GmbH

Figure A1-7: ***Aporrectodea longa*** **abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



Source: ECT Oekotoxikologie GmbH

Figure A1-8: ***Aporrectodea longa*** **biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



Source: ECT Oekotoxikologie GmbH

Figure A1-9: ***Aporrectodea rosea*** **abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**

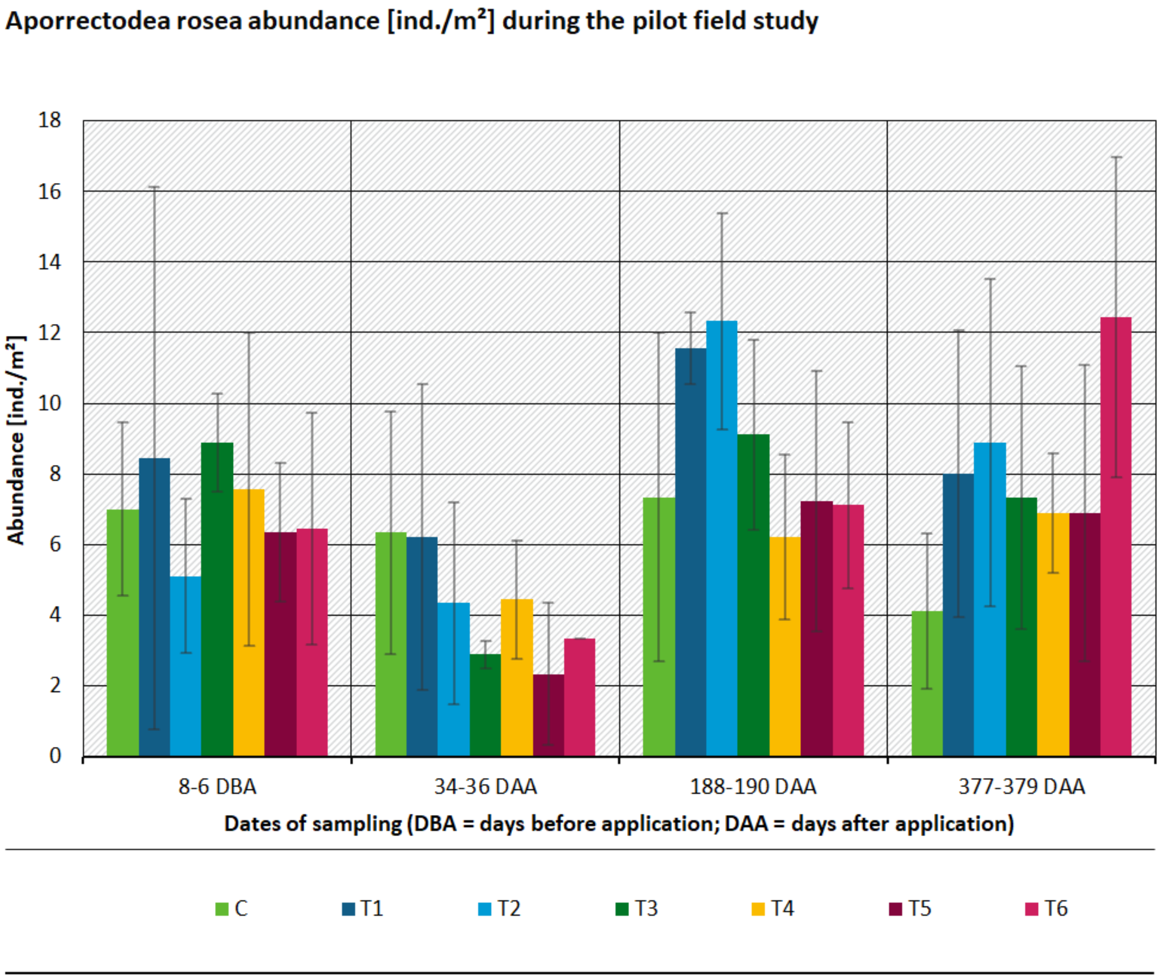


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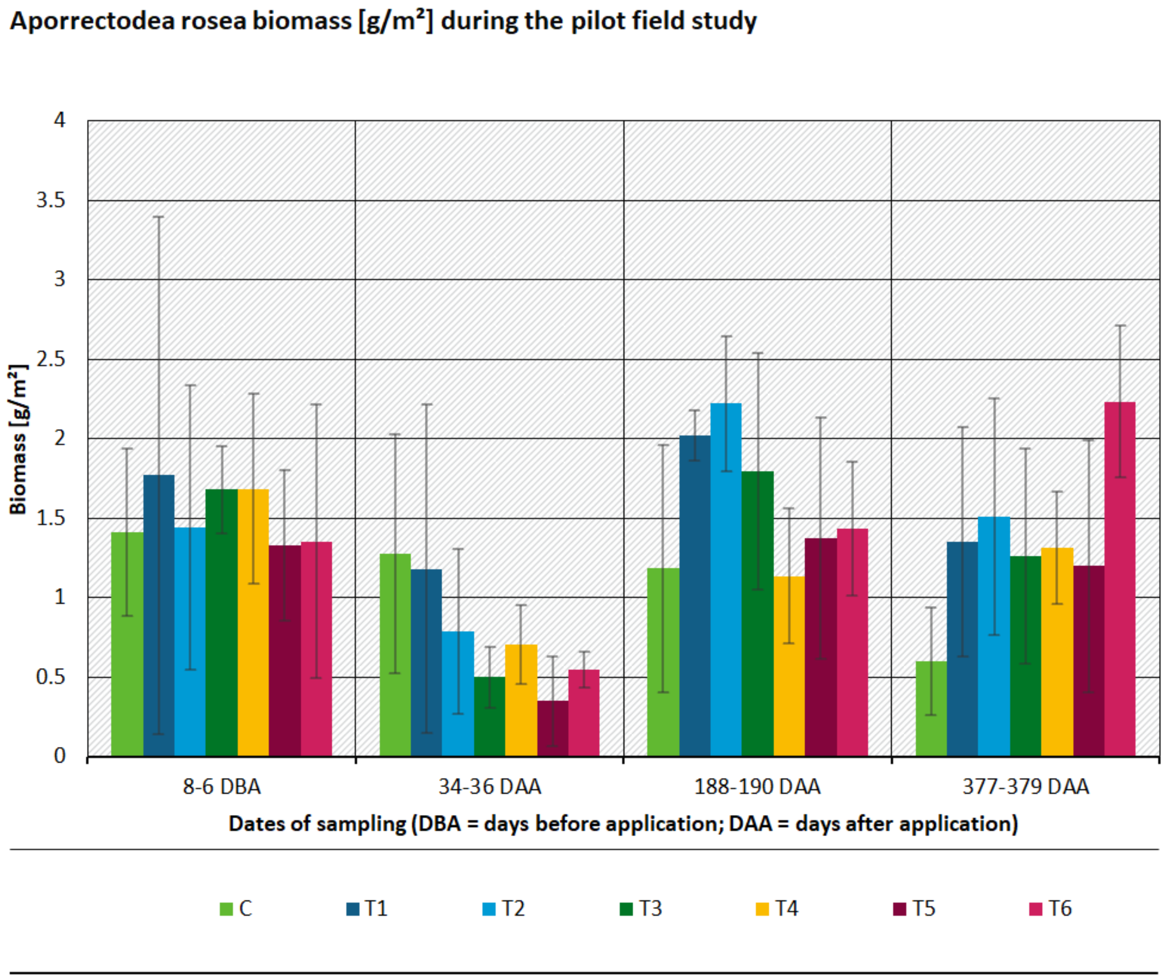
Figure A1-10: *Aporrectodea rosea* **biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



Source: ECT Oekotoxikologie GmbH

Figure A1-11: *Lumbricus* spp. abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)

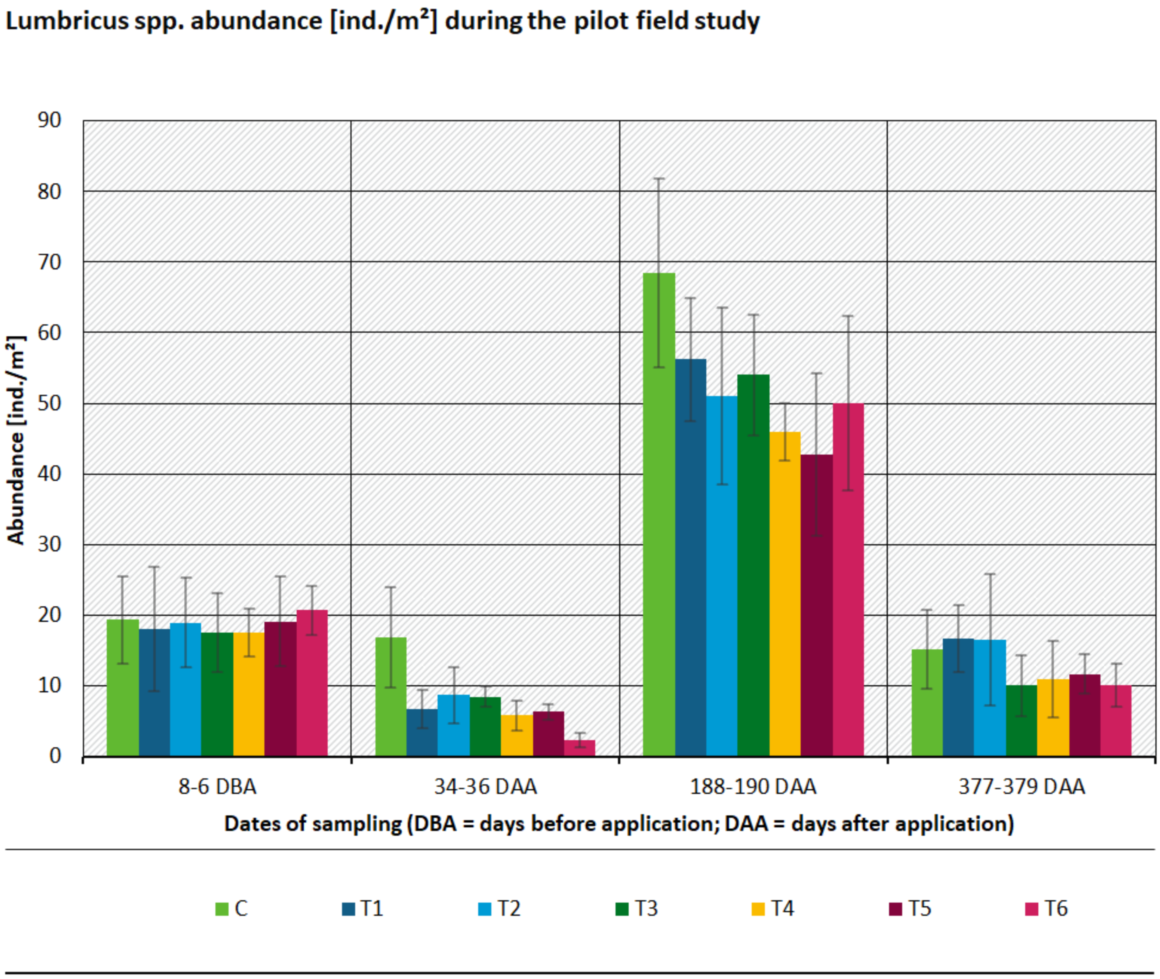


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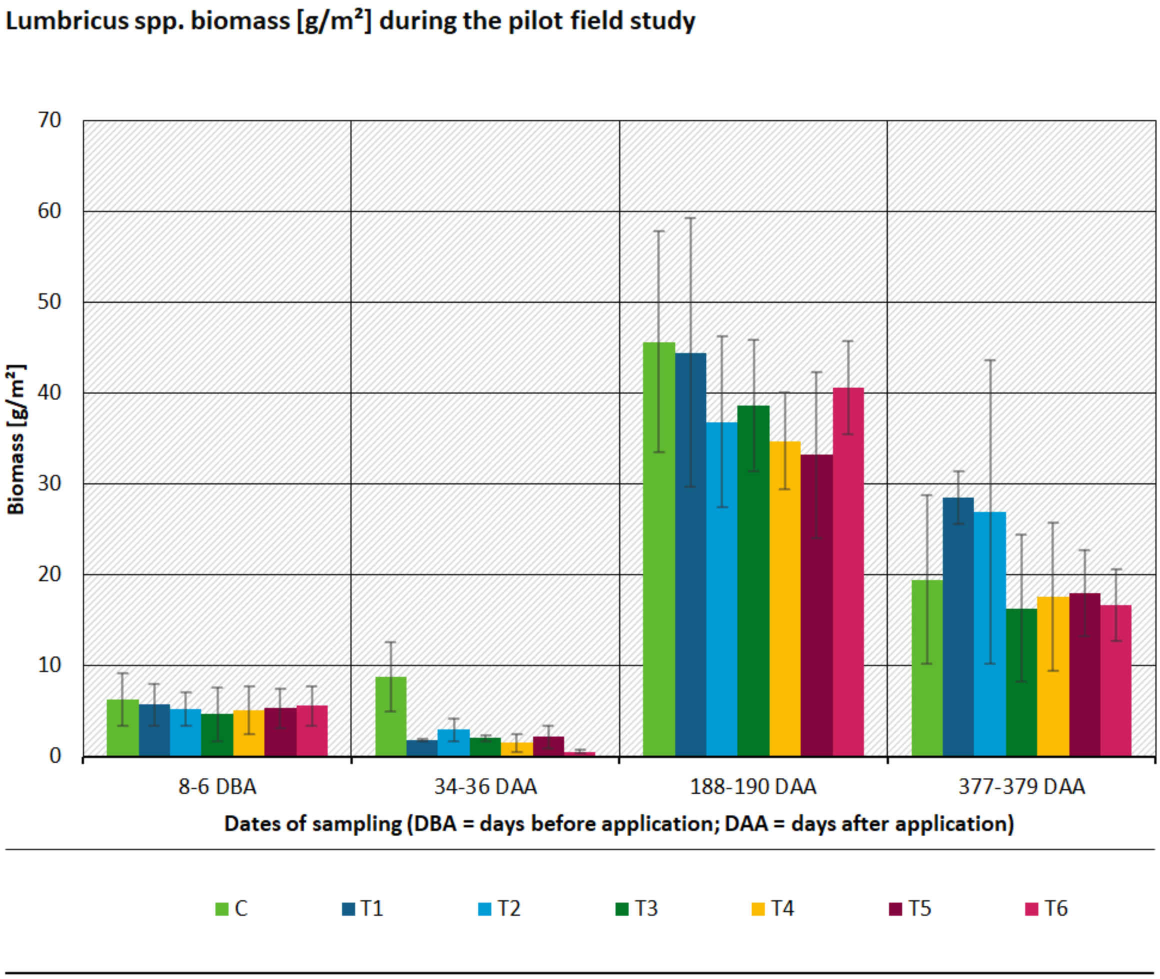
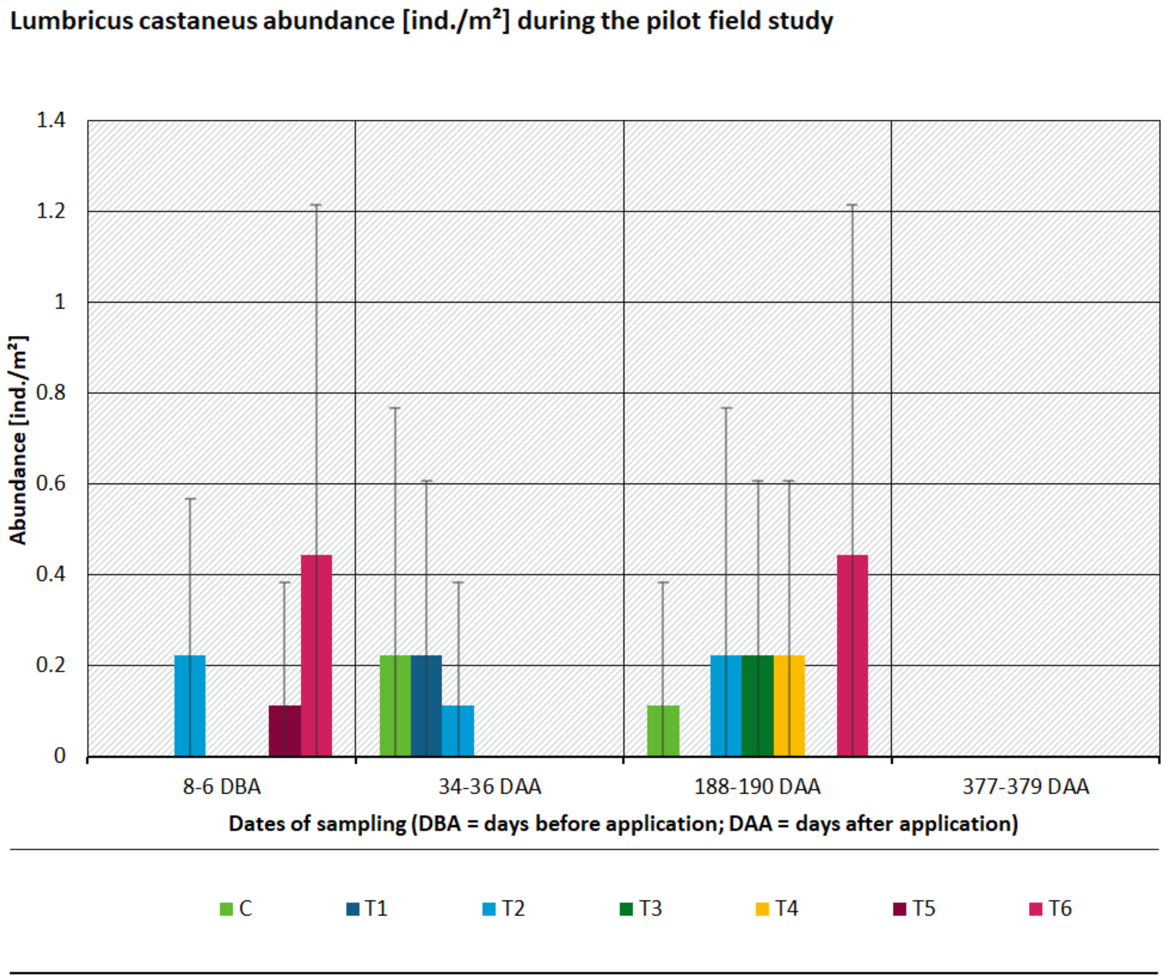
Figure A1-12: ***Lumbricus*** **spp. biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



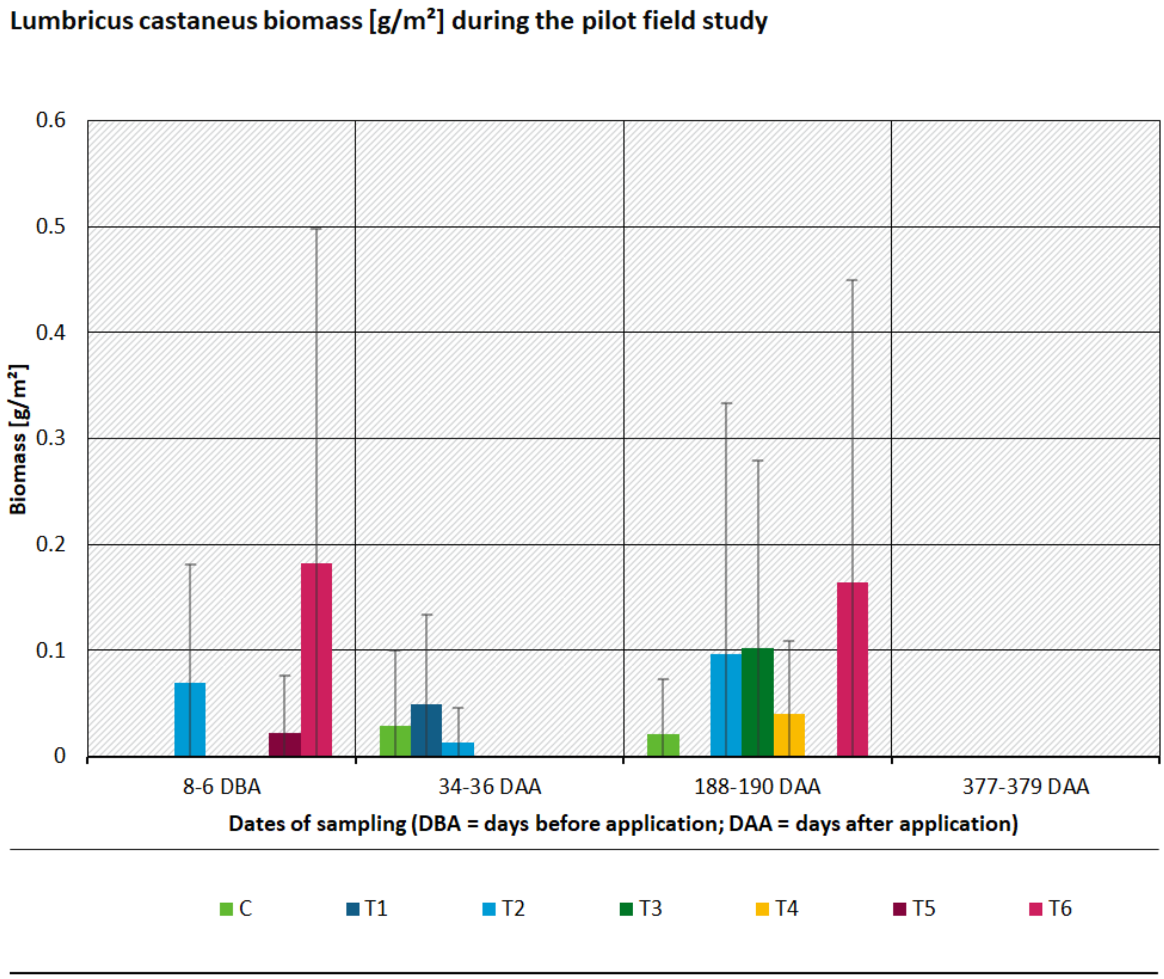
Source: ECT Oekotoxikologie GmbH

Figure A1-13: ***Lumbricus castaneus*** **abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



Source: ECT Oekotoxikologie GmbH

Figure A1-14: ***Lumbricus castaneus*** **biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



Source: ECT Oekotoxikologie GmbH

Figure A1-15: *Lumbricus terrestris* abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)

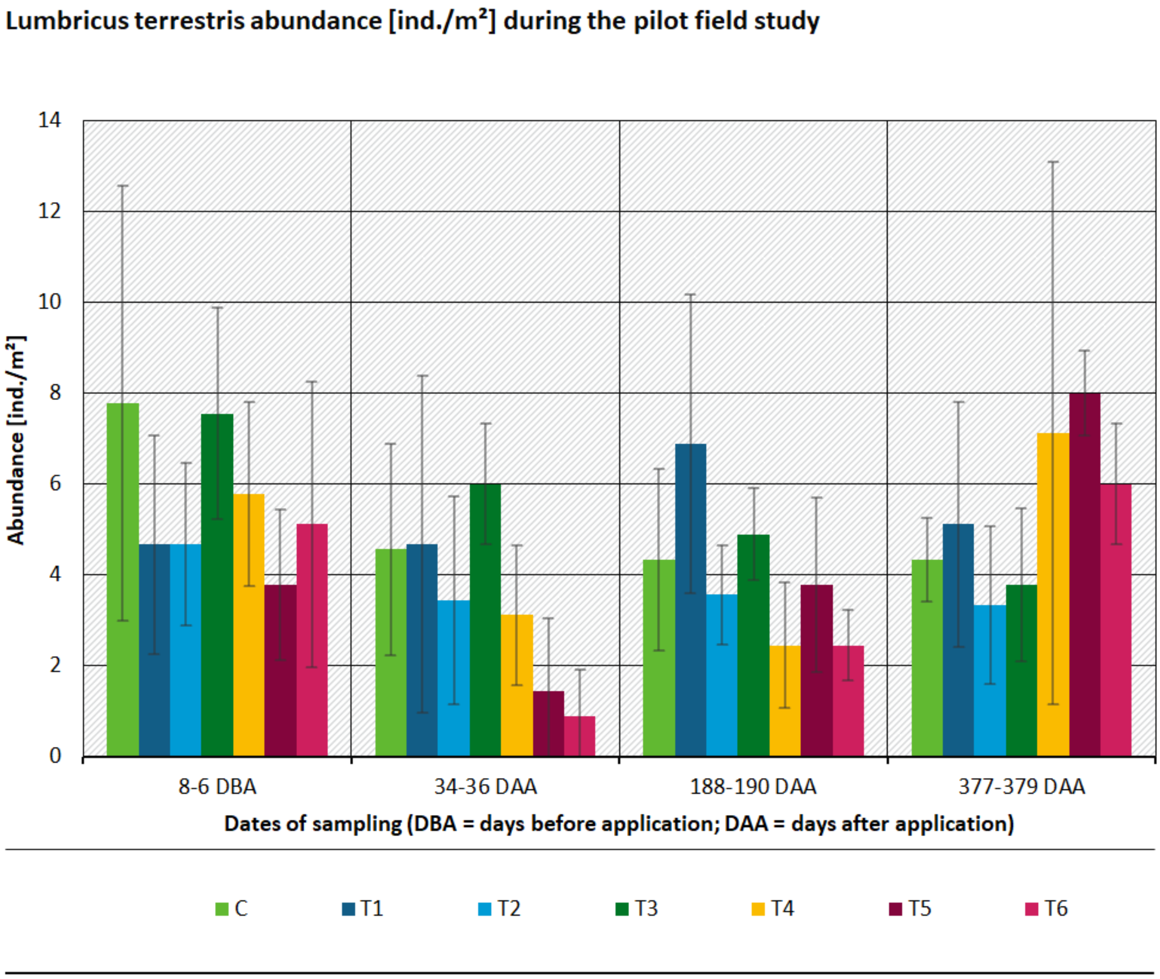


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Figure A1-16: ***Lumbricus terrestris*** **biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**

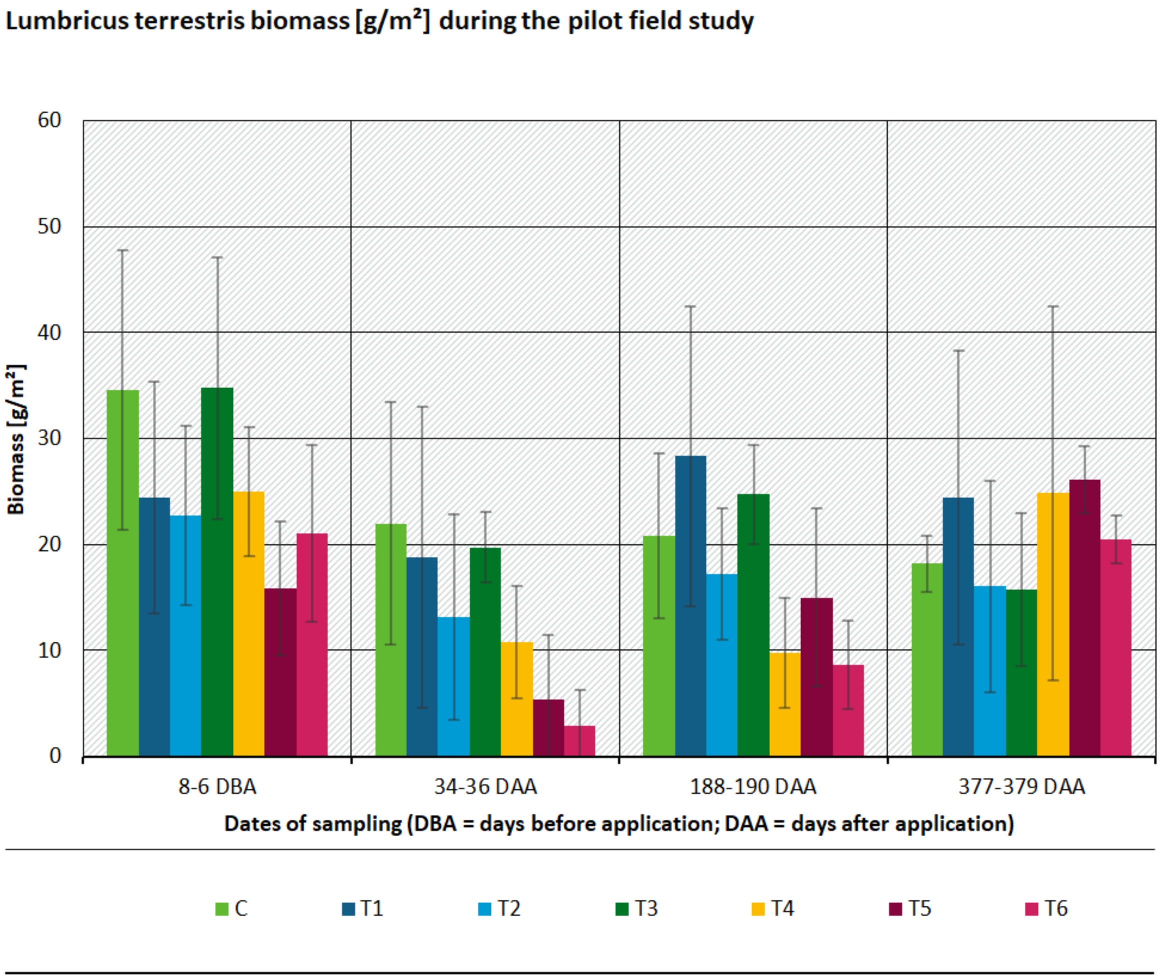

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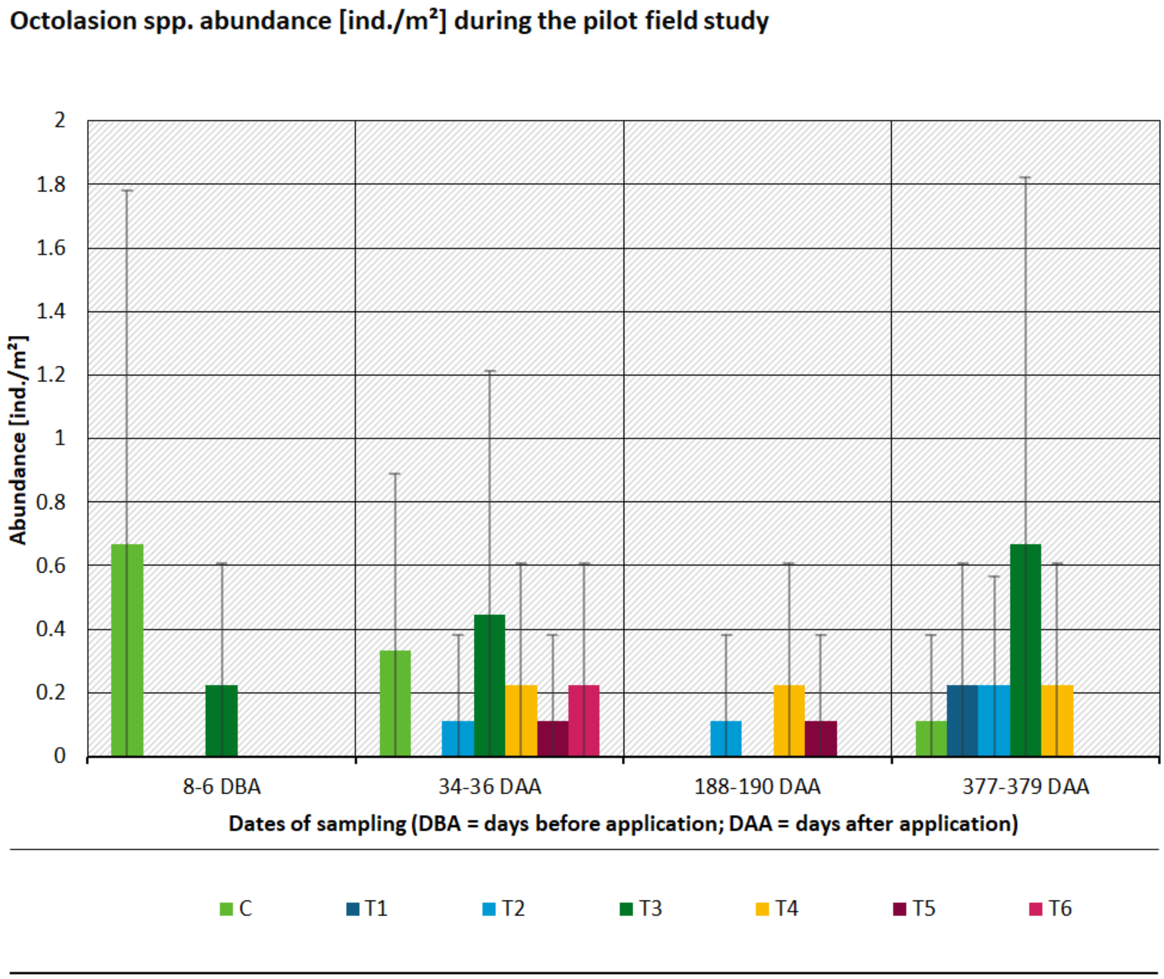
Figure A1-17: *Octolasion* spp. abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



Source: ECT Oekotoxikologie GmbH

Figure A1-18: ***Octolasion*** **spp. biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**

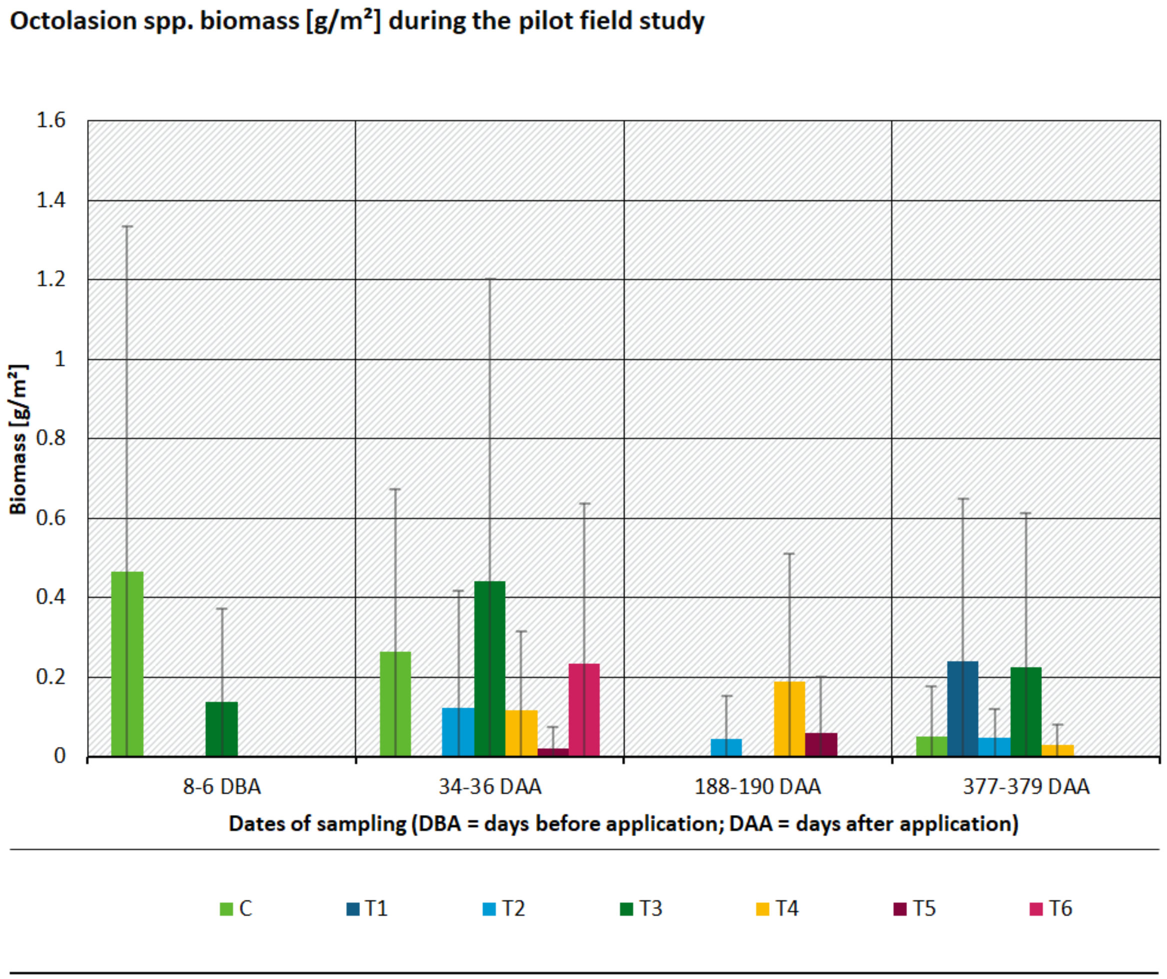


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Figure A1-19: ***Octolasion cyaneum*** **abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**

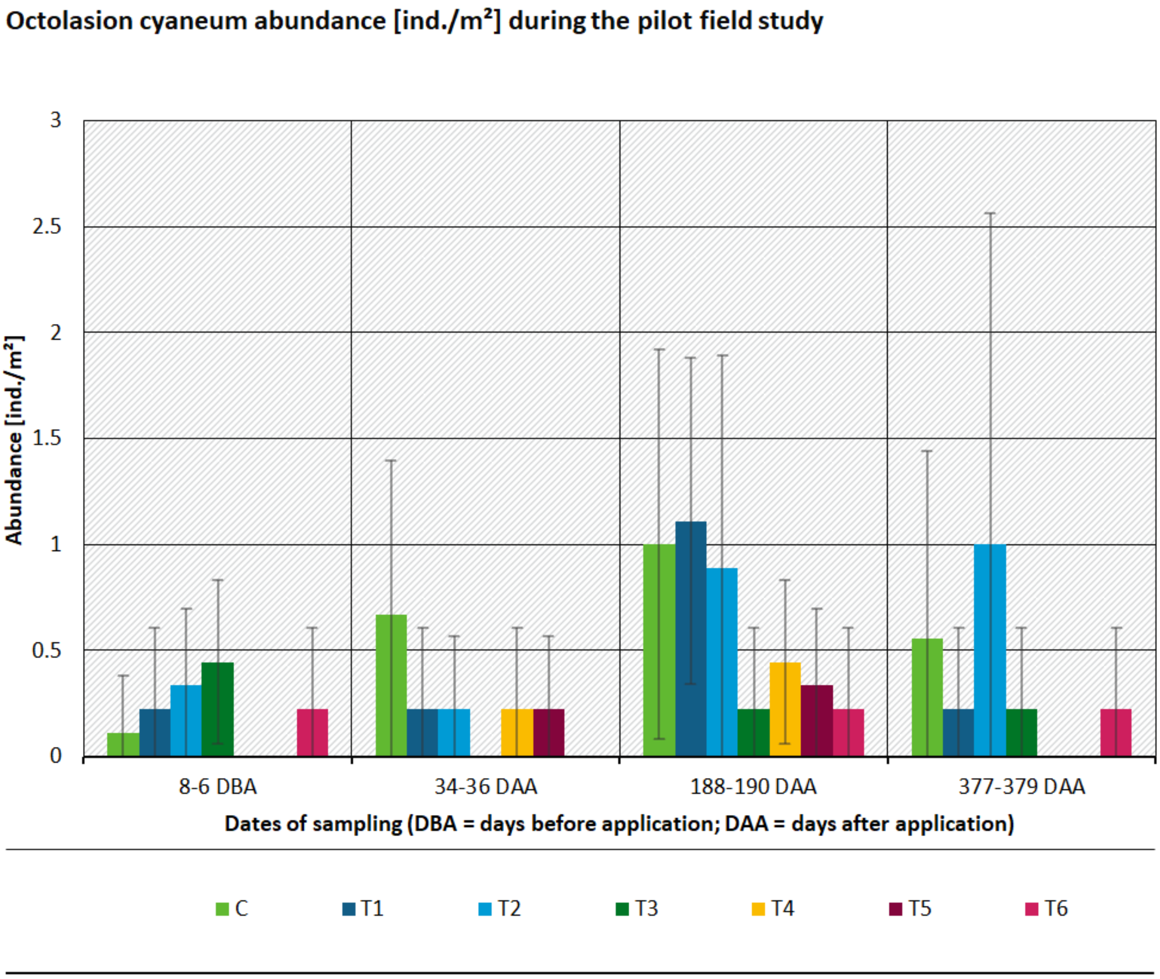

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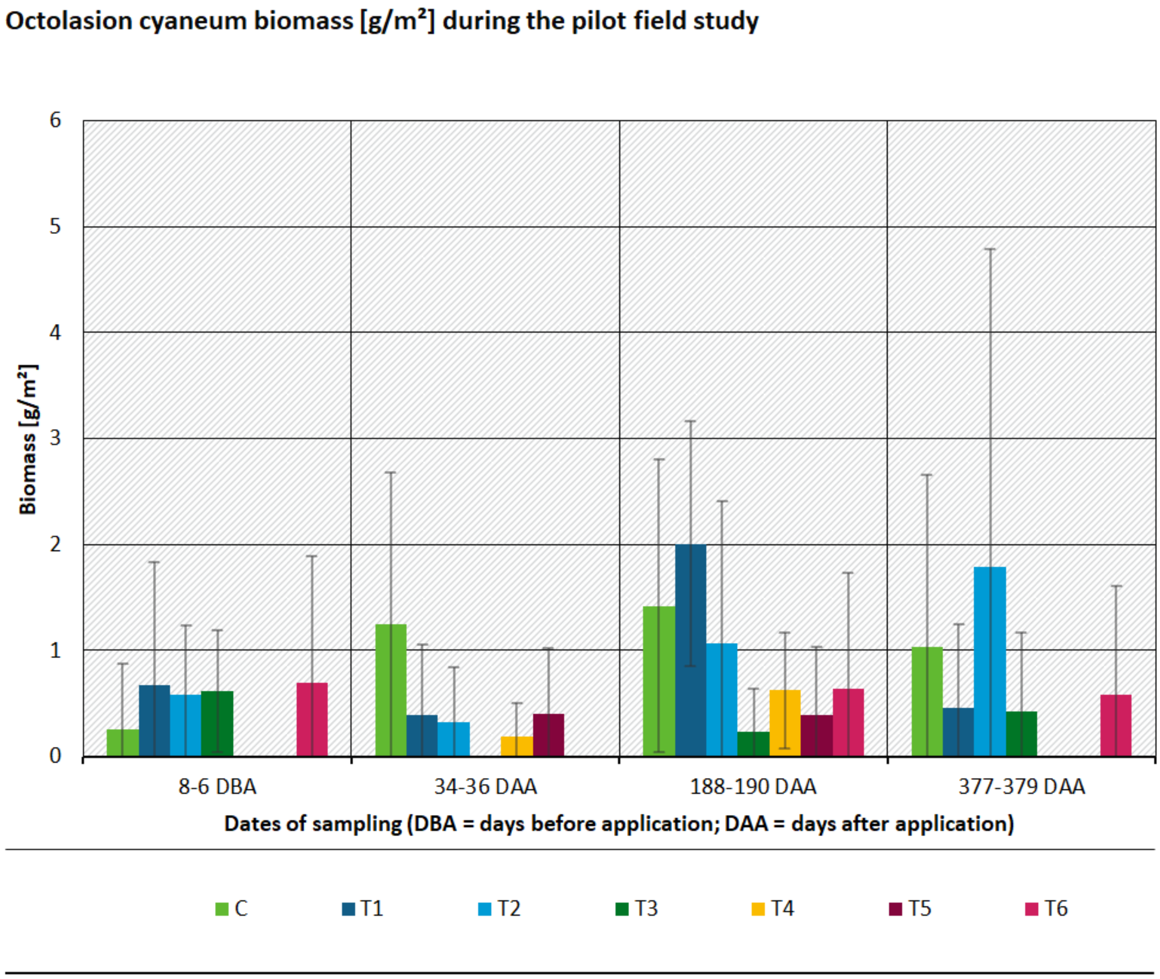
Figure A1-20: ***Octolasion cyaneum*** **biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



Source: ECT Oekotoxikologie GmbH

Figure A1-21: *Octolasion tyrtaeum* abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)

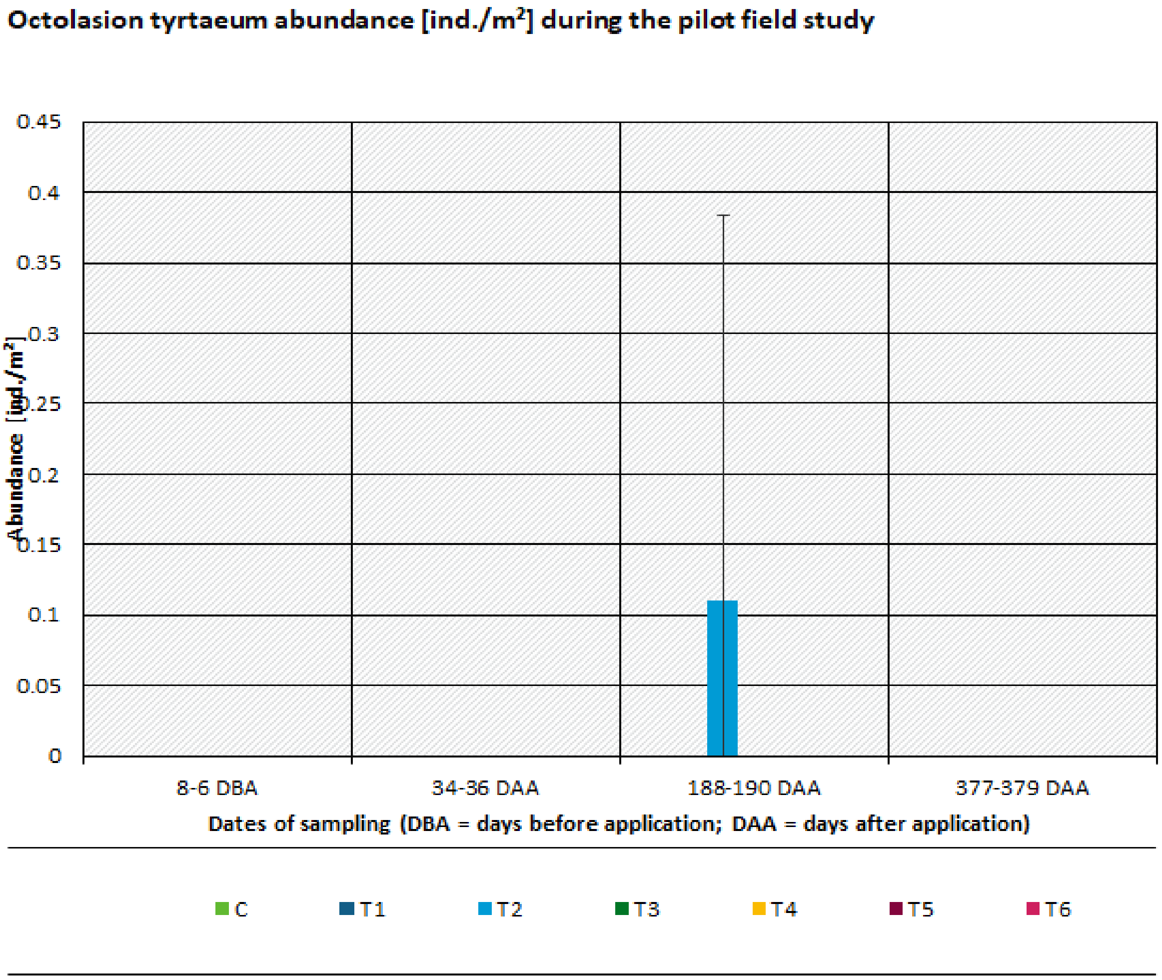


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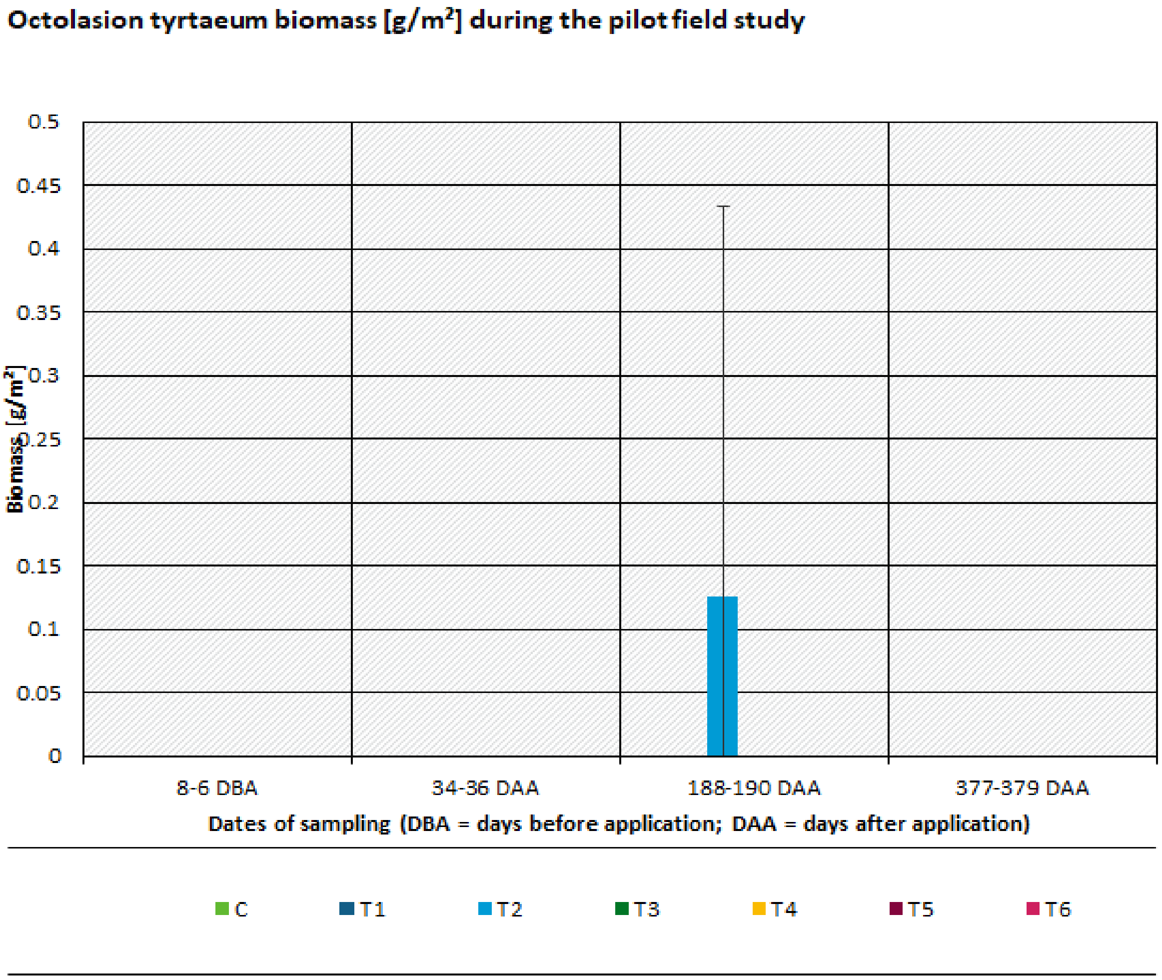
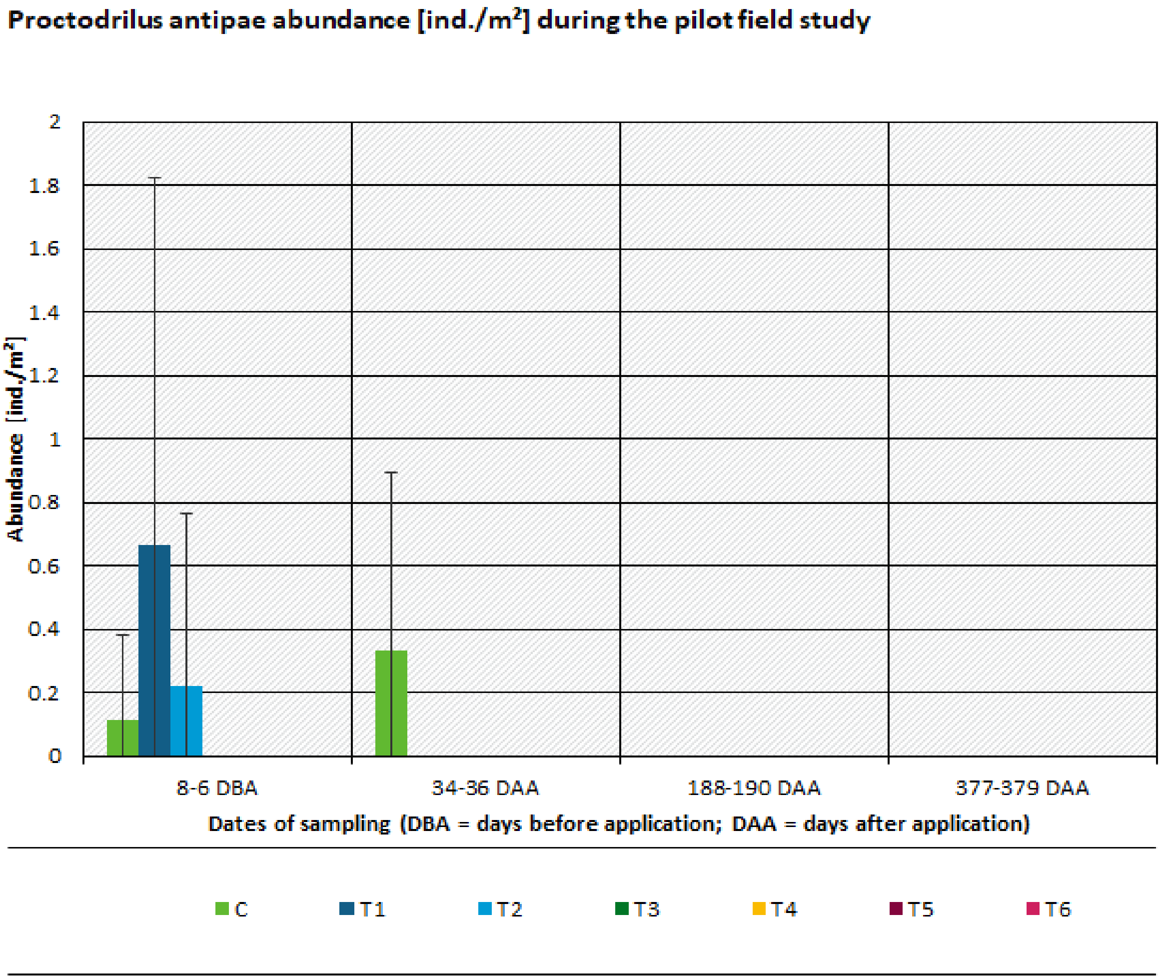
Figure A1-22: *Octolasion tyrtaeum* biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



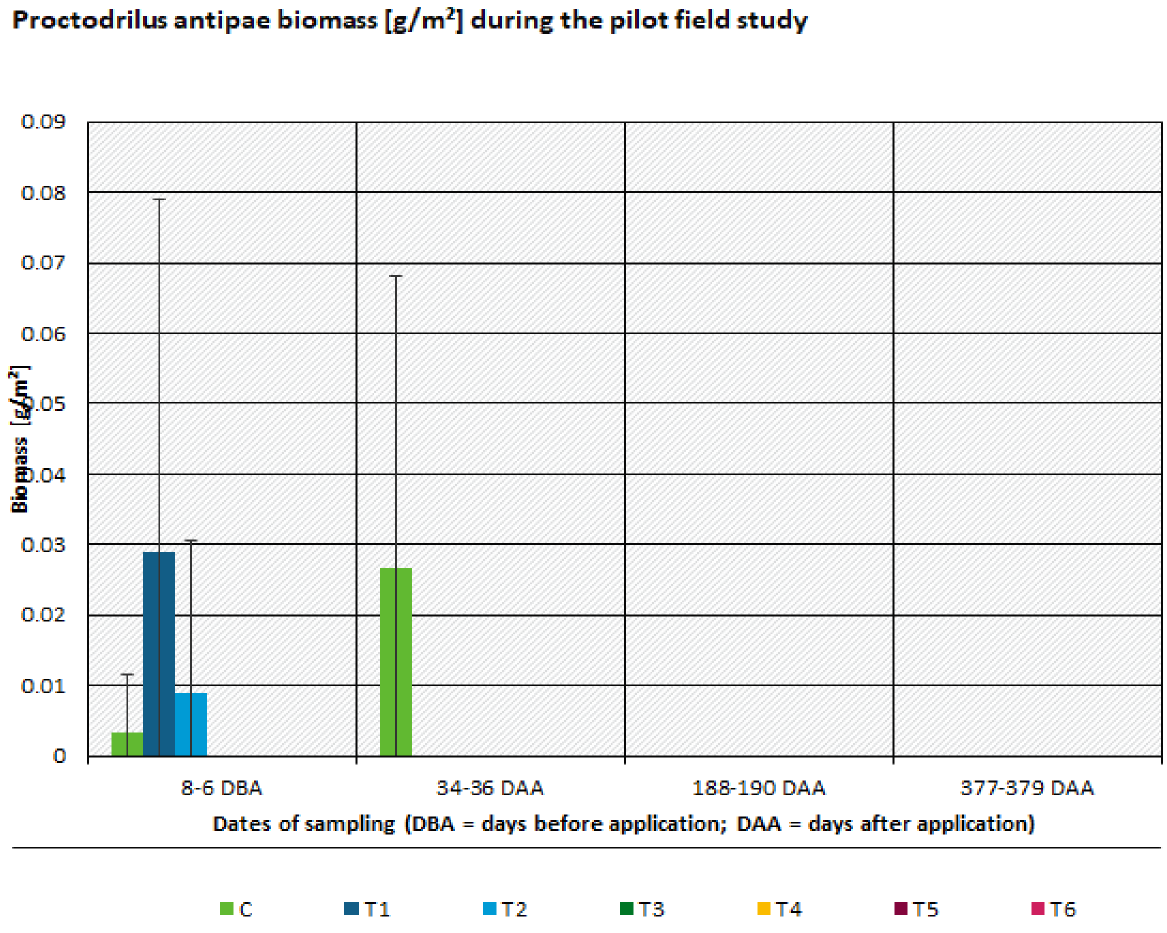
Source: ECT Oekotoxikologie GmbH

Figure A1-23: *Proctodrilus antipae* abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



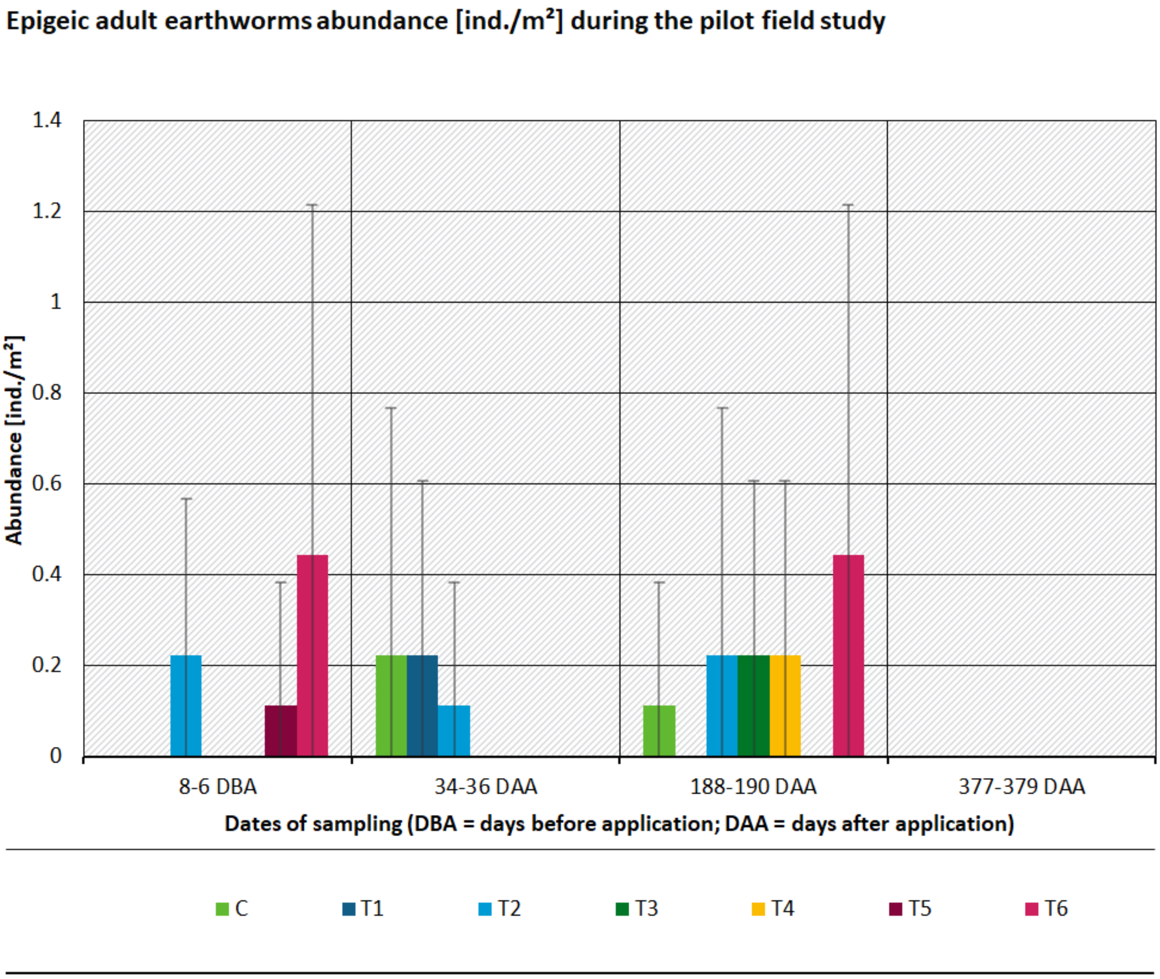
Source: ECT Oekotoxikologie GmbH

Figure A1-24: *Proctodrilus antipae* biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



Source: ECT Oekotoxikologie GmbH

Figure A1-25: Epigeic adult earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



Source: ECT Oekotoxikologie GmbH

Figure A1-26: Epigeic adult earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)

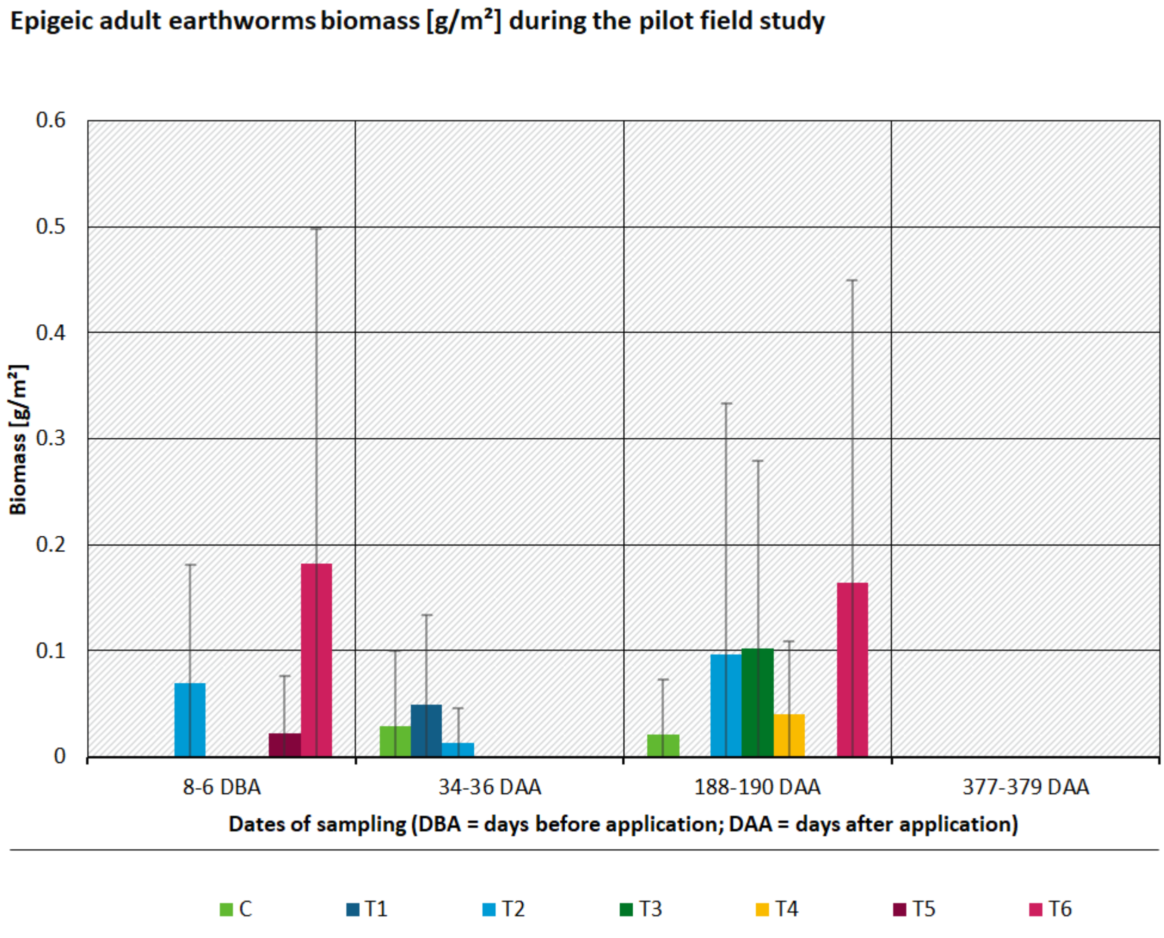


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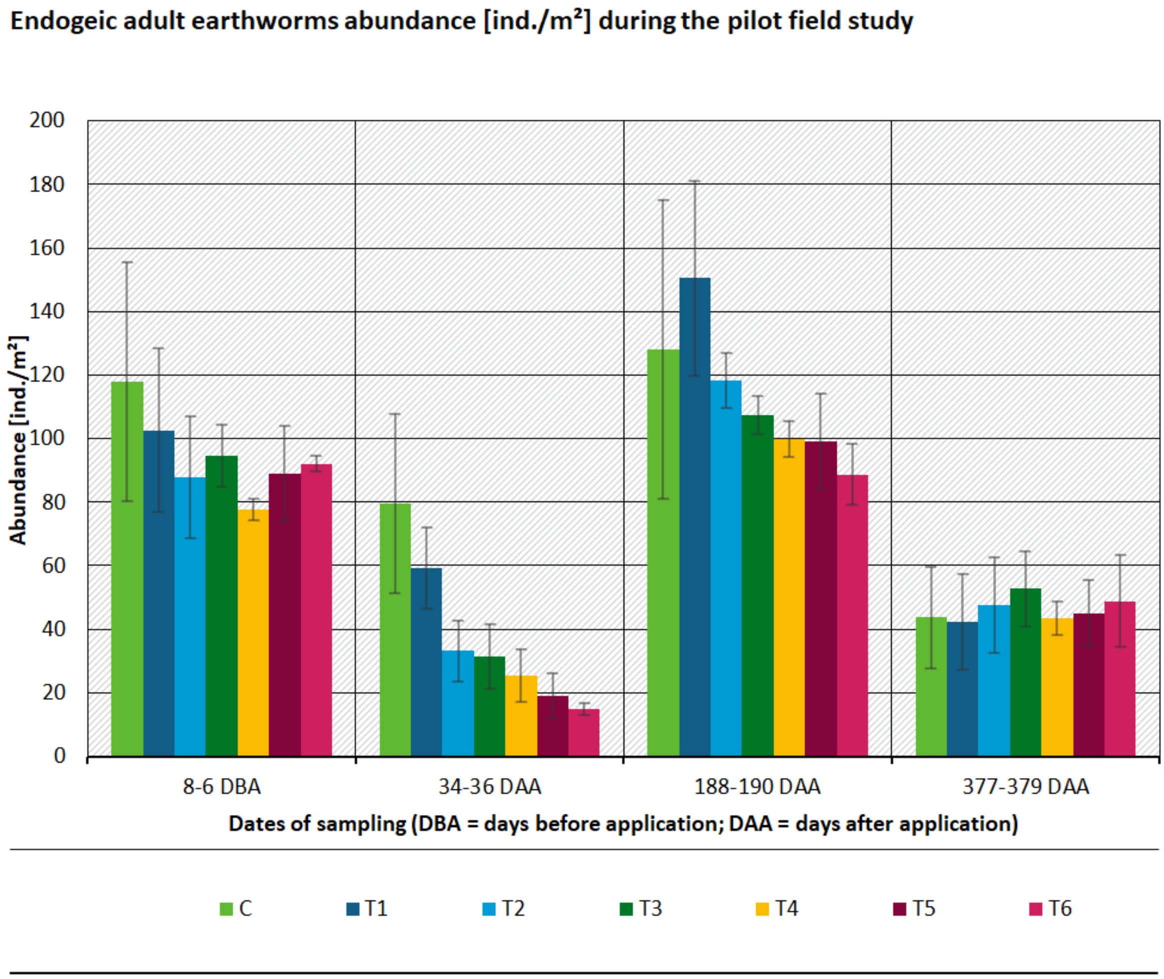
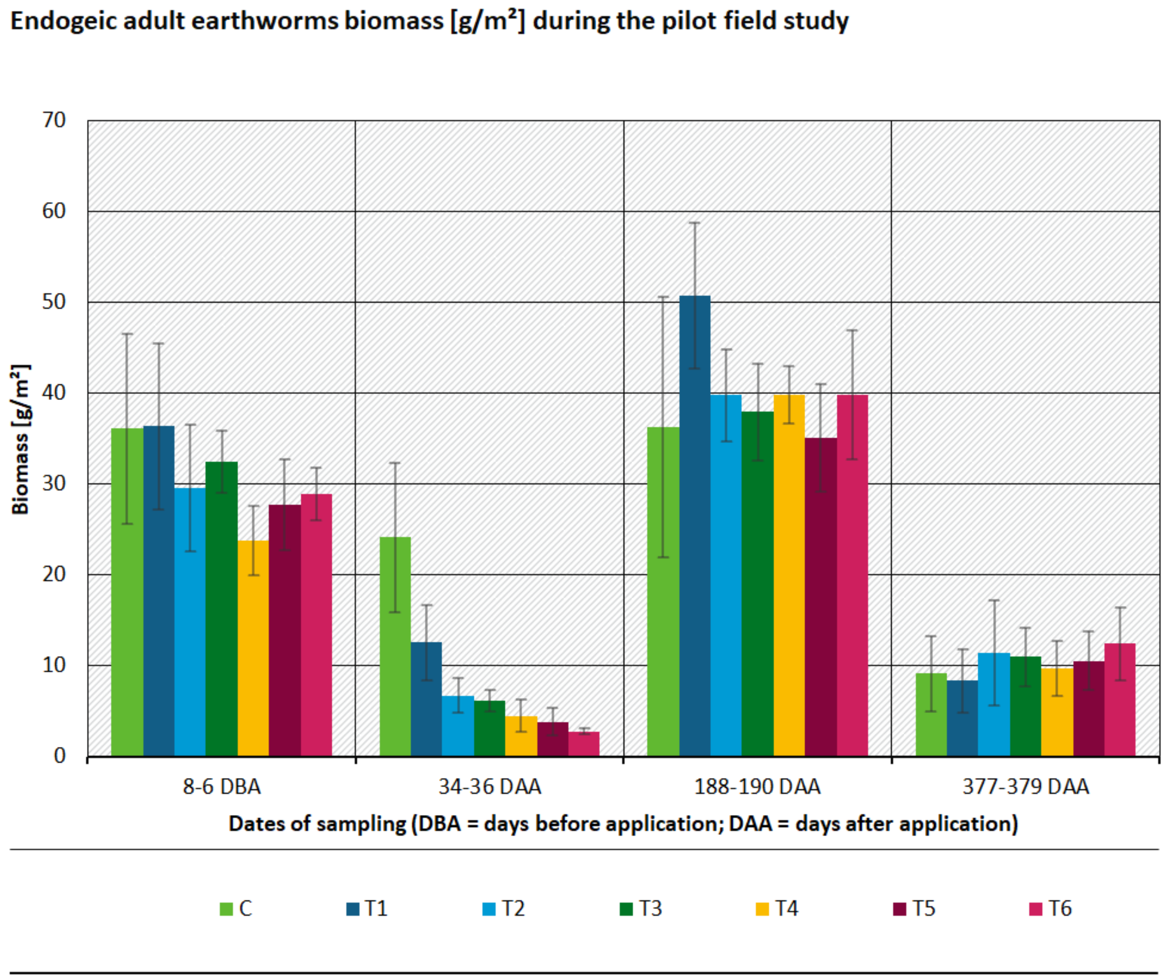
Figure A1-27: Endogeic adult earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



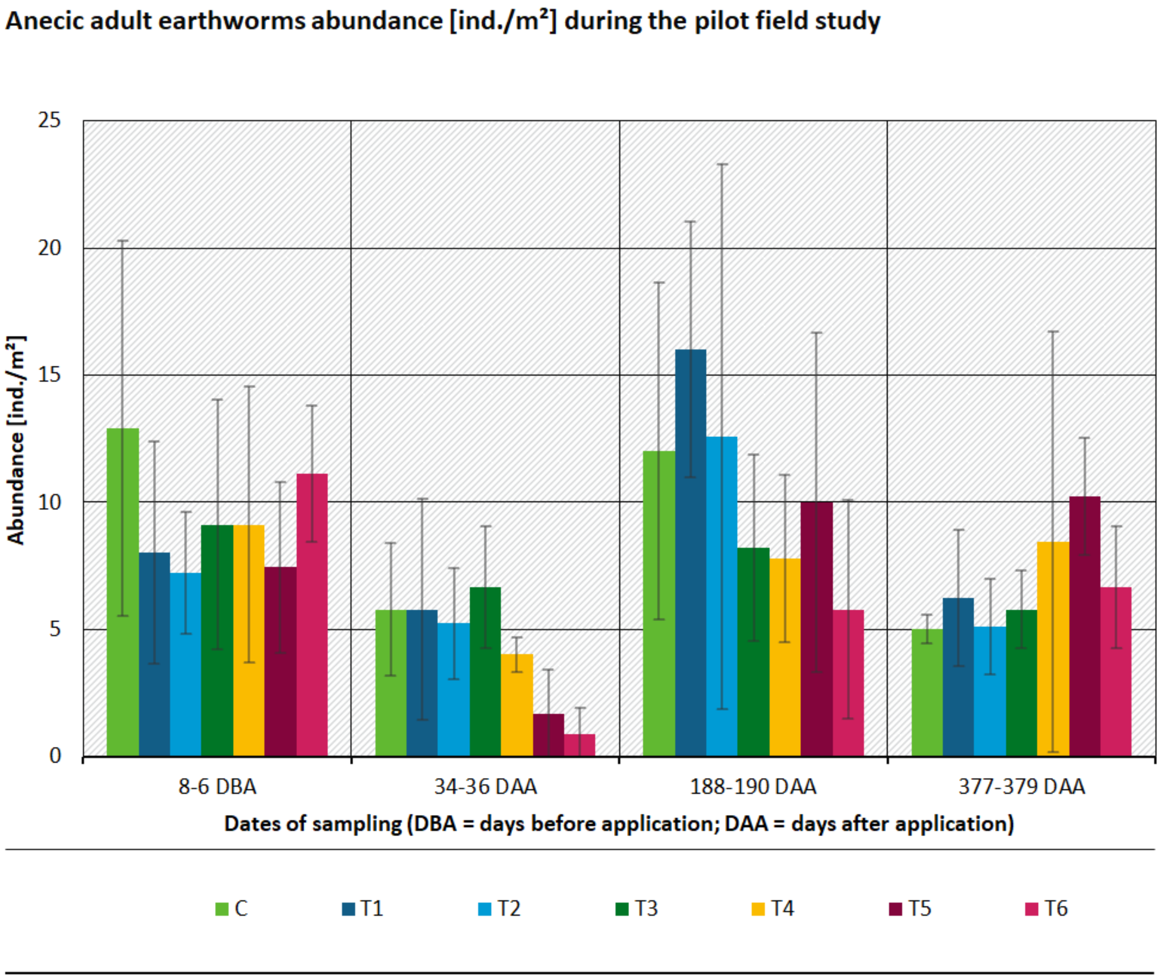
Source: ECT Oekotoxikologie GmbH

Figure A1-28: Endogeic adult earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



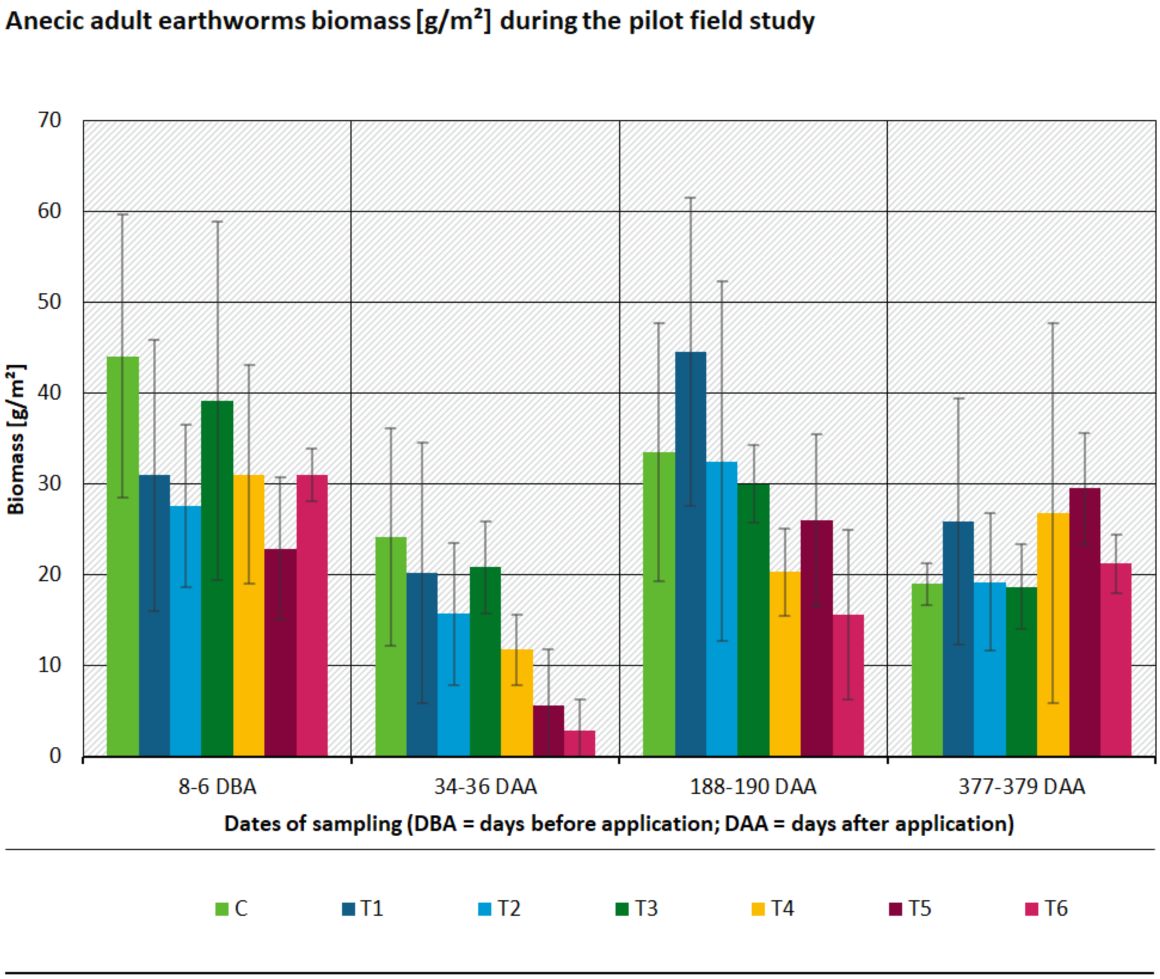
Source: ECT Oekotoxikologie GmbH

Figure A1-29: Anecic adult earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



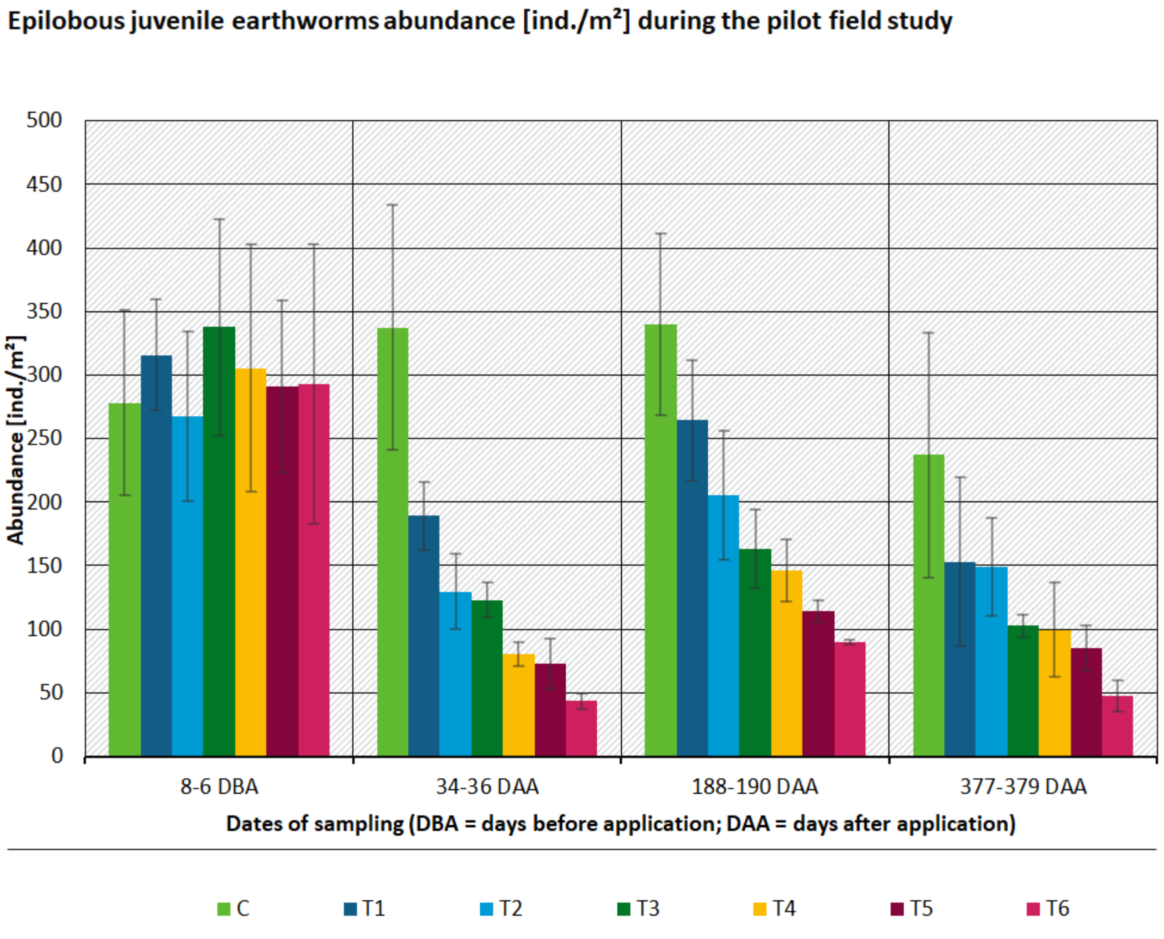
Source: ECT Oekotoxikologie GmbH

Figure A1-30: Anecic adult earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



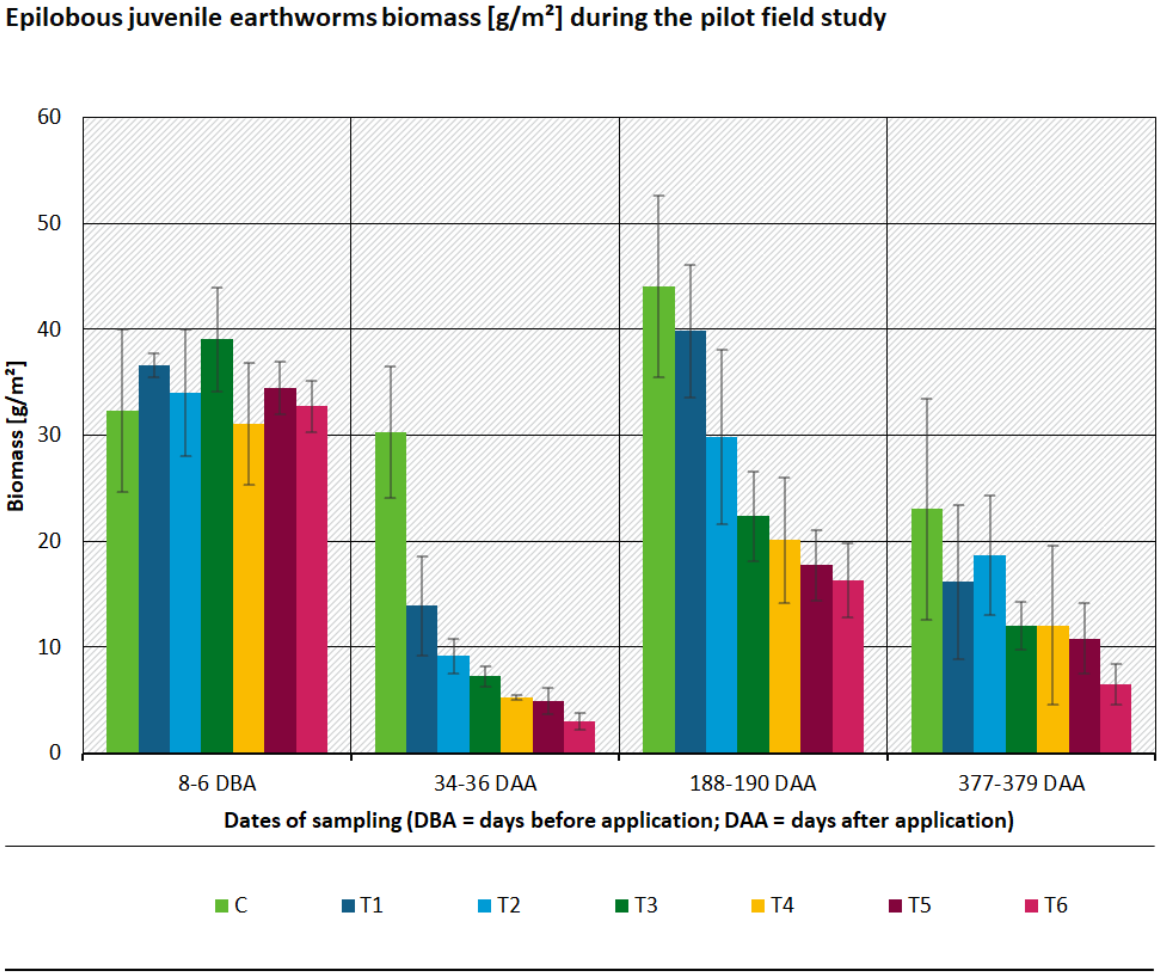
Source: ECT Oekotoxikologie GmbH

Figure A1-31: Epilobous juvenile earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



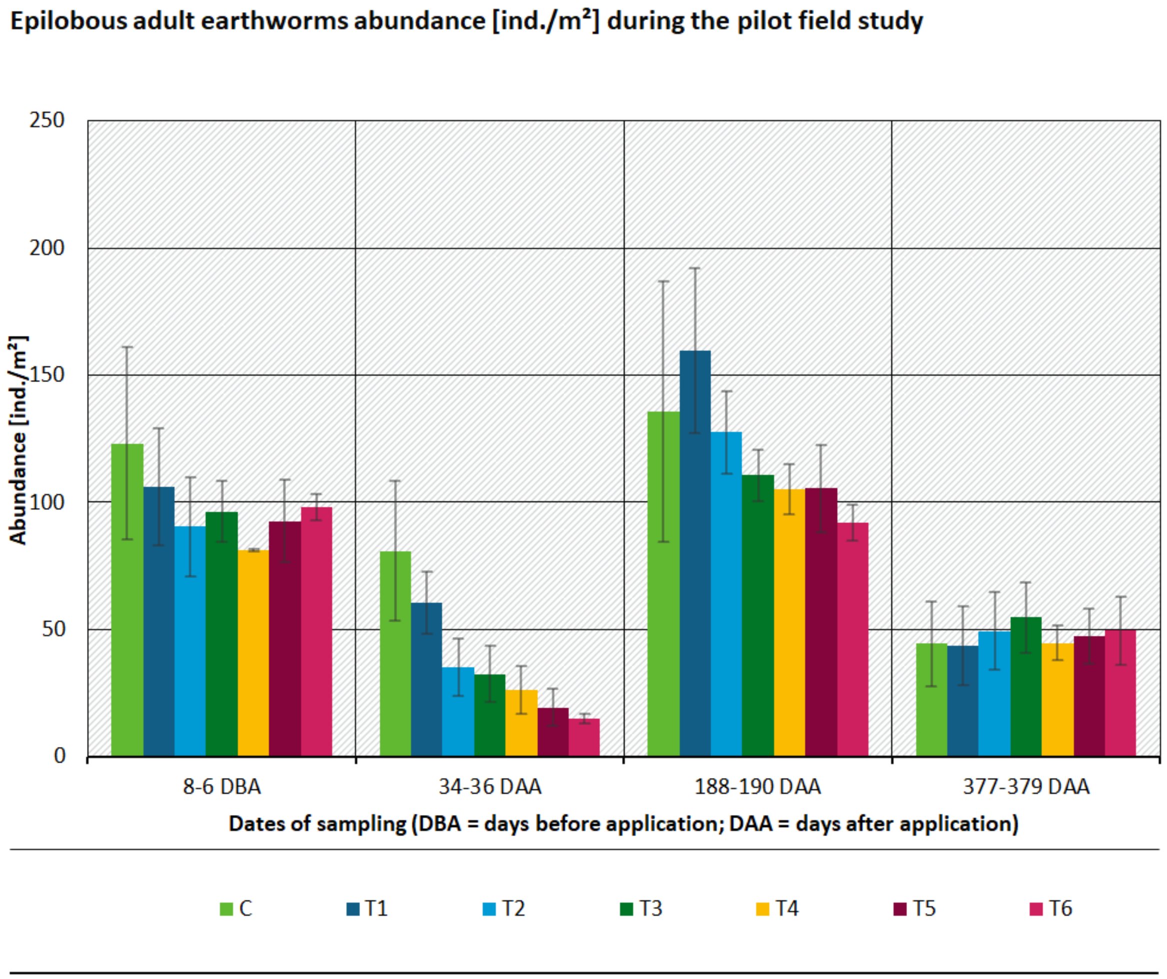
Source: ECT Oekotoxikologie GmbH

Figure A1-32: Epilobous juvenile earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



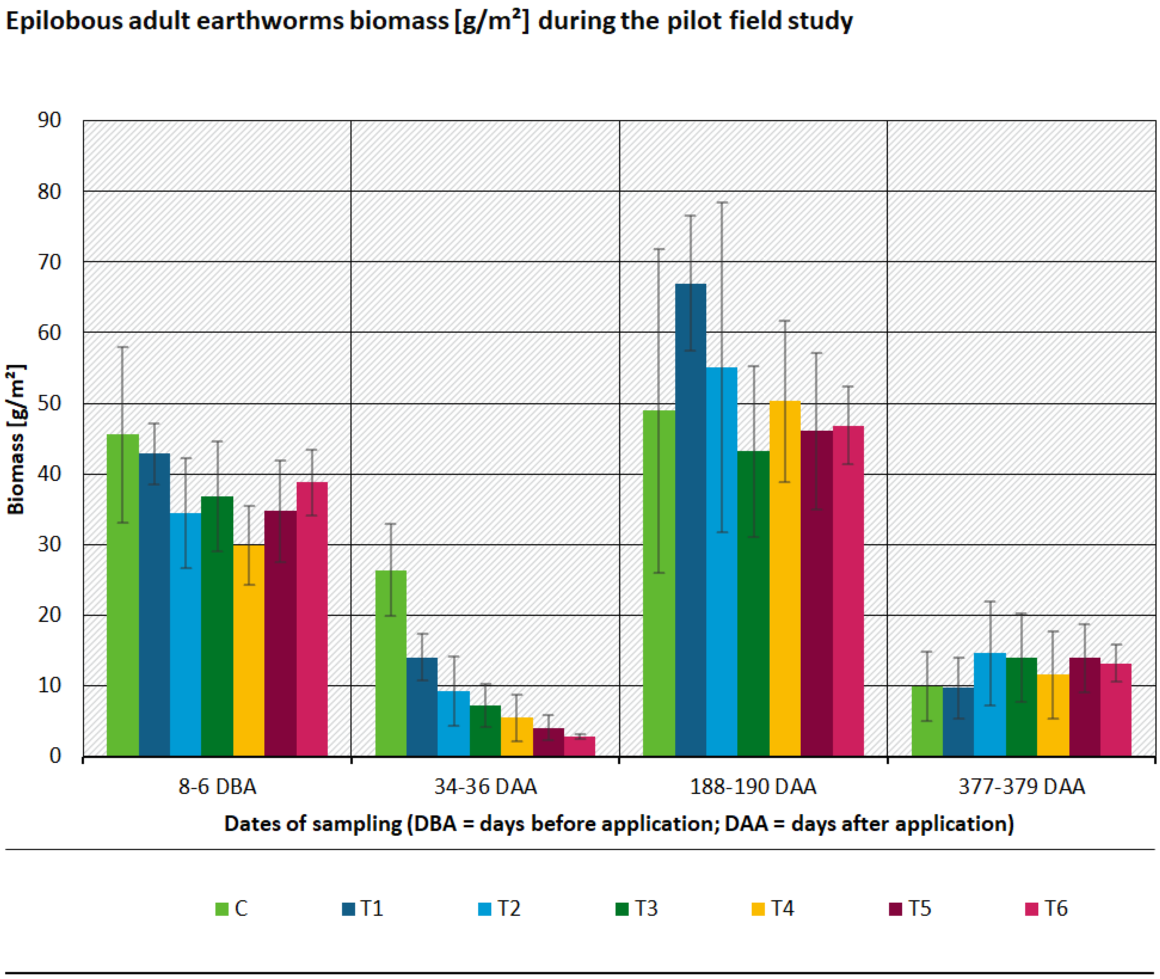
Source: ECT Oekotoxikologie GmbH

Figure A1-33: **Epilobous adult earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



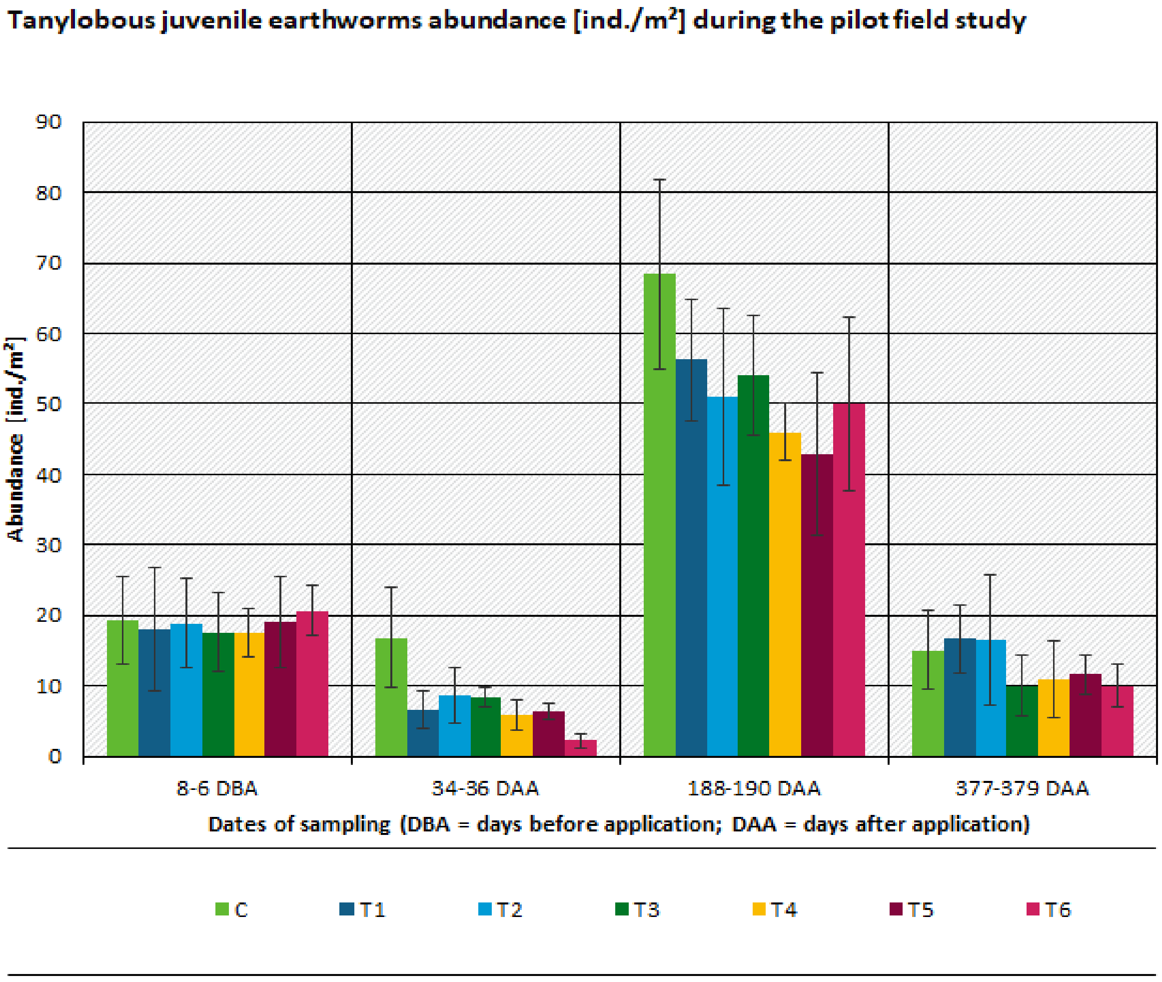
Source: ECT Oekotoxikologie GmbH

Figure A1-34: Epilobous adult earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



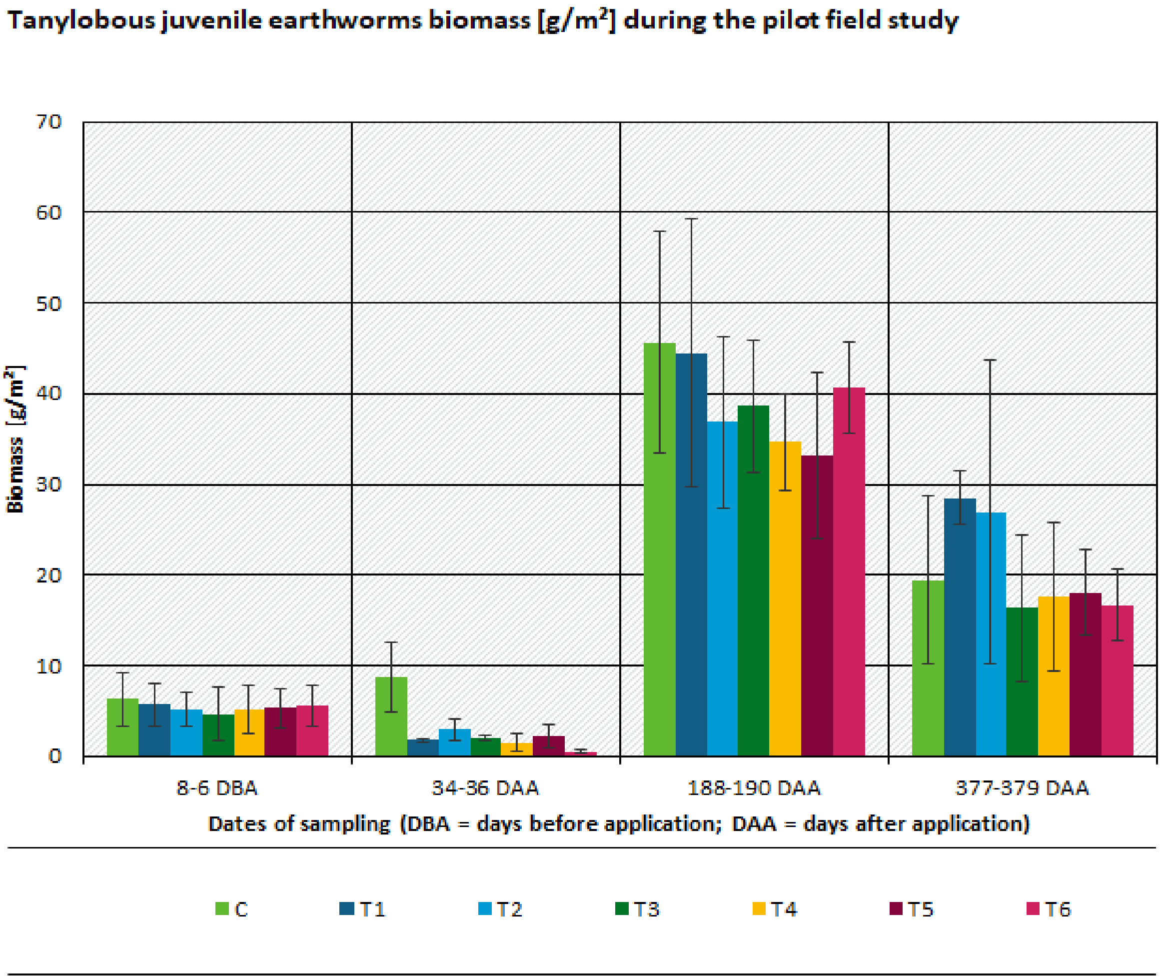
Source: ECT Oekotoxikologie GmbH

Figure A1-35: Tanylobous juvenile earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



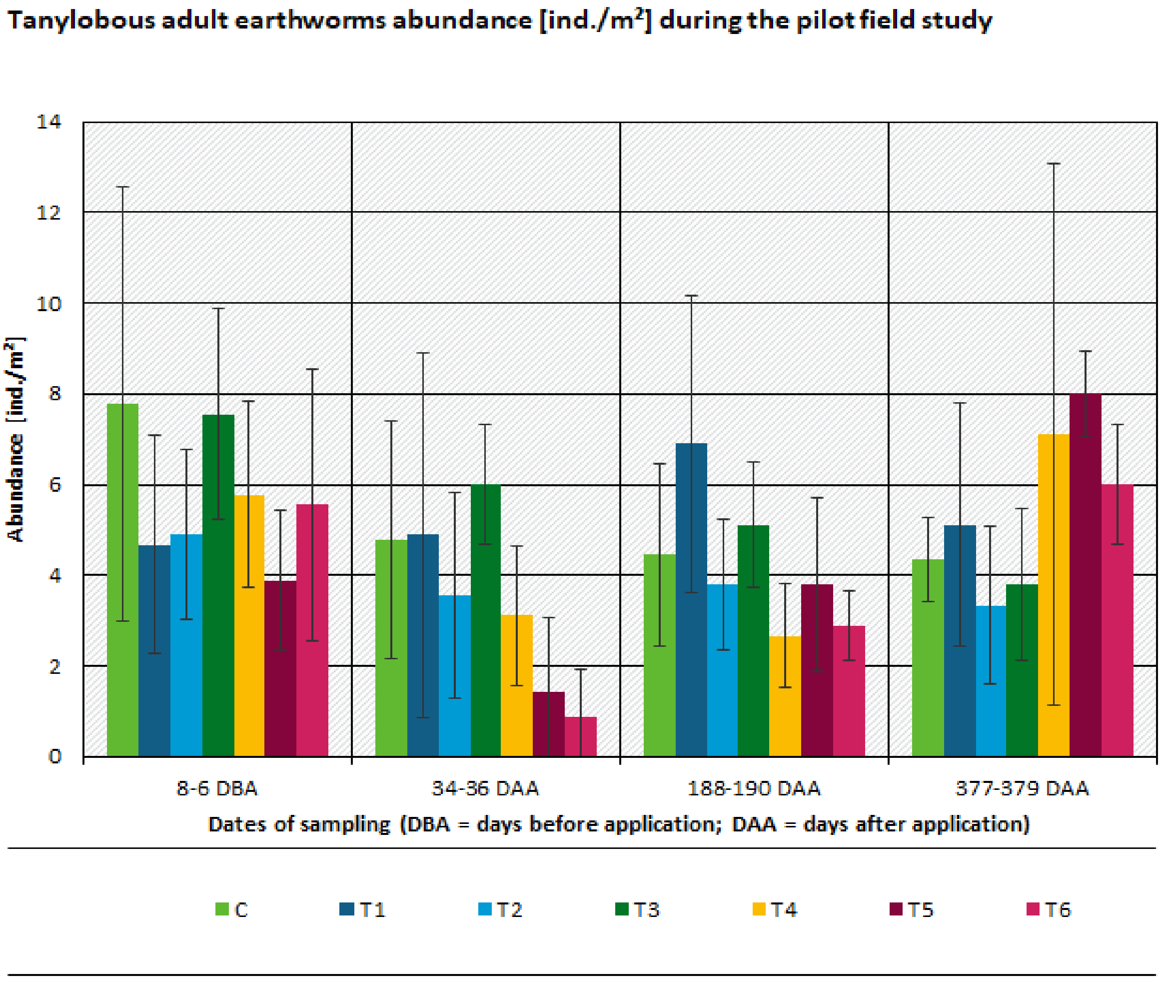
Source: ECT Oekotoxikologie GmbH

Figure A1-36: Tanylobous juvenile earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



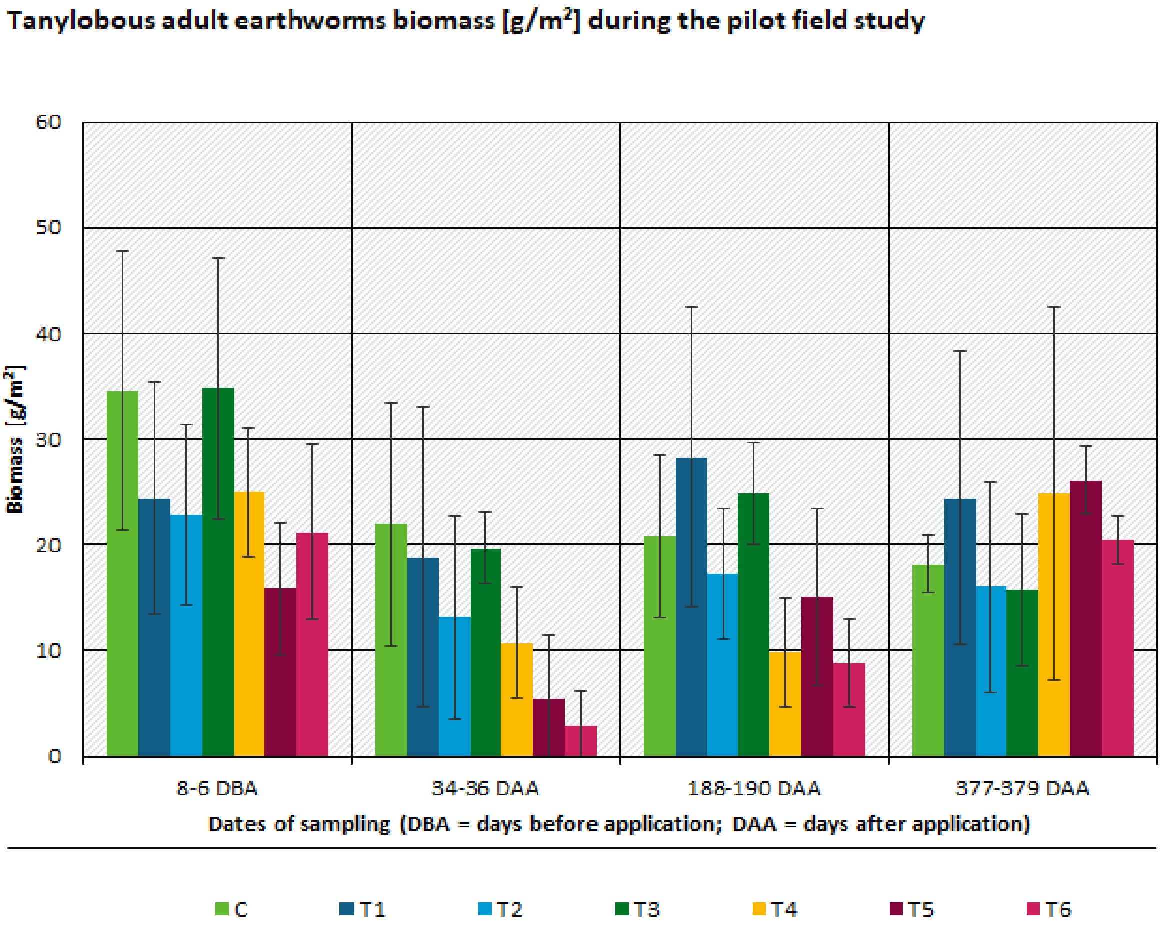
Source: ECT Oekotoxikologie GmbH

Figure A1-37: Tanylobous adult earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



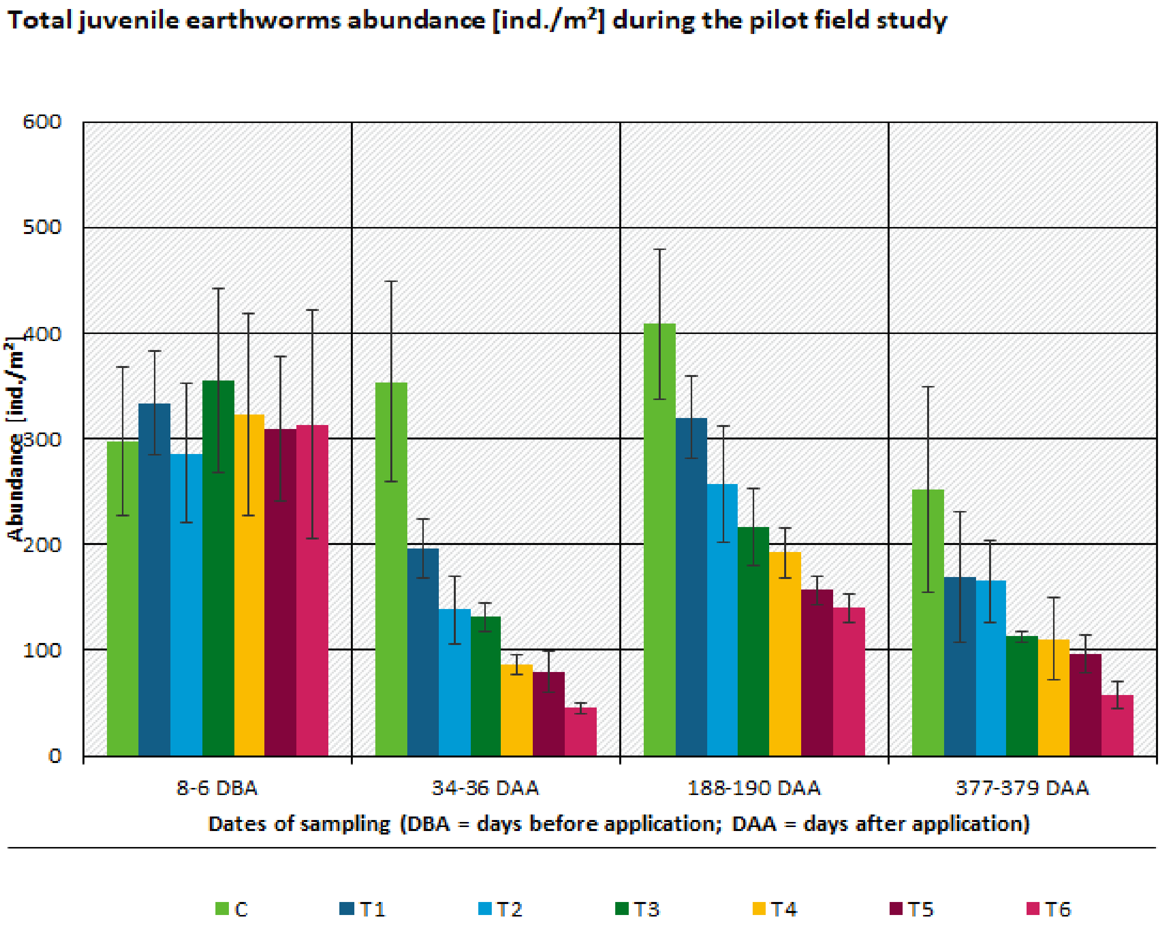
Source: ECT Oekotoxikologie GmbH

Figure A1-38: **Tanylobous adult earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)**



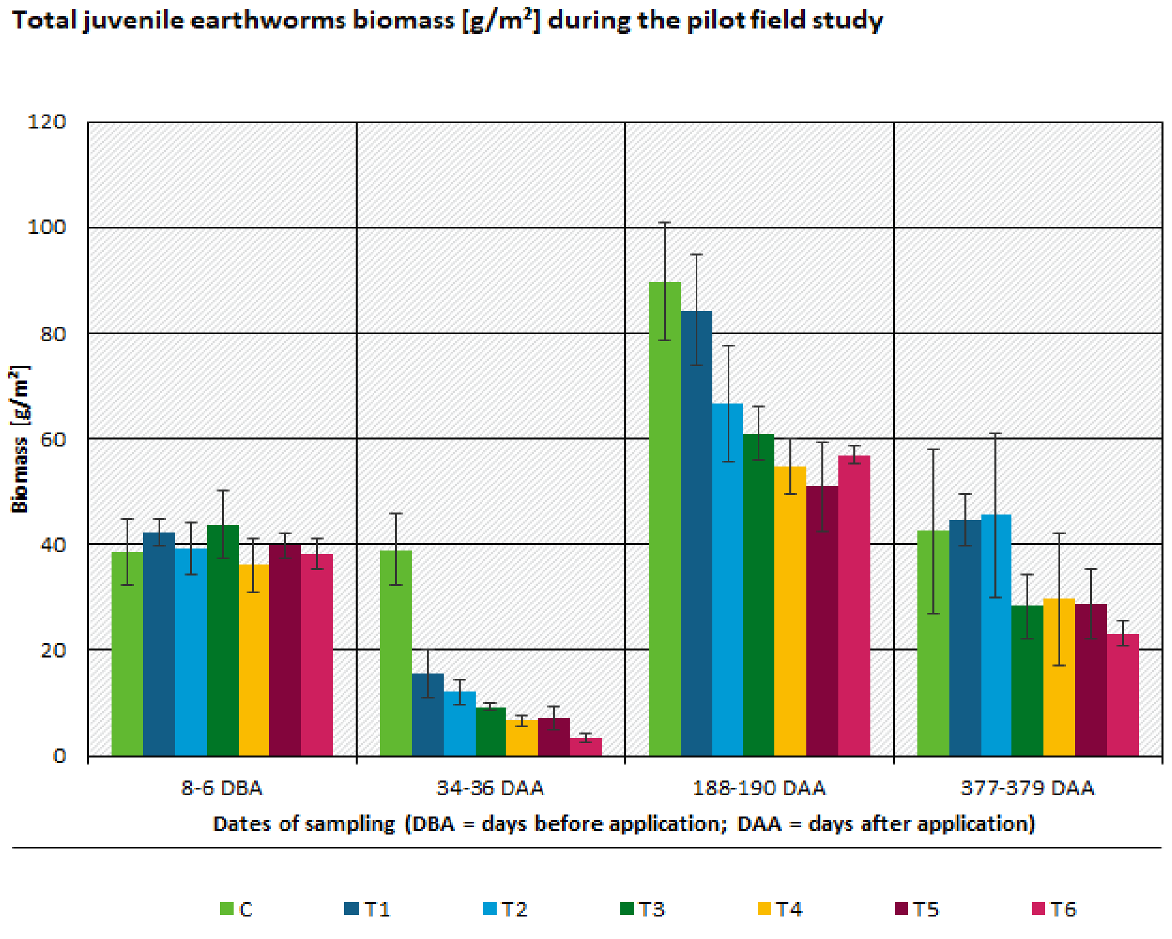
Source: ECT Oekotoxikologie GmbH

Figure A1-39: Total juvenile earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



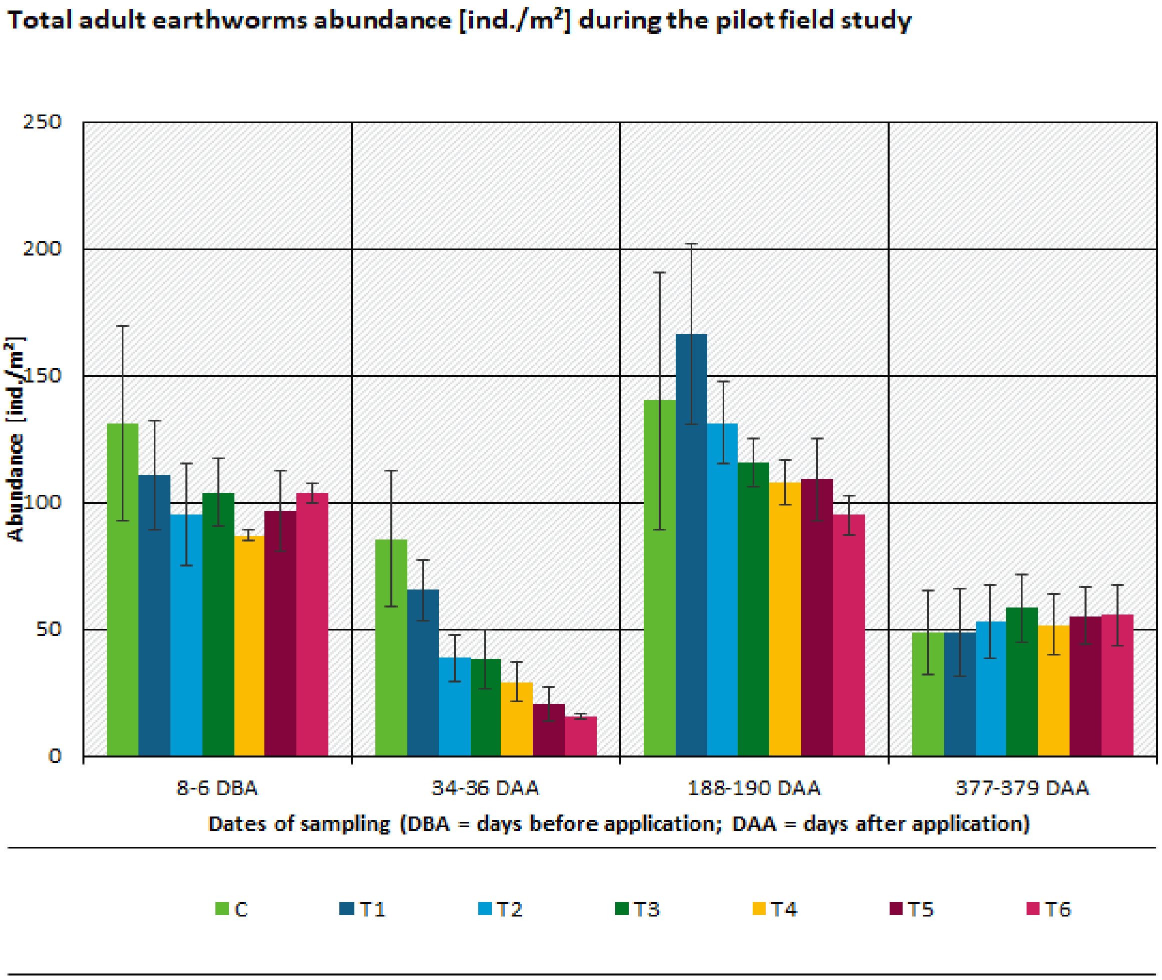
Source: ECT Oekotoxikologie GmbH

Figure A1-40: Total juvenile earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



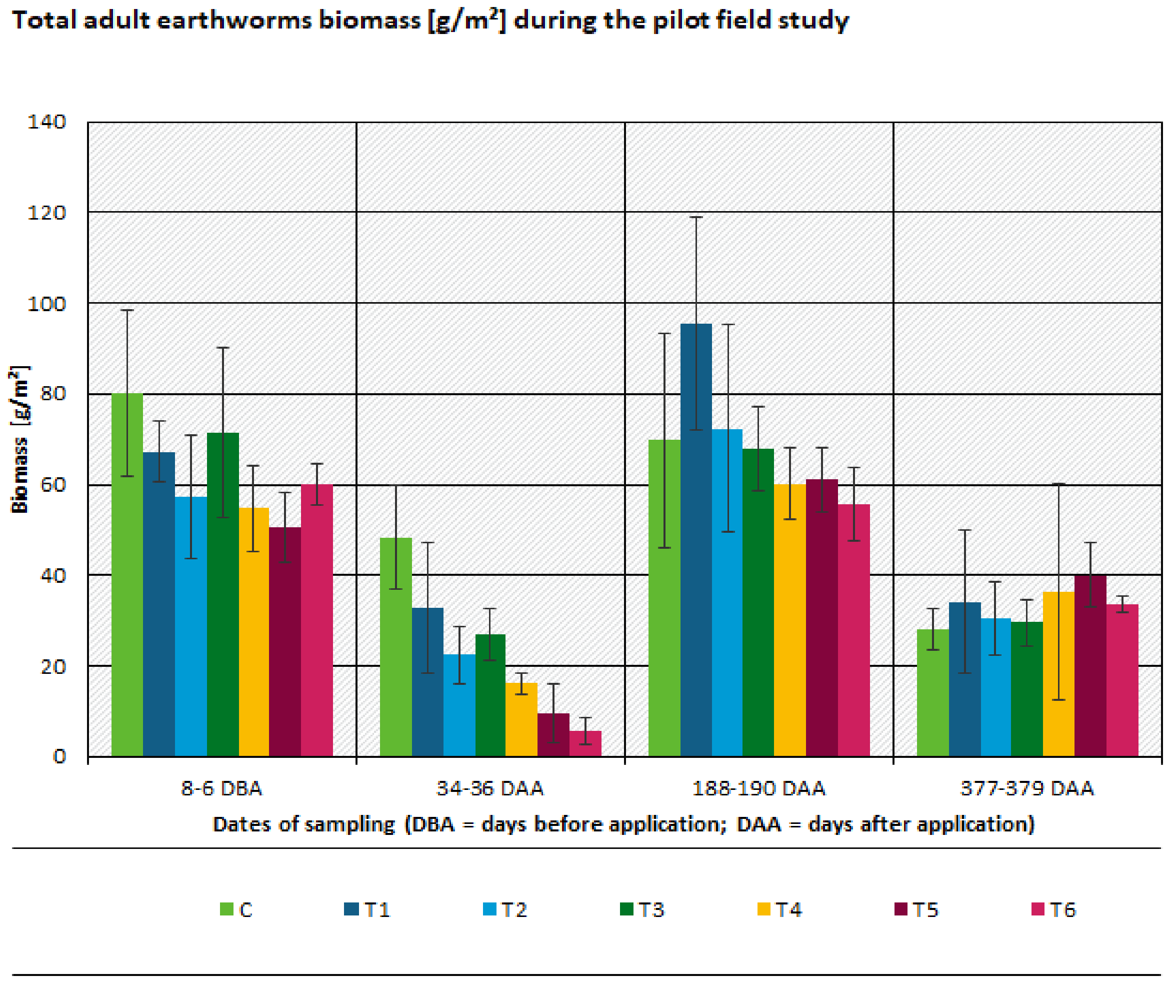
Source: ECT Oekotoxikologie GmbH

Figure A1-41: Total adult earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



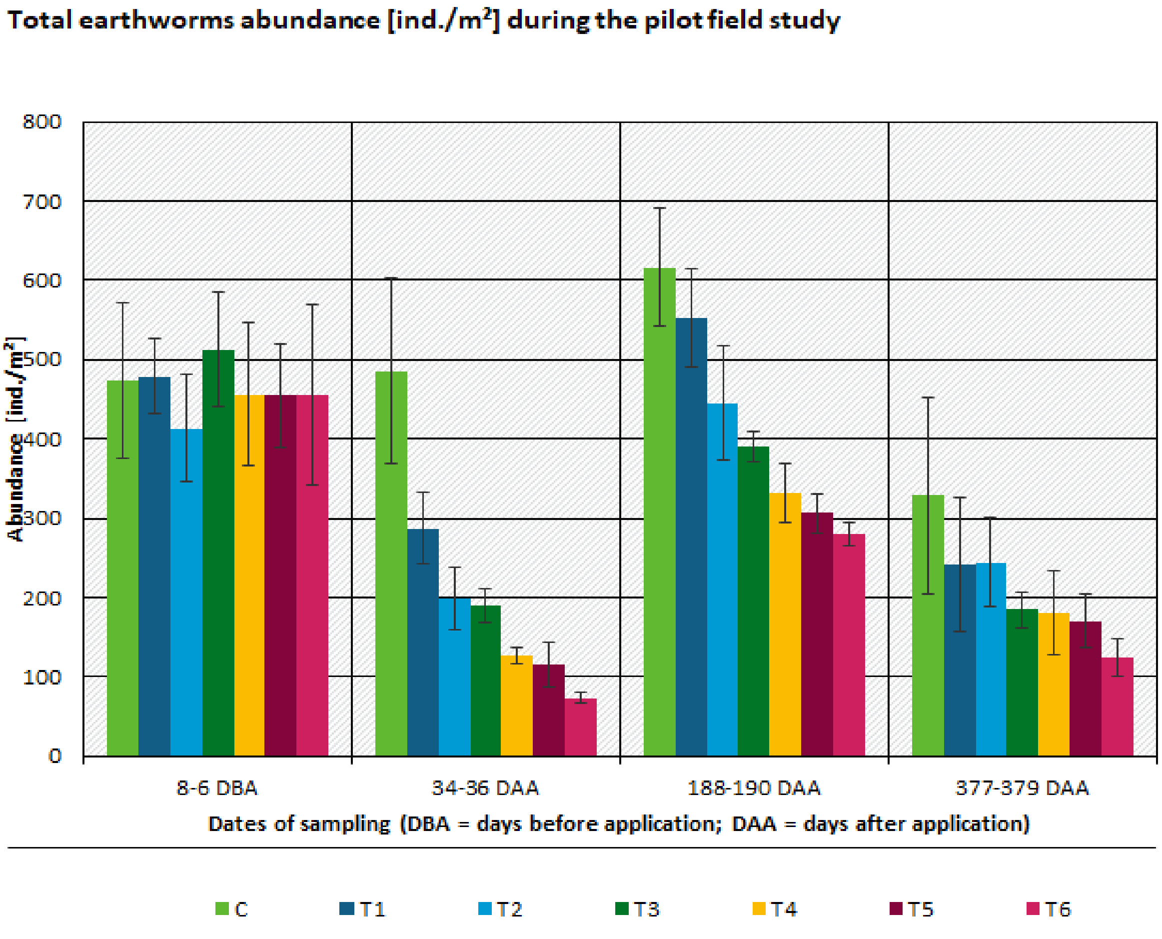
Source: ECT Oekotoxikologie GmbH

Figure A1-42: Total adult earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



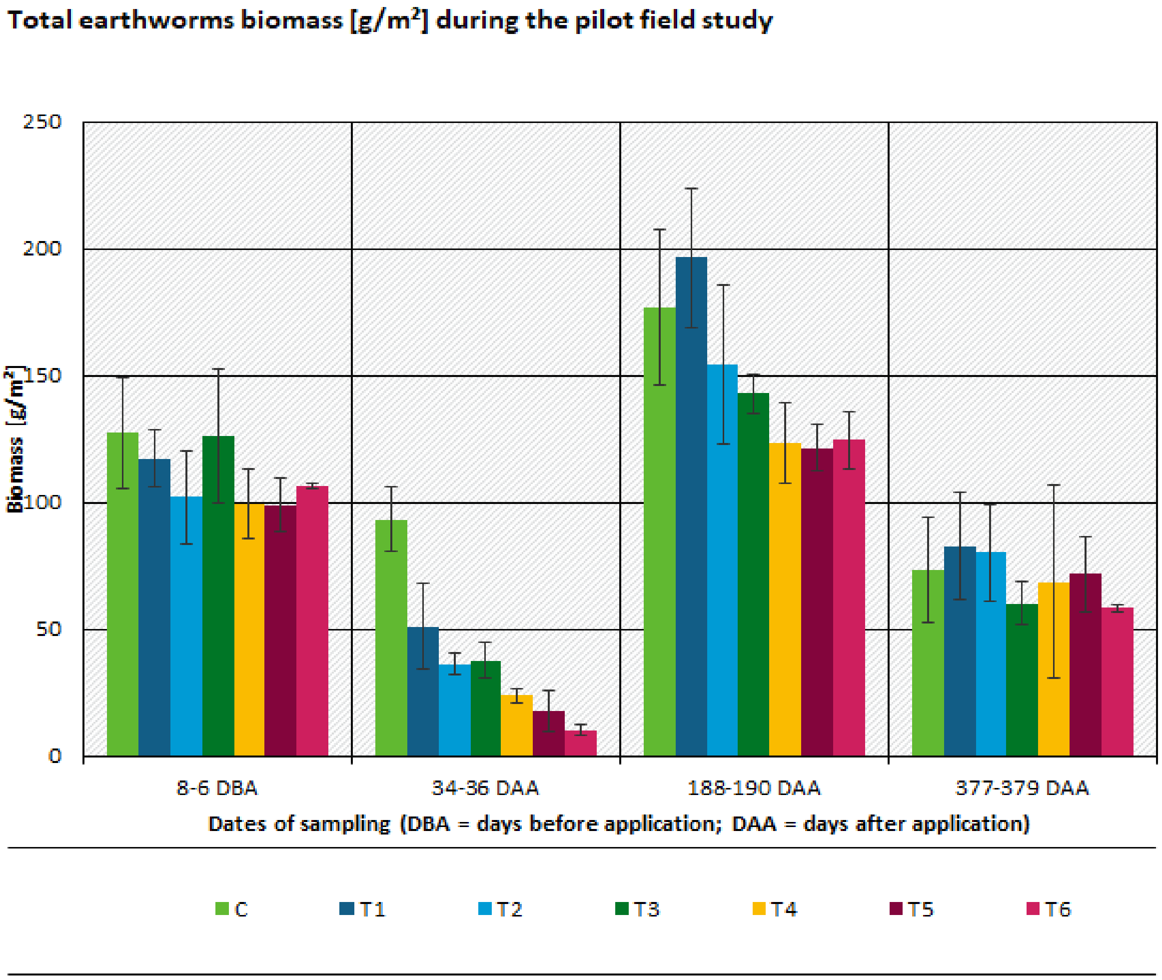
Source: ECT Oekotoxikologie GmbH

Figure A1-43: Total earthworms abundance [ind./m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



Source: ECT Oekotoxikologie GmbH

Figure A1-44: Total earthworms biomass [g/m²] during the pilot field study. C = control; T1 - T6: treatment rates with carbendazim (T1 = 0.6, T2 = 1.8, T3 = 3.2, T4 = 5.8, T5= 10.5, T6 = 31.5 kg a.s./ha)



Source: ECT Oekotoxikologie GmbH

A.1.2 8-6 DBA (pre-sampling): mean abundance & biomass of earthworms per plot and treatment

Table A1-2: Abundance of earthworms [ind/m²] in the control plots (Ca – Cf) 8-6 DBA

Taxon	Ca	Cb	Cc	Cd	Ce	Cf	Mean	SD
Aporrectodea sp. sensu lato[a]	401.3	193.3	219.3	292.7	265.3	292.7	277.4	72.7
Allolobophora chlorotica	148.0	109.3	84.0	124.0	48.0	97.3	101.8	34.4
Aporrectodea caliginosa	14.7	8.0	6.0	8.7	10.7	6.0	9.0	3.3
Aporrectodea longa	2.0	10.7	4.7	1.3	0.7	11.3	5.1	4.8
Aporrectodea rosea	9.3	7.3	3.3	8.7	4.7	8.7	7.0	2.4
Lumbricus spp.	18.0	23.3	23.3	24.7	18.7	8.0	19.3	6.2
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	6.7	16.7	5.3	6.7	8.7	2.7	7.8	4.8
Octolasion spp.	0.0	0.0	0.0	0.0	1.3	2.7	0.7	1.1
Octolasion cyaneum	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.3
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.7	0.0	0.0	0.0	0.1	0.3
Undetermined	52.0	60.0	40.0	32.0	41.3	46.7	45.3	9.8
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**172.0**	**124.7**	**94.0**	**141.3**	**63.3**	**112.7**	**118.0**	**37.7**
Anecic adults	**8.7**	**27.3**	**10.0**	**8.0**	**9.3**	**14.0**	**12.9**	**7.4**
Epilobous juveniles	**401.3**	**193.3**	**219.3**	**292.7**	**266.7**	**295.3**	**278.1**	**72.8**
Epilobous adults	**174.0**	**135.3**	**98.7**	**142.7**	**64.0**	**124.0**	**123.1**	**38.0**
Tanylobous juveniles	**18.0**	**23.3**	**23.3**	**24.7**	**18.7**	**8.0**	**19.3**	**6.2**
Tanylobous adults	**6.7**	**16.7**	**5.3**	**6.7**	**8.7**	**2.7**	**7.8**	**4.8**
Total juveniles	**419.3**	**216.7**	**242.7**	**317.3**	**285.3**	**303.3**	**297.4**	**70.7**
Total adults	**180.7**	**152.0**	**104.0**	**149.3**	**72.7**	**126.7**	**130.9**	**38.5**
Total earthworms	**652.0**	**428.7**	**386.7**	**498.7**	**399.3**	**476.7**	**473.7**	**97.5**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-3: Biomass of earthworms [g/m²] in the control plots (Ca – Cf) 8-6 DBA

Taxon	Ca	Cb	Cc	Cd	Ce	Cf	Mean	SD
Aporrectodea sp. sensu lato[a]	40.77	25.31	31.04	28.29	25.01	40.77	31.86	7.24
Allolobophora chlorotica	38.27	28.81	22.79	33.68	12.35	25.11	26.84	9.06
Aporrectodea caliginosa	11.14	7.31	4.98	8.04	9.01	4.92	7.57	2.40
Aporrectodea longa	2.23	17.99	11.33	2.48	1.08	21.81	9.49	8.94
Aporrectodea rosea	1.86	1.49	0.55	1.78	1.00	1.77	1.41	0.53
Lumbricus spp.	5.19	5.47	9.49	8.99	6.85	1.61	6.27	2.89
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	34.74	57.29	29.40	29.93	38.57	17.54	34.58	13.19
Octolasion spp.	0.00	0.00	0.00	0.00	0.63	2.16	0.47	0.87
Octolasion cyaneum	0.00	0.00	0.00	0.00	0.00	1.53	0.25	0.62
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.02	0.00	0.00	0.00	0.003	0.008
Undetermined	9.35	16.61	8.21	5.61	4.17	7.49	8.57	4.35
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**51.27**	**37.61**	**28.35**	**43.50**	**22.37**	**33.33**	**36.07**	**10.43**
Anecic adults	**36.97**	**75.27**	**40.73**	**32.41**	**39.65**	**39.35**	**44.06**	**15.57**
Epilobous juveniles	**40.77**	**25.31**	**31.04**	**28.29**	**25.64**	**42.93**	**32.33**	**7.69**
Epilobous adults	**53.51**	**55.59**	**39.67**	**45.98**	**23.45**	**55.15**	**45.56**	**12.49**
Tanylobous juveniles	**5.19**	**5.47**	**9.49**	**8.99**	**6.85**	**1.61**	**6.27**	**2.89**
Tanylobous adults	**34.74**	**57.29**	**29.40**	**29.93**	**38.57**	**17.54**	**34.58**	**13.19**
Total juveniles	**45.96**	**30.77**	**40.53**	**37.28**	**32.49**	**44.53**	**38.60**	**6.22**
Total adults	**88.25**	**112.88**	**69.07**	**75.91**	**62.01**	**72.69**	**80.14**	**18.23**
Total earthworms	**143.55**	**160.26**	**117.82**	**118.81**	**98.67**	**124.71**	**127.30**	**21.63**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-4: Abundance of earthworms [ind/m²] in the plots treated with 0.6 kg carbendazim/ha (T1a – T1c) 8-6 DBA

Taxon	T1a	T1b	T1c	Mean	SD
Aporrectodea sp. sensu lato[a]	266.7	330.0	350.7	315.8	43.8
Allolobophora chlorotica	83.3	98.7	69.3	83.8	14.7
Aporrectodea caliginosa	11.3	12.0	5.3	9.6	3.7
Aporrectodea longa	4.7	0.0	5.3	3.3	2.9
Aporrectodea rosea	4.0	17.3	4.0	8.4	7.7
Lumbricus spp.	11.3	28.0	14.7	18.0	8.8
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	2.7	4.0	7.3	4.7	2.4
Octolasion spp.	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.7	0.0	0.0	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	2.0	0.0	0.7	1.2
Undetermined	39.3	20.7	43.3	34.4	12.1
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**99.3**	**130.0**	**78.7**	**102.7**	**25.8**
Anecic adults	**7.3**	**4.0**	**12.7**	**8.0**	**4.4**
Epilobous juveniles	**266.7**	**330.0**	**350.7**	**315.8**	**43.8**
Epilobous adults	**104.0**	**130.0**	**84.0**	**106.0**	**23.1**
Tanylobous juveniles	**11.3**	**28.0**	**14.7**	**18.0**	**8.8**
Tanylobous adults	**2.7**	**4.0**	**7.3**	**4.7**	**2.4**
Total juveniles	**278.0**	**358.0**	**365.3**	**333.8**	**48.4**
Total adults	**106.7**	**134.0**	**91.3**	**110.7**	**21.6**
Total earthworms	**424.0**	**512.7**	**500.0**	**478.9**	**48.0**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-5: Biomass of earthworms [g/m²] in the plots treated with 0.6 kg carbendazim/ha (T1a – T1c) 8-6 DBA

Taxon	T1a	T1b	T1c	Mean	SD
Aporrectodea sp. sensu lato[a]	36.01	35.96	37.88	36.62	1.10
Allolobophora chlorotica	21.89	26.26	19.08	22.41	3.62
Aporrectodea caliginosa	12.52	15.14	6.75	11.47	4.29
Aporrectodea longa	8.52	0.00	10.97	6.50	5.76
Aporrectodea rosea	0.60	3.63	1.08	1.77	1.63
Lumbricus spp.	3.25	7.91	5.84	5.66	2.33
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	16.48	19.89	36.97	24.44	10.98
Octolasion spp.	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	2.01	0.00	0.00	0.67	1.16
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.09	0.00	0.03	0.05
Undetermined	7.79	4.27	11.29	7.78	3.51
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**37.03**	**45.11**	**26.91**	**36.35**	**9.12**
Anecic adults	**25.00**	**19.89**	**47.94**	**30.94**	**14.94**
Epilobous juveniles	**36.01**	**35.96**	**37.88**	**36.62**	**1.10**
Epilobous adults	**45.55**	**45.11**	**37.89**	**42.85**	**4.30**
Tanylobous juveniles	**3.25**	**7.91**	**5.84**	**5.66**	**2.33**
Tanylobous adults	**16.48**	**19.89**	**36.97**	**24.44**	**10.98**
Total juveniles	**39.25**	**43.87**	**43.72**	**42.28**	**2.62**
Total adults	**62.03**	**65.00**	**74.85**	**67.29**	**6.71**
Total earthworms	**109.07**	**113.14**	**129.87**	**117.36**	**11.02**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-6: Abundance of earthworms [ind/m²] in the plots treated with 1.8 kg carbendazim/ha (T2a – T2f) 8-6 DBA

Taxon	T2a	T2b	T2c	T2d	T2e	T2f	Mean	SD
Aporrectodea sp. sensu lato[a]	377.3	252.7	310.7	249.3	221.3	194.0	267.6	66.3
Allolobophora chlorotica	58.7	56.7	88.7	86.0	102.7	60.7	75.6	19.4
Aporrectodea caliginosa	4.7	5.3	11.3	2.0	8.7	7.3	6.6	3.3
Aporrectodea longa	2.0	2.7	0.0	1.3	5.3	4.0	2.6	1.9
Aporrectodea rosea	6.7	8.0	6.0	2.7	2.7	4.7	5.1	2.2
Lumbricus spp.	15.3	21.3	20.7	26.0	8.0	22.0	18.9	6.3
Lumbricus castaneus	0.0	0.0	0.0	0.7	0.7	0.0	0.2	0.3
Lumbricus terrestris	6.0	5.3	5.3	4.0	6.0	1.3	4.7	1.8
Octolasion spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.0	0.7	0.7	0.7	0.0	0.0	0.3	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	1.3	0.2	0.5
Undetermined	34.7	28.0	25.3	36.7	44.7	18.0	31.2	9.4
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.7**	**0.7**	**0.0**	**0.2**	**0.3**
Endogeic adults	**70.0**	**70.7**	**106.7**	**91.3**	**114.0**	**74.0**	**87.8**	**19.3**
Anecic adults	**8.0**	**8.0**	**5.3**	**5.3**	**11.3**	**5.3**	**7.2**	**2.4**
Epilobous juveniles	**377.3**	**252.7**	**310.7**	**249.3**	**221.3**	**194.0**	**267.6**	**66.3**
Epilobous adults	**72.0**	**73.3**	**106.7**	**92.7**	**119.3**	**78.0**	**90.3**	**19.4**
Tanylobous juveniles	**15.3**	**21.3**	**20.7**	**26.0**	**8.0**	**22.0**	**18.9**	**6.3**
Tanylobous adults	**6.0**	**5.3**	**5.3**	**4.7**	**6.7**	**1.3**	**4.9**	**1.9**
Total juveniles	**392.7**	**274.0**	**331.3**	**275.3**	**229.3**	**216.0**	**286.4**	**66.0**
Total adults	**78.0**	**78.7**	**112.0**	**97.3**	**126.0**	**79.3**	**95.2**	**20.3**
Total earthworms	**505.3**	**380.7**	**468.7**	**409.3**	**400.0**	**313.3**	**412.9**	**67.5**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-7: Biomass of earthworms [g/m²] in the plots treated with 1.8 kg carbendazim/ha (T2a – T2f) 8-6 DBA

Taxon	T2a	T2b	T2c	T2d	T2e	T2f	Mean	SD
Aporrectodea sp. sensu lato[a]	37.23	27.97	35.78	36.35	41.21	25.51	34.01	5.99
Allolobophora chlorotica	17.08	15.24	24.63	24.39	27.49	16.29	20.85	5.24
Aporrectodea caliginosa	3.96	4.97	11.31	2.81	9.55	7.56	6.69	3.34
Aporrectodea longa	3.76	6.20	0.00	1.69	10.21	7.19	4.84	3.76
Aporrectodea rosea	1.23	1.85	1.19	0.68	0.65	3.04	1.44	0.90
Lumbricus spp.	3.86	6.73	4.89	7.36	2.45	6.02	5.22	1.85
Lumbricus castaneus	0.00	0.00	0.00	0.15	0.26	0.00	0.07	0.11
Lumbricus terrestris	27.05	22.20	28.89	19.61	31.06	7.81	22.77	8.47
Octolasion spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.00	0.97	1.15	1.38	0.00	0.00	0.58	0.65
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.05	0.01	0.02
Undetermined	5.23	4.29	3.19	9.01	8.33	3.90	5.66	2.44
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.15**	**0.26**	**0.00**	**0.07**	**0.11**
Endogeic adults	**22.27**	**23.03**	**38.29**	**29.25**	**37.69**	**26.95**	**29.58**	**7.00**
Anecic adults	**30.81**	**28.40**	**28.89**	**21.30**	**41.27**	**14.99**	**27.61**	**8.92**
Epilobous juveniles	**37.23**	**27.97**	**35.78**	**36.35**	**41.21**	**25.51**	**34.01**	**5.99**
Epilobous adults	**26.03**	**29.23**	**38.29**	**30.95**	**47.91**	**34.13**	**34.42**	**7.83**
Tanylobous juveniles	**3.86**	**6.73**	**4.89**	**7.36**	**2.45**	**6.02**	**5.22**	**1.85**
Tanylobous adults	**27.05**	**22.20**	**28.89**	**19.76**	**31.32**	**7.81**	**22.84**	**8.51**
Total juveniles	**41.09**	**34.69**	**40.67**	**43.71**	**43.66**	**31.53**	**39.23**	**5.00**
Total adults	**53.07**	**51.43**	**67.17**	**50.71**	**79.23**	**41.94**	**57.26**	**13.49**
Total earthworms	**99.39**	**90.41**	**111.04**	**103.43**	**131.22**	**77.37**	**102.14**	**18.36**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-8: Abundance of earthworms [ind/m²] in the plots treated with 3.2 kg carbendazim/ha (T3a – T3c) 8-6 DBA

Taxon	T3a	T3b	T3c	Mean	SD
Aporrectodea sp. sensu lato[a]	413.3	354.0	244.7	337.3	85.6
Allolobophora chlorotica	66.0	77.3	88.7	77.3	11.3
Aporrectodea caliginosa	7.3	9.3	7.3	8.0	1.2
Aporrectodea longa	0.0	0.0	4.7	1.6	2.7
Aporrectodea rosea	10.0	9.3	7.3	8.9	1.4
Lumbricus spp.	14.7	24.0	14.0	17.6	5.6
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	7.3	5.3	10.0	7.6	2.3
Octolasion spp.	0.0	0.0	0.7	0.2	0.4
Octolasion cyaneum	0.7	0.7	0.0	0.4	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	35.3	72.7	51.3	53.1	18.7
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**84.0**	**96.7**	**103.3**	**94.7**	**9.8**
Anecic adults	**7.3**	**5.3**	**14.7**	**9.1**	**4.9**
Epilobous juveniles	**413.3**	**354.0**	**245.3**	**337.6**	**85.2**
Epilobous adults	**84.0**	**96.7**	**108.0**	**96.2**	**12.0**
Tanylobous juveniles	**14.7**	**24.0**	**14.0**	**17.6**	**5.6**
Tanylobous adults	**7.3**	**5.3**	**10.0**	**7.6**	**2.3**
Total juveniles	**428.0**	**378.0**	**259.3**	**355.1**	**86.6**
Total adults	**91.3**	**102.0**	**118.0**	**103.8**	**13.4**
Total earthworms	**554.7**	**552.7**	**428.7**	**512.0**	**72.2**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-9: Biomass of earthworms [g/m²] in the plots treated with 3.2 kg carbendazim/ha (T3a – T3c) 8-6 DBA

Taxon	T3a	T3b	T3c	Mean	SD
Aporrectodea sp. sensu lato[a]	33.75	39.89	43.08	38.90	4.74
Allolobophora chlorotica	18.27	19.46	23.40	20.38	2.68
Aporrectodea caliginosa	8.12	14.31	7.00	9.81	3.94
Aporrectodea longa	0.00	0.00	13.11	4.37	7.57
Aporrectodea rosea	1.89	1.78	1.37	1.68	0.27
Lumbricus spp.	2.57	8.05	3.31	4.64	2.97
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	31.71	24.21	48.38	34.77	12.37
Octolasion spp.	0.00	0.00	0.41	0.14	0.23
Octolasion cyaneum	1.15	0.69	0.00	0.61	0.58
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	6.22	11.37	15.25	10.94	4.53
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**29.43**	**36.23**	**31.77**	**32.48**	**3.46**
Anecic adults	**31.71**	**24.21**	**61.49**	**39.14**	**19.72**
Epilobous juveniles	**33.75**	**39.89**	**43.49**	**39.04**	**4.92**
Epilobous adults	**29.43**	**36.23**	**44.88**	**36.85**	**7.74**
Tanylobous juveniles	**2.57**	**8.05**	**3.31**	**4.64**	**2.97**
Tanylobous adults	**31.71**	**24.21**	**48.38**	**34.77**	**12.37**
Total juveniles	**36.32**	**47.94**	**46.79**	**43.68**	**6.40**
Total adults	**61.13**	**60.45**	**93.26**	**71.61**	**18.75**
Total earthworms	**103.67**	**119.75**	**155.30**	**126.24**	**26.42**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-10: Abundance of earthworms [ind/m²] in the plots treated with 5.8 kg carbendazim/ha (T4a – T4c) 8-6 DBA

Taxon	T4a	T4b	T4c	Mean	SD
Aporrectodea sp. sensu lato[a]	410.0	288.0	218.7	305.6	96.9
Allolobophora chlorotica	64.0	67.3	66.7	66.0	1.8
Aporrectodea caliginosa	4.0	6.7	2.0	4.2	2.3
Aporrectodea longa	0.0	2.7	7.3	3.3	3.7
Aporrectodea rosea	12.7	4.7	5.3	7.6	4.4
Lumbricus spp.	16.7	14.7	21.3	17.6	3.4
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	5.3	4.0	8.0	5.8	2.0
Octolasion spp.	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.0	0.0	0.0	0.0	0.0
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	40.0	56.7	43.3	46.7	8.8
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**80.7**	**78.7**	**74.0**	**77.8**	**3.4**
Anecic adults	**5.3**	**6.7**	**15.3**	**9.1**	**5.4**
Epilobous juveniles	**410.0**	**288.0**	**218.7**	**305.6**	**96.9**
Epilobous adults	**80.7**	**81.3**	**81.3**	**81.1**	**0.4**
Tanylobous juveniles	**16.7**	**14.7**	**21.3**	**17.6**	**3.4**
Tanylobous adults	**5.3**	**4.0**	**8.0**	**5.8**	**2.0**
Total juveniles	**426.7**	**302.7**	**240.0**	**323.1**	**95.0**
Total adults	**86.0**	**85.3**	**89.3**	**86.9**	**2.1**
Total earthworms	**552.7**	**444.7**	**372.7**	**456.7**	**90.6**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-11: Biomass of earthworms [g/m²] in the plots treated with 5.8 kg carbendazim/ha (T4a – T4c) 8-6 DBA

Taxon	T4a	T4b	T4c	Mean	SD
Aporrectodea sp. sensu lato[a]	31.25	36.80	25.20	31.08	5.80
Allolobophora chlorotica	16.39	19.60	17.06	17.68	1.69
Aporrectodea caliginosa	4.61	7.01	1.57	4.40	2.73
Aporrectodea longa	0.00	5.01	13.15	6.05	6.64
Aporrectodea rosea	2.34	1.16	1.55	1.68	0.60
Lumbricus spp.	2.45	5.22	7.68	5.12	2.62
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	23.21	19.97	31.75	24.98	6.08
Octolasion spp.	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.00	0.00	0.00	0.00	0.00
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	3.46	10.90	10.47	8.28	4.18
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**23.35**	**27.77**	**20.19**	**23.77**	**3.81**
Anecic adults	**23.21**	**24.98**	**44.90**	**31.03**	**12.05**
Epilobous juveniles	**31.25**	**36.80**	**25.20**	**31.08**	**5.80**
Epilobous adults	**23.35**	**32.78**	**33.34**	**29.82**	**5.61**
Tanylobous juveniles	**2.45**	**5.22**	**7.68**	**5.12**	**2.62**
Tanylobous adults	**23.21**	**19.97**	**31.75**	**24.98**	**6.08**
Total juveniles	**33.70**	**42.02**	**32.88**	**36.20**	**5.06**
Total adults	**46.55**	**52.75**	**65.09**	**54.80**	**9.43**
Total earthworms	**83.71**	**105.67**	**108.44**	**99.28**	**13.55**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-12: Abundance of earthworms [ind/m²] in the plots treated with 10.5 kg carbendazim/ha (T5a – T5f) 8-6 DBA

Taxon	T5a	T5b	T5c	T5d	T5e	T5f	Mean	SD
Aporrectodea sp. sensu lato[a]	331.3	211.3	379.3	258.0	336.0	228.0	290.7	67.6
Allolobophora chlorotica	88.7	96.0	61.3	76.7	72.7	65.3	76.8	13.4
Aporrectodea caliginosa	6.7	3.3	4.0	9.3	7.3	4.0	5.8	2.4
Aporrectodea longa	0.7	8.7	2.7	2.0	4.7	3.3	3.7	2.8
Aporrectodea rosea	6.7	9.3	6.7	6.7	5.3	3.3	6.3	2.0
Lumbricus spp.	24.7	10.0	16.7	16.7	18.7	28.0	19.1	6.4
Lumbricus castaneus	0.0	0.0	0.0	0.7	0.0	0.0	0.1	0.3
Lumbricus terrestris	3.3	2.7	3.3	2.0	6.7	4.7	3.8	1.7
Octolasion spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	54.7	37.3	36.7	42.0	62.0	56.7	48.2	10.9
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.7**	**0.0**	**0.0**	**0.1**	**0.3**
Endogeic adults	**102.0**	**108.7**	**72.0**	**92.7**	**85.3**	**72.7**	**88.9**	**15.1**
Anecic adults	**4.0**	**11.3**	**6.0**	**4.0**	**11.3**	**8.0**	**7.4**	**3.4**
Epilobous juveniles	**331.3**	**211.3**	**379.3**	**258.0**	**336.0**	**228.0**	**290.7**	**67.6**
Epilobous adults	**102.7**	**117.3**	**74.7**	**94.7**	**90.0**	**76.0**	**92.6**	**16.3**
Tanylobous juveniles	**24.7**	**10.0**	**16.7**	**16.7**	**18.7**	**28.0**	**19.1**	**6.4**
Tanylobous adults	**3.3**	**2.7**	**3.3**	**2.7**	**6.7**	**4.7**	**3.9**	**1.5**
Total juveniles	**356.0**	**221.3**	**396.0**	**274.7**	**354.7**	**256.0**	**309.8**	**68.6**
Total adults	**106.0**	**120.0**	**78.0**	**97.3**	**96.7**	**80.7**	**96.4**	**15.7**
Total earthworms	**516.7**	**378.7**	**510.7**	**414.0**	**513.3**	**393.3**	**454.4**	**65.7**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-13: Biomass of earthworms [g/m²] in the plots treated with 10.5 kg carbendazim/ha (T5a – T5f) 8-6 DBA

Taxon	T5a	T5b	T5c	T5d	T5e	T5f	Mean	SD
Aporrectodea sp. sensu lato[a]	34.75	36.55	33.18	31.01	37.87	33.56	34.49	2.47
Allolobophora chlorotica	24.04	27.27	16.43	21.01	18.73	17.54	20.84	4.15
Aporrectodea caliginosa	6.53	3.81	3.90	8.78	6.01	4.07	5.52	1.98
Aporrectodea longa	1.72	13.82	6.60	6.67	8.23	5.21	7.04	3.98
Aporrectodea rosea	1.21	2.21	1.39	1.17	1.21	0.79	1.33	0.47
Lumbricus spp.	8.43	2.45	4.20	5.80	3.89	6.97	5.29	2.20
Lumbricus castaneus	0.00	0.00	0.00	0.13	0.00	0.00	0.02	0.05
Lumbricus terrestris	11.61	13.17	15.70	8.79	26.54	19.22	15.84	6.33
Octolasion spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	10.40	10.02	4.21	6.78	10.13	9.63	8.53	2.50
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.13**	**0.00**	**0.00**	**0.02**	**0.05**
Endogeic adults	**31.79**	**33.29**	**21.73**	**30.96**	**25.95**	**22.40**	**27.68**	**5.01**
Anecic adults	**13.33**	**26.99**	**22.30**	**15.47**	**34.77**	**24.43**	**22.88**	**7.84**
Epilobous juveniles	**34.75**	**36.55**	**33.18**	**31.01**	**37.87**	**33.56**	**34.49**	**2.47**
Epilobous adults	**33.51**	**47.11**	**28.33**	**37.63**	**34.18**	**27.61**	**34.73**	**7.14**
Tanylobous juveniles	**8.43**	**2.45**	**4.20**	**5.80**	**3.89**	**6.97**	**5.29**	**2.20**
Tanylobous adults	**11.61**	**13.17**	**15.70**	**8.93**	**26.54**	**19.22**	**15.86**	**6.30**
Total juveniles	**43.18**	**39.01**	**37.38**	**36.81**	**41.75**	**40.53**	**39.78**	**2.50**
Total adults	**45.11**	**60.28**	**44.03**	**46.56**	**60.72**	**46.83**	**50.59**	**7.75**
Total earthworms	**98.69**	**109.31**	**85.62**	**90.15**	**112.60**	**96.99**	**98.89**	**10.51**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-14: Abundance of earthworms [ind/m²] in the plots treated with 31.5 kg carbendazim/ha (T6a – T6c) 8-6 DBA

Taxon	T6a	T6b	T6c	Mean	SD
Aporrectodea sp. sensu lato[a]	416.0	256.7	205.3	292.7	109.9
Allolobophora chlorotica	74.0	78.0	87.3	79.8	6.8
Aporrectodea caliginosa	11.3	2.7	2.7	5.6	5.0
Aporrectodea longa	2.0	4.7	11.3	6.0	4.8
Aporrectodea rosea	8.7	8.0	2.7	6.4	3.3
Lumbricus spp.	19.3	18.0	24.7	20.7	3.5
Lumbricus castaneus	0.0	1.3	0.0	0.4	0.8
Lumbricus terrestris	8.7	4.0	2.7	5.1	3.2
Octolasion spp.	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.0	0.7	0.0	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	44.7	32.7	38.7	38.7	6.0
Epigeic adults	**0.0**	**1.3**	**0.0**	**0.4**	**0.8**
Endogeic adults	**94.0**	**89.3**	**92.7**	**92.0**	**2.4**
Anecic adults	**10.7**	**8.7**	**14.0**	**11.1**	**2.7**
Epilobous juveniles	**416.0**	**256.7**	**205.3**	**292.7**	**109.9**
Epilobous adults	**96.0**	**94.0**	**104.0**	**98.0**	**5.3**
Tanylobous juveniles	**19.3**	**18.0**	**24.7**	**20.7**	**3.5**
Tanylobous adults	**8.7**	**5.3**	**2.7**	**5.6**	**3.0**
Total juveniles	**435.3**	**274.7**	**230.0**	**313.3**	**108.0**
Total adults	**104.7**	**99.3**	**106.7**	**103.6**	**3.8**
Total earthworms	**584.7**	**406.7**	**375.3**	**455.6**	**112.9**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

Table A1-15: Biomass of earthworms [g/m²] in the plots treated with 31.5 kg carbendazim/ha (T6a – T6c) 8-6 DBA

Taxon	T6a	T6b	T6c	Mean	SD
Aporrectodea sp. sensu lato[a]	32.42	35.33	30.43	32.73	2.46
Allolobophora chlorotica	18.34	21.89	22.59	20.94	2.28
Aporrectodea caliginosa	11.35	3.49	2.84	5.89	4.73
Aporrectodea longa	3.97	7.52	18.34	9.94	7.48
Aporrectodea rosea	1.89	1.81	0.36	1.35	0.86
Lumbricus spp.	3.11	6.03	7.48	5.54	2.22
Lumbricus castaneus	0.00	0.55	0.00	0.18	0.32
Lumbricus terrestris	29.67	20.42	13.00	21.03	8.35
Octolasion spp.	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.00	2.08	0.00	0.69	1.20
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	4.93	6.89	12.76	8.19	4.08
Epigeic adults	**0.00**	**0.55**	**0.00**	**0.18**	**0.32**
Endogeic adults	**31.57**	**29.27**	**25.79**	**28.88**	**2.91**
Anecic adults	**33.64**	**27.94**	**31.34**	**30.97**	**2.87**
Epilobous juveniles	**32.42**	**35.33**	**30.43**	**32.73**	**2.46**
Epilobous adults	**35.55**	**36.79**	**44.13**	**38.82**	**4.64**
Tanylobous juveniles	**3.11**	**6.03**	**7.48**	**5.54**	**2.22**
Tanylobous adults	**29.67**	**20.97**	**13.00**	**21.21**	**8.34**
Total juveniles	**35.53**	**41.37**	**37.91**	**38.27**	**2.93**
Total adults	**65.21**	**57.76**	**57.13**	**60.04**	**4.50**
Total earthworms	**105.67**	**106.01**	**107.81**	**106.50**	**1.15**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DBA = days before application, SD = standard deviation.

A.1.3 34-36 DAA: mean abundance & biomass of earthworms per plot and treatment

Table A1-16: Abundance of earthworms [ind/m²] in the control plots (Ca – Cf) 34-36 DAA

Taxon	Ca	Cb	Cc	Cd	Ce	Cf	Mean	SD
Aporrectodea sp. sensu lato[a]	444.7	156.0	352.0	346.0	344.0	378.7	336.9	96.4
Allolobophora chlorotica	81.3	52.0	46.0	108.0	42.7	67.3	66.2	25.1
Aporrectodea caliginosa	10.0	3.3	3.3	9.3	3.3	6.0	5.9	3.1
Aporrectodea longa	0.0	2.0	2.0	0.7	2.0	0.7	1.2	0.9
Aporrectodea rosea	9.3	3.3	2.7	6.7	11.3	4.7	6.3	3.4
Lumbricus spp.	6.7	16.7	18.0	26.7	21.3	11.3	16.8	7.1
Lumbricus castaneus	0.0	0.0	1.3	0.0	0.0	0.0	0.2	0.5
Lumbricus terrestris	6.7	6.7	6.7	2.7	2.7	2.0	4.6	2.3
Octolasion spp.	0.0	0.0	0.7	0.0	1.3	0.0	0.3	0.6
Octolasion cyaneum	0.0	0.7	0.7	0.0	0.7	2.0	0.7	0.7
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.7	1.3	0.3	0.6
Undetermined	58.7	32.0	46.7	57.3	53.3	30.7	46.4	12.4
Epigeic adults	**0.0**	**0.0**	**1.3**	**0.0**	**0.0**	**0.0**	**0.2**	**0.5**
Endogeic adults	**100.7**	**59.3**	**52.7**	**124.0**	**58.7**	**81.3**	**79.4**	**28.3**
Anecic adults	**6.7**	**8.7**	**8.7**	**3.3**	**4.7**	**2.7**	**5.8**	**2.6**
Epilobous juveniles	**444.7**	**156.0**	**352.7**	**346.0**	**345.3**	**378.7**	**337.2**	**96.4**
Epilobous adults	**100.7**	**61.3**	**54.7**	**124.7**	**60.7**	**82.0**	**80.7**	**27.5**
Tanylobous juveniles	**6.7**	**16.7**	**18.0**	**26.7**	**21.3**	**11.3**	**16.8**	**7.1**
Tanylobous adults	**6.7**	**6.7**	**8.0**	**2.7**	**2.7**	**2.0**	**4.8**	**2.6**
Total juveniles	**451.3**	**172.7**	**370.7**	**372.7**	**366.7**	**390.0**	**354.0**	**94.3**
Total adults	**107.3**	**68.0**	**62.7**	**127.3**	**63.3**	**84.0**	**85.4**	**26.6**
Total earthworms	**617.3**	**272.7**	**480.0**	**557.3**	**483.3**	**504.7**	**485.9**	**116.8**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-17: **Biomass of earthworms [g/m²] in the control plots (Ca – Cf) 34-36 DAA**

Taxon	**Ca**	**Cb**	**Cc**	**Cd**	**Ce**	**Cf**	**Mean**	**SD**
Aporrectodea sp. sensu lato[a]	32.79	18.19	35.48	31.62	31.50	30.61	30.03	6.04
Allolobophora chlorotica	18.98	12.60	11.46	26.59	9.93	15.24	15.80	6.17
Aporrectodea caliginosa	8.79	3.15	3.78	9.44	4.39	4.97	5.75	2.68
Aporrectodea longa	0.00	4.73	3.49	0.43	3.90	0.90	2.24	2.03
Aporrectodea rosea	1.88	0.63	0.70	1.37	2.43	0.64	1.27	0.75
Lumbricus spp.	1.93	10.49	11.23	10.71	11.49	6.48	8.72	3.80
Lumbricus castaneus	0.00	0.00	0.17	0.00	0.00	0.00	0.03	0.07
Lumbricus terrestris	33.73	28.14	34.39	14.23	12.51	8.75	21.96	11.45
Octolasion spp.	0.00	0.00	0.71	0.00	0.86	0.00	0.26	0.41
Octolasion cyaneum	0.00	1.27	0.89	0.00	1.45	3.89	1.25	1.43
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.07	0.09	0.03	0.04
Undetermined	4.29	5.32	7.21	5.07	9.57	4.43	5.98	2.05
Epigeic adults	**0.00**	**0.00**	**0.17**	**0.00**	**0.00**	**0.00**	**0.03**	**0.07**
Endogeic adults	**29.65**	**17.66**	**16.83**	**37.39**	**18.27**	**24.83**	**24.10**	**8.20**
Anecic adults	**33.73**	**32.87**	**37.88**	**14.67**	**16.41**	**9.65**	**24.20**	**11.97**
Epilobous juveniles	**32.79**	**18.19**	**36.19**	**31.62**	**32.36**	**30.61**	**30.29**	**6.23**
Epilobous adults	**29.65**	**22.39**	**20.31**	**37.83**	**22.17**	**25.73**	**26.34**	**6.52**
Tanylobous juveniles	**1.93**	**10.49**	**11.23**	**10.71**	**11.49**	**6.48**	**8.72**	**3.80**
Tanylobous adults	**33.73**	**28.14**	**34.57**	**14.23**	**12.51**	**8.75**	**21.99**	**11.48**
Total juveniles	**34.73**	**28.67**	**47.43**	**42.33**	**43.85**	**37.09**	**39.02**	**6.84**
Total adults	**63.38**	**50.53**	**54.88**	**52.06**	**34.67**	**34.47**	**48.33**	**11.55**
Total earthworms	**102.40**	**84.52**	**109.51**	**99.47**	**88.09**	**75.99**	**93.33**	**12.55**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-18: Abundance of earthworms [ind/m²] in the plots treated with 0.6 kg carbendazim/ha (T1a – T1c) 34-36 DAA

Taxon	T1a	T1b	T1c	Mean	SD
Aporrectodea sp. sensu lato[a]	182.0	218.7	166.7	189.1	26.7
Allolobophora chlorotica	42.7	64.0	45.3	50.7	11.6
Aporrectodea caliginosa	3.3	2.7	0.7	2.2	1.4
Aporrectodea longa	0.7	0.7	2.0	1.1	0.8
Aporrectodea rosea	10.7	6.0	2.0	6.2	4.3
Lumbricus spp.	9.3	6.7	4.0	6.7	2.7
Lumbricus castaneus	0.0	0.0	0.7	0.2	0.4
Lumbricus terrestris	0.7	5.3	8.0	4.7	3.7
Octolasion spp.	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.0	0.7	0.0	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	18.7	34.0	26.7	26.4	7.7
Epigeic adults	**0.0**	**0.0**	**0.7**	**0.2**	**0.4**
Endogeic adults	**56.7**	**73.3**	**48.0**	**59.3**	**12.9**
Anecic adults	**1.3**	**6.0**	**10.0**	**5.8**	**4.3**
Epilobous juveniles	**182.0**	**218.7**	**166.7**	**189.1**	**26.7**
Epilobous adults	**57.3**	**74.0**	**50.0**	**60.4**	**12.3**
Tanylobous juveniles	**9.3**	**6.7**	**4.0**	**6.7**	**2.7**
Tanylobous adults	**0.7**	**5.3**	**8.7**	**4.9**	**4.0**
Total juveniles	**191.3**	**225.3**	**170.7**	**195.8**	**27.6**
Total adults	**58.0**	**79.3**	**58.7**	**65.3**	**12.1**
Total earthworms	**268.0**	**338.7**	**256.0**	**287.6**	**44.7**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-19: **Biomass of earthworms [g/m²] in the plots treated with 0.6 kg carbendazim/ha (T1a – T1c) 34-36 DAA**

Taxon	T1a	T1b	T1c	Mean	SD
Aporrectodea sp. sensu lato[a]	13.43	18.81	9.45	13.89	4.70
Allolobophora chlorotica	8.06	11.94	7.61	9.20	2.38
Aporrectodea caliginosa	2.45	2.56	0.34	1.78	1.25
Aporrectodea longa	1.32	0.69	2.33	1.44	0.83
Aporrectodea rosea	2.33	0.89	0.32	1.18	1.03
Lumbricus spp.	1.67	1.65	1.90	1.74	0.14
Lumbricus castaneus	0.00	0.00	0.15	0.05	0.08
Lumbricus terrestris	2.43	26.09	27.82	18.78	14.18
Octolasion spp.	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.00	1.15	0.00	0.38	0.67
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	2.07	3.47	2.24	2.59	0.76
Epigeic adults	**0.00**	**0.00**	**0.15**	**0.05**	**0.08**
Endogeic adults	**12.84**	**16.54**	**8.27**	**12.55**	**4.14**
Anecic adults	**3.75**	**26.77**	**30.15**	**20.22**	**14.36**
Epilobous juveniles	**13.43**	**18.81**	**9.45**	**13.89**	**4.70**
Epilobous adults	**14.16**	**17.23**	**10.59**	**13.99**	**3.32**
Tanylobous juveniles	**1.67**	**1.65**	**1.90**	**1.74**	**0.14**
Tanylobous adults	**2.43**	**26.09**	**27.97**	**18.83**	**14.23**
Total juveniles	**15.09**	**20.46**	**11.35**	**15.63**	**4.58**
Total adults	**16.59**	**43.31**	**38.56**	**32.82**	**14.25**
Total earthworms	**33.75**	**67.24**	**52.15**	**51.05**	**16.77**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-20: Abundance of earthworms [ind/m²] in the plots treated with 1.8 kg carbendazim/ha (T2a – T2f) 34-36 DAA

Taxon	T2a	T2b	T2c	T2d	T2e	T2f	Mean	SD
Aporrectodea sp. sensu lato[a]	134.7	123.3	144.7	100.7	96.7	176.7	129.4	29.8
Allolobophora chlorotica	14.7	19.3	28.7	39.3	28.7	35.3	27.7	9.3
Aporrectodea caliginosa	2.0	0.0	2.0	0.0	0.7	1.3	1.0	0.9
Aporrectodea longa	0.0	0.0	0.7	2.7	6.7	0.7	1.8	2.6
Aporrectodea rosea	2.7	4.0	2.7	2.7	10.0	4.0	4.3	2.9
Lumbricus spp.	5.3	4.0	11.3	9.3	7.3	14.7	8.7	4.0
Lumbricus castaneus	0.0	0.0	0.0	0.7	0.0	0.0	0.1	0.3
Lumbricus terrestris	6.0	6.7	2.0	2.7	1.3	2.0	3.4	2.3
Octolasion spp.	0.0	0.0	0.7	0.0	0.0	0.0	0.1	0.3
Octolasion cyaneum	0.0	0.7	0.0	0.0	0.0	0.7	0.2	0.3
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	22.0	14.7	35.3	11.3	16.7	29.3	21.6	9.2
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.7**	**0.0**	**0.0**	**0.1**	**0.3**
Endogeic adults	**19.3**	**24.0**	**33.3**	**42.0**	**39.3**	**41.3**	**33.2**	**9.6**
Anecic adults	**6.0**	**6.7**	**2.7**	**5.3**	**8.0**	**2.7**	**5.2**	**2.2**
Epilobous juveniles	**134.7**	**123.3**	**145.3**	**100.7**	**96.7**	**176.7**	**129.6**	**29.8**
Epilobous adults	**19.3**	**24.0**	**34.0**	**44.7**	**46.0**	**42.0**	**35.0**	**11.2**
Tanylobous juveniles	**5.3**	**4.0**	**11.3**	**9.3**	**7.3**	**14.7**	**8.7**	**4.0**
Tanylobous adults	**6.0**	**6.7**	**2.0**	**3.3**	**1.3**	**2.0**	**3.6**	**2.3**
Total juveniles	**140.0**	**127.3**	**156.7**	**110.0**	**104.0**	**191.3**	**138.2**	**32.4**
Total adults	**25.3**	**30.7**	**36.0**	**48.0**	**47.3**	**44.0**	**38.6**	**9.4**
Total earthworms	**187.3**	**172.7**	**228.0**	**169.3**	**168.0**	**264.7**	**198.3**	**39.6**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-21: Biomass of earthworms [g/m²] in the plots treated with 1.8 kg carbendazim/ha (T2a – T2f) 34-36 DAA

Taxon	T2a	T2b	T2c	T2d	T2e	T2f	Mean	SD
Aporrectodea sp. sensu lato[a]	8.53	6.99	9.29	8.00	9.56	11.77	9.03	1.63
Allolobophora chlorotica	2.47	3.14	4.34	7.57	4.85	6.66	4.84	1.98
Aporrectodea caliginosa	1.45	0.00	1.70	0.00	0.45	0.77	0.73	0.72
Aporrectodea longa	0.00	0.00	0.82	3.62	10.16	0.66	2.54	3.97
Aporrectodea rosea	0.49	0.69	0.45	0.49	1.81	0.78	0.79	0.52
Lumbricus spp.	1.04	3.38	3.09	3.35	1.97	4.66	2.91	1.26
Lumbricus castaneus	0.00	0.00	0.00	0.08	0.00	0.00	0.01	0.03
Lumbricus terrestris	22.13	27.43	7.12	12.16	2.55	7.38	13.13	9.67
Octolasion spp.	0.00	0.00	0.73	0.00	0.00	0.00	0.12	0.30
Octolasion cyaneum	0.00	0.79	0.00	0.00	0.00	1.17	0.33	0.52
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	2.00	1.03	2.73	0.91	2.16	2.77	1.93	0.81
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.08**	**0.00**	**0.00**	**0.01**	**0.03**
Endogeic adults	**4.42**	**4.63**	**6.49**	**8.07**	**7.11**	**9.38**	**6.68**	**1.94**
Anecic adults	**22.13**	**27.43**	**7.94**	**15.78**	**12.71**	**8.04**	**15.67**	**7.83**
Epilobous juveniles	**8.53**	**6.99**	**10.02**	**8.00**	**9.56**	**11.77**	**9.15**	**1.68**
Epilobous adults	**4.42**	**4.63**	**7.31**	**11.69**	**17.27**	**10.04**	**9.23**	**4.89**
Tanylobous juveniles	**1.04**	**3.38**	**3.09**	**3.35**	**1.97**	**4.66**	**2.91**	**1.26**
Tanylobous adults	**22.13**	**27.43**	**7.12**	**12.24**	**2.55**	**7.38**	**13.14**	**9.67**
Total juveniles	**9.57**	**10.37**	**13.11**	**11.35**	**11.53**	**16.43**	**12.06**	**2.45**
Total adults	**26.55**	**32.06**	**14.43**	**23.93**	**19.82**	**17.42**	**22.37**	**6.45**
Total earthworms	**38.12**	**43.47**	**30.28**	**36.18**	**33.51**	**36.62**	**36.36**	**4.45**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-22: Abundance of earthworms [ind/m²] in the plots treated with 3.2 kg carbendazim/ha (T3a – T3c) 34-36 DAA

Taxon	T3a	T3b	T3c	Mean	SD
Aporrectodea sp. sensu lato[a]	136.7	109.3	121.3	122.4	13.7
Allolobophora chlorotica	14.0	26.7	35.3	25.3	10.7
Aporrectodea caliginosa	4.0	4.0	2.0	3.3	1.2
Aporrectodea longa	0.0	0.0	2.0	0.7	1.2
Aporrectodea rosea	2.7	2.7	3.3	2.9	0.4
Lumbricus spp.	8.0	7.3	10.0	8.4	1.4
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	6.0	4.7	7.3	6.0	1.3
Octolasion spp.	0.0	0.0	1.3	0.4	0.8
Octolasion cyaneum	0.0	0.0	0.0	0.0	0.0
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	18.0	15.3	30.0	21.1	7.8
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**20.7**	**33.3**	**40.7**	**31.6**	**10.1**
Anecic adults	**6.0**	**4.7**	**9.3**	**6.7**	**2.4**
Epilobous juveniles	**136.7**	**109.3**	**122.7**	**122.9**	**13.7**
Epilobous adults	**20.7**	**33.3**	**42.7**	**32.2**	**11.0**
Tanylobous juveniles	**8.0**	**7.3**	**10.0**	**8.4**	**1.4**
Tanylobous adults	**6.0**	**4.7**	**7.3**	**6.0**	**1.3**
Total juveniles	**144.7**	**116.7**	**132.7**	**131.3**	**14.0**
Total adults	**26.7**	**38.0**	**50.0**	**38.2**	**11.7**
Total earthworms	**189.3**	**170.0**	**212.7**	**190.7**	**21.4**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-23: Biomass of earthworms [g/m²] in the plots treated with 3.2 kg carbendazim/ha (T3a – T3c) 34-36 DAA

Taxon	T3a	T3b	T3c	Mean	SD
Aporrectodea sp. sensu lato[a]	7.27	6.25	6.85	6.79	0.51
Allolobophora chlorotica	2.00	4.31	5.47	3.93	1.77
Aporrectodea caliginosa	2.54	1.39	1.11	1.68	0.76
Aporrectodea longa	0.00	0.00	3.29	1.10	1.90
Aporrectodea rosea	0.35	0.43	0.71	0.50	0.19
Lumbricus spp.	1.73	2.41	1.90	2.02	0.35
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	19.90	16.30	22.94	19.71	3.32
Octolasion spp.	0.00	0.00	1.32	0.44	0.76
Octolasion cyaneum	0.00	0.00	0.00	0.00	0.00
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	1.09	1.36	1.99	1.48	0.47
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**4.89**	**6.13**	**7.29**	**6.10**	**1.20**
Anecic adults	**19.90**	**16.30**	**26.23**	**20.81**	**5.03**
Epilobous juveniles	**7.27**	**6.25**	**8.17**	**7.23**	**0.96**
Epilobous adults	**4.89**	**6.13**	**10.58**	**7.20**	**2.99**
Tanylobous juveniles	**1.73**	**2.41**	**1.90**	**2.02**	**0.35**
Tanylobous adults	**19.90**	**16.30**	**22.94**	**19.71**	**3.32**
Total juveniles	**9.00**	**8.66**	**10.07**	**9.24**	**0.73**
Total adults	**24.79**	**22.43**	**33.52**	**26.91**	**5.84**
Total earthworms	**34.87**	**32.45**	**45.58**	**37.64**	**6.99**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-24: Abundance of earthworms [ind/m²] in the plots treated with 5.8 kg carbendazim/ha (T4a – T4c) 34-36 DAA

Taxon	T4a	T4b	T4c	Mean	SD
Aporrectodea sp. sensu lato[a]	80.7	88.7	71.3	80.2	8.7
Allolobophora chlorotica	10.7	26.7	22.0	19.8	8.2
Aporrectodea caliginosa	0.7	2.0	0.0	0.9	1.0
Aporrectodea longa	0.0	2.0	0.7	0.9	1.0
Aporrectodea rosea	6.0	4.7	2.7	4.4	1.7
Lumbricus spp.	3.3	7.3	6.7	5.8	2.1
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	4.0	1.3	4.0	3.1	1.5
Octolasion spp.	0.0	0.7	0.0	0.2	0.4
Octolasion cyaneum	0.0	0.7	0.0	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	14.0	4.7	16.0	11.6	6.0
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**17.3**	**34.0**	**24.7**	**25.3**	**8.4**
Anecic adults	**4.0**	**3.3**	**4.7**	**4.0**	**0.7**
Epilobous juveniles	**80.7**	**89.3**	**71.3**	**80.4**	**9.0**
Epilobous adults	**17.3**	**36.0**	**25.3**	**26.2**	**9.4**
Tanylobous juveniles	**3.3**	**7.3**	**6.7**	**5.8**	**2.1**
Tanylobous adults	**4.0**	**1.3**	**4.0**	**3.1**	**1.5**
Total juveniles	**84.0**	**96.7**	**78.0**	**86.2**	**9.5**
Total adults	**21.3**	**37.3**	**29.3**	**29.3**	**8.0**
Total earthworms	**119.3**	**138.7**	**123.3**	**127.1**	**10.2**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-25: Biomass of earthworms [g/m²] in the plots treated with 5.8 kg carbendazim/ha (T4a – T4c) 34-36 DAA

Taxon	T4a	T4b	T4c	Mean	SD
Aporrectodea sp. sensu lato[a]	5.05	5.14	5.11	5.10	0.04
Allolobophora chlorotica	1.77	4.21	3.55	3.18	1.27
Aporrectodea caliginosa	0.24	0.95	0.00	0.40	0.49
Aporrectodea longa	0.00	2.69	0.25	0.98	1.49
Aporrectodea rosea	0.93	0.75	0.43	0.70	0.25
Lumbricus spp.	0.37	1.83	2.21	1.47	0.97
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	13.34	4.64	14.27	10.75	5.31
Octolasion spp.	0.00	0.35	0.00	0.12	0.20
Octolasion cyaneum	0.00	0.55	0.00	0.18	0.32
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	1.19	0.46	1.40	1.02	0.49
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**2.93**	**6.46**	**3.99**	**4.46**	**1.81**
Anecic adults	**13.34**	**7.33**	**14.53**	**11.73**	**3.86**
Epilobous juveniles	**5.05**	**5.49**	**5.11**	**5.22**	**0.23**
Epilobous adults	**2.93**	**9.15**	**4.24**	**5.44**	**3.28**
Tanylobous juveniles	**0.37**	**1.83**	**2.21**	**1.47**	**0.97**
Tanylobous adults	**13.34**	**4.64**	**14.27**	**10.75**	**5.31**
Total juveniles	**5.43**	**7.32**	**7.33**	**6.69**	**1.10**
Total adults	**16.27**	**13.79**	**18.51**	**16.19**	**2.36**
Total earthworms	**22.89**	**21.57**	**27.24**	**23.90**	**2.97**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-26: **Abundance of earthworms [ind/m²] in the plots treated with 10.5 kg carbendazim/ha (T5a – T5f) 34-36 DAA**

Taxon	T5a	T5b	T5c	T5d	T5e	T5f	Mean	SD
Aporrectodea sp. sensu lato[a]	86.7	72.0	100.7	69.3	42.7	67.3	73.1	19.6
Allolobophora chlorotica	22.7	23.3	14.7	15.3	8.7	9.3	15.7	6.3
Aporrectodea caliginosa	0.0	0.7	2.0	0.7	0.0	0.7	0.7	0.7
Aporrectodea longa	0.0	0.7	0.7	0.0	0.0	0.0	0.2	0.3
Aporrectodea rosea	4.7	0.7	1.3	4.7	0.0	2.7	2.3	2.0
Lumbricus spp.	5.3	6.0	6.0	7.3	5.3	8.0	6.3	1.1
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	1.3	0.0	4.0	0.7	2.7	0.0	1.4	1.6
Octolasion spp.	0.0	0.0	0.7	0.0	0.0	0.0	0.1	0.3
Octolasion cyaneum	0.0	0.0	0.7	0.7	0.0	0.0	0.2	0.3
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	15.3	13.3	12.0	24.7	6.7	18.0	15.0	6.1
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**27.3**	**24.7**	**18.7**	**21.3**	**8.7**	**12.7**	**18.9**	**7.1**
Anecic adults	**1.3**	**0.7**	**4.7**	**0.7**	**2.7**	**0.0**	**1.7**	**1.7**
Epilobous juveniles	**86.7**	**72.0**	**101.3**	**69.3**	**42.7**	**67.3**	**73.2**	**19.8**
Epilobous adults	**27.3**	**25.3**	**19.3**	**21.3**	**8.7**	**12.7**	**19.1**	**7.2**
Tanylobous juveniles	**5.3**	**6.0**	**6.0**	**7.3**	**5.3**	**8.0**	**6.3**	**1.1**
Tanylobous adults	**1.3**	**0.0**	**4.0**	**0.7**	**2.7**	**0.0**	**1.4**	**1.6**
Total juveniles	**92.0**	**78.0**	**107.3**	**76.7**	**48.0**	**75.3**	**79.6**	**19.8**
Total adults	**28.7**	**25.3**	**23.3**	**22.0**	**11.3**	**12.7**	**20.6**	**7.0**
Total earthworms	**136.0**	**116.7**	**142.7**	**123.3**	**66.0**	**106.0**	**115.1**	**27.4**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-27: Biomass of earthworms [g/m²] in the plots treated with 10.5 kg carbendazim/ha (T5a – T5f) 34-36 DAA

Taxon	T5a	T5b	T5c	T5d	T5e	T5f	Mean	SD
Aporrectodea sp. sensu lato[a]	5.93	4.65	6.34	5.16	2.84	4.38	4.88	1.25
Allolobophora chlorotica	3.61	3.84	2.35	2.43	1.47	1.56	2.55	1.00
Aporrectodea caliginosa	0.00	0.53	1.36	0.66	0.00	0.38	0.49	0.51
Aporrectodea longa	0.00	0.67	0.77	0.00	0.00	0.00	0.24	0.37
Aporrectodea rosea	0.57	0.13	0.24	0.75	0.00	0.40	0.35	0.28
Lumbricus spp.	1.45	1.47	3.93	1.28	1.01	3.57	2.12	1.28
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	5.04	0.00	15.53	2.49	9.25	0.00	5.38	6.07
Octolasion spp.	0.00	0.00	0.13	0.00	0.00	0.00	0.02	0.05
Octolasion cyaneum	0.00	0.00	1.11	1.28	0.00	0.00	0.40	0.62
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	1.05	1.42	1.27	3.03	0.39	1.14	1.38	0.88
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**4.18**	**4.49**	**5.07**	**5.12**	**1.47**	**2.34**	**3.78**	**1.52**
Anecic adults	**5.04**	**0.67**	**16.30**	**2.49**	**9.25**	**0.00**	**5.63**	**6.22**
Epilobous juveniles	**5.93**	**4.65**	**6.47**	**5.16**	**2.84**	**4.38**	**4.90**	**1.28**
Epilobous adults	**4.18**	**5.17**	**5.84**	**5.12**	**1.47**	**2.34**	**4.02**	**1.74**
Tanylobous juveniles	**1.45**	**1.47**	**3.93**	**1.28**	**1.01**	**3.57**	**2.12**	**1.28**
Tanylobous adults	**5.04**	**0.00**	**15.53**	**2.49**	**9.25**	**0.00**	**5.38**	**6.07**
Total juveniles	**7.37**	**6.13**	**10.39**	**6.44**	**3.85**	**7.95**	**7.02**	**2.17**
Total adults	**9.22**	**5.17**	**21.37**	**7.61**	**10.73**	**2.34**	**9.40**	**6.57**
Total earthworms	**17.64**	**12.71**	**33.03**	**17.07**	**14.97**	**11.43**	**17.81**	**7.83**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-28: Abundance of earthworms [ind/m²] in the plots treated with 31.5 kg carbendazim/ha (T6a – T6c) 34-36 DAA

Taxon	T6a	T6b	T6c	Mean	SD
Aporrectodea sp. sensu lato[a]	49.3	43.3	37.3	43.3	6.0
Allolobophora chlorotica	12.7	11.3	8.7	10.9	2.0
Aporrectodea caliginosa	0.0	1.3	0.7	0.7	0.7
Aporrectodea longa	0.0	0.0	0.0	0.0	0.0
Aporrectodea rosea	3.3	3.3	3.3	3.3	0.0
Lumbricus spp.	1.3	2.0	3.3	2.2	1.0
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	0.7	0.0	2.0	0.9	1.0
Octolasion spp.	0.0	0.7	0.0	0.2	0.4
Octolasion cyaneum	0.0	0.0	0.0	0.0	0.0
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	12.0	10.7	10.7	11.1	0.8
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**16.0**	**16.0**	**12.7**	**14.9**	**1.9**
Anecic adults	**0.7**	**0.0**	**2.0**	**0.9**	**1.0**
Epilobous juveniles	**49.3**	**44.0**	**37.3**	**43.6**	**6.0**
Epilobous adults	**16.0**	**16.0**	**12.7**	**14.9**	**1.9**
Tanylobous juveniles	**1.3**	**2.0**	**3.3**	**2.2**	**1.0**
Tanylobous adults	**0.7**	**0.0**	**2.0**	**0.9**	**1.0**
Total juveniles	**50.7**	**46.0**	**40.7**	**45.8**	**5.0**
Total adults	**16.7**	**16.0**	**14.7**	**15.8**	**1.0**
Total earthworms	**79.3**	**72.7**	**66.0**	**72.7**	**6.7**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-29: Biomass of earthworms [g/m²] in the plots treated with 31.5 kg carbendazim/ha (T6a – T6c) 34-36 DAA

Taxon	T6a	T6b	T6c	Mean	SD
Aporrectodea sp. sensu lato[a]	3.30	2.87	2.09	2.75	0.61
Allolobophora chlorotica	2.11	1.89	1.39	1.80	0.37
Aporrectodea caliginosa	0.00	0.77	0.49	0.42	0.39
Aporrectodea longa	0.00	0.00	0.00	0.00	0.00
Aporrectodea rosea	0.64	0.42	0.58	0.55	0.11
Lumbricus spp.	0.30	0.60	0.60	0.50	0.17
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	1.97	0.00	6.63	2.87	3.41
Octolasion spp.	0.00	0.70	0.00	0.23	0.40
Octolasion cyaneum	0.00	0.00	0.00	0.00	0.00
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	0.71	1.70	1.22	1.21	0.50
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**2.75**	**3.08**	**2.46**	**2.76**	**0.31**
Anecic adults	**1.97**	**0.00**	**6.63**	**2.87**	**3.41**
Epilobous juveniles	**3.30**	**3.57**	**2.09**	**2.98**	**0.79**
Epilobous adults	**2.75**	**3.08**	**2.46**	**2.76**	**0.31**
Tanylobous juveniles	**0.30**	**0.60**	**0.60**	**0.50**	**0.17**
Tanylobous adults	**1.97**	**0.00**	**6.63**	**2.87**	**3.41**
Total juveniles	**3.60**	**4.17**	**2.69**	**3.48**	**0.75**
Total adults	**4.71**	**3.08**	**9.09**	**5.63**	**3.11**
Total earthworms	**9.02**	**8.95**	**13.00**	**10.32**	**2.32**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

A.1.4 188-190 DAA: mean abundance & biomass of earthworms per plot and treatment

Table A1-30: Abundance of earthworms [ind/m²] in the control plots (Ca – Cf) 188-190 DAA

Taxon	Ca	Cb	Cc	Cd	Ce	Cf	Mean	SD
Aporrectodea sp. sensu lato[a]	345.3	238.0	385.3	268.0	394.7	408.7	340.0	71.2
Allolobophora chlorotica	70.7	196.0	128.0	118.7	83.3	90.7	114.6	45.4
Aporrectodea caliginosa	3.3	4.7	6.0	4.7	7.3	4.7	5.1	1.4
Aporrectodea longa	1.3	14.7	10.7	0.7	4.7	14.0	7.7	6.3
Aporrectodea rosea	0.7	9.3	5.3	6.0	8.0	14.7	7.3	4.7
Lumbricus spp.	58.0	68.7	92.7	72.0	64.0	55.3	68.4	13.4
Lumbricus castaneus	0.0	0.0	0.7	0.0	0.0	0.0	0.1	0.3
Lumbricus terrestris	2.7	2.0	4.0	6.0	4.0	7.3	4.3	2.0
Octolasion spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.0	2.0	0.7	0.0	2.0	1.3	1.0	0.9
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	49.3	60.7	75.3	58.7	88.7	73.3	67.7	14.1
Epigeic adults	**0.0**	**0.0**	**0.7**	**0.0**	**0.0**	**0.0**	**0.1**	**0.3**
Endogeic adults	**74.7**	**212.0**	**140.0**	**129.3**	**100.7**	**111.3**	**128.0**	**47.1**
Anecic adults	**4.0**	**16.7**	**14.7**	**6.7**	**8.7**	**21.3**	**12.0**	**6.6**
Epilobous juveniles	**345.3**	**238.0**	**385.3**	**268.0**	**394.7**	**408.7**	**340.0**	**71.2**
Epilobous adults	**76.0**	**226.7**	**150.7**	**130.0**	**105.3**	**125.3**	**135.7**	**51.2**
Tanylobous juveniles	**58.0**	**68.7**	**92.7**	**72.0**	**64.0**	**55.3**	**68.4**	**13.4**
Tanylobous adults	**2.7**	**2.0**	**4.7**	**6.0**	**4.0**	**7.3**	**4.4**	**2.0**
Total juveniles	**403.3**	**306.7**	**478.0**	**340.0**	**458.7**	**464.0**	**408.4**	**71.4**
Total adults	**78.7**	**228.7**	**155.3**	**136.0**	**109.3**	**132.7**	**140.1**	**50.7**
Total earthworms	**531.3**	**596.0**	**708.7**	**534.7**	**656.7**	**670.0**	**616.2**	**73.9**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-31: Biomass of earthworms [g/m²] in the control plots (Ca – Cf) 188-190 DAA

Taxon	Ca	Cb	Cc	Cd	Ce	Cf	Mean	SD
Aporrectodea sp. sensu lato[a]	39.20	45.17	51.43	31.41	42.17	55.15	44.09	8.55
Allolobophora chlorotica	15.73	53.12	30.26	30.41	20.62	23.13	28.88	13.16
Aporrectodea caliginosa	3.03	4.78	5.30	4.01	6.49	5.30	4.82	1.19
Aporrectodea longa	1.46	24.32	20.43	1.74	6.46	21.45	12.64	10.55
Aporrectodea rosea	0.11	1.50	0.94	0.91	1.16	2.47	1.18	0.78
Lumbricus spp.	43.58	37.21	60.19	57.96	46.82	28.19	45.66	12.20
Lumbricus castaneus	0.00	0.00	0.13	0.00	0.00	0.00	0.02	0.05
Lumbricus terrestris	12.49	11.64	24.17	23.99	20.65	31.97	20.82	7.74
Octolasion spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.00	3.04	1.09	0.00	3.08	1.32	1.42	1.38
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	10.81	17.89	18.77	13.03	21.01	21.97	17.25	4.44
Epigeic adults	**0.00**	**0.00**	**0.13**	**0.00**	**0.00**	**0.00**	**0.02**	**0.05**
Endogeic adults	**18.87**	**62.44**	**37.59**	**35.34**	**31.35**	**32.21**	**36.30**	**14.36**
Anecic adults	**13.95**	**35.96**	**44.60**	**25.73**	**27.11**	**53.41**	**33.46**	**14.21**
Epilobous juveniles	**39.20**	**45.17**	**51.43**	**31.41**	**42.17**	**55.15**	**44.09**	**8.55**
Epilobous adults	**20.33**	**86.76**	**58.01**	**37.08**	**37.81**	**53.66**	**48.94**	**22.88**
Tanylobous juveniles	**43.58**	**37.21**	**60.19**	**57.96**	**46.82**	**28.19**	**45.66**	**12.20**
Tanylobous adults	**12.49**	**11.64**	**24.30**	**23.99**	**20.65**	**31.97**	**20.84**	**7.75**
Total juveniles	**82.78**	**82.38**	**111.62**	**89.37**	**88.99**	**83.33**	**89.75**	**11.16**
Total adults	**32.82**	**98.40**	**82.31**	**61.07**	**58.45**	**85.63**	**69.78**	**23.65**
Total earthworms	**126.41**	**198.67**	**212.70**	**163.47**	**168.46**	**190.93**	**176.77**	**30.83**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-32: Abundance of earthworms [ind/m²] in the plots treated with 0.6 kg carbendazim/ha (T1a – T1c) 188-190 DAA

Taxon	T1a	T1b	T1c	Mean	SD
Aporrectodea sp. sensu lato[a]	314.0	219.3	259.3	264.2	47.5
Allolobophora chlorotica	118.7	94.0	161.3	124.7	34.1
Aporrectodea caliginosa	8.7	19.3	11.3	13.1	5.6
Aporrectodea longa	10.7	6.0	10.7	9.1	2.7
Aporrectodea rosea	12.7	10.7	11.3	11.6	1.0
Lumbricus spp.	49.3	66.0	53.3	56.2	8.7
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	4.7	5.3	10.7	6.9	3.3
Octolasion spp.	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.7	2.0	0.7	1.1	0.8
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	63.3	57.3	74.0	64.9	8.4
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**140.7**	**126.0**	**184.7**	**150.4**	**30.5**
Anecic adults	**15.3**	**11.3**	**21.3**	**16.0**	**5.0**
Epilobous juveniles	**314.0**	**219.3**	**259.3**	**264.2**	**47.5**
Epilobous adults	**151.3**	**132.0**	**195.3**	**159.6**	**32.5**
Tanylobous juveniles	**49.3**	**66.0**	**53.3**	**56.2**	**8.7**
Tanylobous adults	**4.7**	**5.3**	**10.7**	**6.9**	**3.3**
Total juveniles	**363.3**	**285.3**	**312.7**	**320.4**	**39.6**
Total adults	**156.0**	**137.3**	**206.0**	**166.4**	**35.5**
Total earthworms	**582.7**	**480.0**	**592.7**	**551.8**	**62.4**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-33: Biomass of earthworms [g/m²] in the plots treated with 0.6 kg carbendazim/ha (T1a – T1c) 188-190 DAA

Taxon	T1a	T1b	T1c	Mean	SD
Aporrectodea sp. sensu lato[a]	41.33	32.99	45.22	39.84	6.25
Allolobophora chlorotica	31.51	25.63	41.88	33.01	8.23
Aporrectodea caliginosa	8.67	19.43	13.03	13.71	5.41
Aporrectodea longa	20.76	9.39	18.62	16.26	6.04
Aporrectodea rosea	2.07	1.84	2.15	2.02	0.16
Lumbricus spp.	31.82	60.71	40.89	44.47	14.77
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	19.75	20.61	44.64	28.34	14.13
Octolasion spp.	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.85	3.17	2.01	2.01	1.16
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	15.80	16.12	17.97	16.63	1.17
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**43.10**	**50.07**	**59.07**	**50.74**	**8.00**
Anecic adults	**40.51**	**30.01**	**63.26**	**44.59**	**17.00**
Epilobous juveniles	**41.33**	**32.99**	**45.22**	**39.84**	**6.25**
Epilobous adults	**63.86**	**59.46**	**77.69**	**67.00**	**9.51**
Tanylobous juveniles	**31.82**	**60.71**	**40.89**	**44.47**	**14.77**
Tanylobous adults	**19.75**	**20.61**	**44.64**	**28.34**	**14.13**
Total juveniles	**73.15**	**93.69**	**86.11**	**84.32**	**10.39**
Total adults	**83.61**	**80.07**	**122.33**	**95.34**	**23.44**
Total earthworms	**172.56**	**189.89**	**226.41**	**196.29**	**27.49**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-34: Abundance of earthworms [ind/m²] in the plots treated with 1.8 kg carbendazim/ha (T2a – T2f) 188-190 DAA

Taxon	T2a	T2b	T2c	T2d	T2e	T2f	Mean	SD
Aporrectodea sp. sensu lato[a]	174.7	240.0	199.3	280.7	136.0	202.0	205.4	50.3
Allolobophora chlorotica	84.0	94.0	91.3	117.3	94.0	88.7	94.9	11.6
Aporrectodea caliginosa	16.0	11.3	9.3	4.7	13.3	6.7	10.2	4.2
Aporrectodea longa	0.0	2.7	3.3	15.3	28.7	4.0	9.0	11.0
Aporrectodea rosea	12.7	14.7	12.0	9.3	8.7	16.7	12.3	3.1
Lumbricus spp.	59.3	65.3	61.3	44.7	36.0	39.3	51.0	12.5
Lumbricus castaneus	0.0	1.3	0.0	0.0	0.0	0.0	0.2	0.5
Lumbricus terrestris	3.3	4.7	4.7	4.0	2.7	2.0	3.6	1.1
Octolasion spp.	0.0	0.7	0.0	0.0	0.0	0.0	0.1	0.3
Octolasion cyaneum	0.0	1.3	0.0	2.7	0.7	0.7	0.9	1.0
Octolasion tyrtaeum	0.0	0.0	0.0	0.7	0.0	0.0	0.1	0.3
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	48.0	72.7	66.7	72.0	46.0	40.0	57.6	14.5
Epigeic adults	**0.0**	**1.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.2**	**0.5**
Endogeic adults	**112.7**	**121.3**	**112.7**	**134.7**	**116.7**	**112.7**	**118.4**	**8.7**
Anecic adults	**3.3**	**7.3**	**8.0**	**19.3**	**31.3**	**6.0**	**12.6**	**10.7**
Epilobous juveniles	**174.7**	**240.7**	**199.3**	**280.7**	**136.0**	**202.0**	**205.6**	**50.4**
Epilobous adults	**112.7**	**124.0**	**116.0**	**150.0**	**145.3**	**116.7**	**127.4**	**16.2**
Tanylobous juveniles	**59.3**	**65.3**	**61.3**	**44.7**	**36.0**	**39.3**	**51.0**	**12.5**
Tanylobous adults	**3.3**	**6.0**	**4.7**	**4.0**	**2.7**	**2.0**	**3.8**	**1.4**
Total juveniles	**234.0**	**306.0**	**260.7**	**325.3**	**172.0**	**241.3**	**256.6**	**54.9**
Total adults	**116.0**	**130.0**	**120.7**	**154.0**	**148.0**	**118.7**	**131.2**	**16.1**
Total earthworms	**398.0**	**508.7**	**448.0**	**551.3**	**366.0**	**400.0**	**445.3**	**71.9**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-35: Biomass of earthworms [g/m²] in the plots treated with 1.8 kg carbendazim/ha (T2a – T2f) 188-190 DAA

Taxon	T2a	T2b	T2c	T2d	T2e	T2f	Mean	SD
Aporrectodea sp. sensu lato[a]	21.06	35.69	21.59	41.41	33.15	25.98	29.81	8.24
Allolobophora chlorotica	22.92	27.50	23.91	31.40	28.02	24.39	26.36	3.20
Aporrectodea caliginosa	15.77	11.55	7.09	4.62	14.49	6.57	10.01	4.58
Aporrectodea longa	0.00	3.85	3.45	23.11	53.69	7.76	15.31	20.48
Aporrectodea rosea	2.64	2.49	1.89	1.85	1.77	2.67	2.22	0.42
Lumbricus spp.	40.71	49.11	45.08	29.38	31.05	25.77	36.85	9.44
Lumbricus castaneus	0.00	0.58	0.00	0.00	0.00	0.00	0.10	0.24
Lumbricus terrestris	17.71	19.01	22.57	23.93	12.17	7.81	17.20	6.18
Octolasion spp.	0.00	0.26	0.00	0.00	0.00	0.00	0.04	0.11
Octolasion cyaneum	0.00	2.37	0.00	3.13	0.43	0.48	1.07	1.34
Octolasion tyrtaeum	0.00	0.00	0.00	0.75	0.00	0.00	0.13	0.31
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	9.61	14.12	16.93	18.57	21.27	11.03	15.26	4.49
Epigeic adults	**0.00**	**0.58**	**0.00**	**0.00**	**0.00**	**0.00**	**0.10**	**0.24**
Endogeic adults	**41.33**	**43.91**	**32.89**	**41.75**	**44.71**	**34.11**	**39.79**	**5.04**
Anecic adults	**17.71**	**22.86**	**26.02**	**47.04**	**65.85**	**15.57**	**32.51**	**19.82**
Epilobous juveniles	**21.06**	**35.96**	**21.59**	**41.41**	**33.15**	**25.98**	**29.86**	**8.27**
Epilobous adults	**41.33**	**47.76**	**36.34**	**64.87**	**98.39**	**41.87**	**55.09**	**23.41**
Tanylobous juveniles	**40.71**	**49.11**	**45.08**	**29.38**	**31.05**	**25.77**	**36.85**	**9.44**
Tanylobous adults	**17.71**	**19.59**	**22.57**	**23.93**	**12.17**	**7.81**	**17.30**	**6.22**
Total juveniles	**61.77**	**85.06**	**66.67**	**70.79**	**64.20**	**51.75**	**66.71**	**11.03**
Total adults	**59.04**	**67.35**	**58.91**	**88.79**	**110.56**	**49.68**	**72.39**	**22.92**
Total earthworms	**130.42**	**166.54**	**142.51**	**178.15**	**196.03**	**112.46**	**154.35**	**31.37**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-36: Abundance of earthworms [ind/m²] in the plots treated with 3.2 kg carbendazim/ha (T3a – T3c) 188-190 DAA

Taxon	T3a	T3b	T3c	Mean	SD
Aporrectodea sp. sensu lato[a]	197.3	136.0	156.0	163.1	31.3
Allolobophora chlorotica	86.0	84.0	84.7	84.9	1.0
Aporrectodea caliginosa	8.0	14.7	16.7	13.1	4.5
Aporrectodea longa	0.0	2.0	8.0	3.3	4.2
Aporrectodea rosea	8.7	6.7	12.0	9.1	2.7
Lumbricus spp.	61.3	56.0	44.7	54.0	8.5
Lumbricus castaneus	0.0	0.7	0.0	0.2	0.4
Lumbricus terrestris	4.7	6.0	4.0	4.9	1.0
Octolasion spp.	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.0	0.0	0.7	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	46.0	67.3	59.3	57.6	10.8
Epigeic adults	**0.0**	**0.7**	**0.0**	**0.2**	**0.4**
Endogeic adults	**102.7**	**105.3**	**114.0**	**107.3**	**5.9**
Anecic adults	**4.7**	**8.0**	**12.0**	**8.2**	**3.7**
Epilobous juveniles	**197.3**	**136.0**	**156.0**	**163.1**	**31.3**
Epilobous adults	**102.7**	**107.3**	**122.0**	**110.7**	**10.1**
Tanylobous juveniles	**61.3**	**56.0**	**44.7**	**54.0**	**8.5**
Tanylobous adults	**4.7**	**6.7**	**4.0**	**5.1**	**1.4**
Total juveniles	**258.7**	**192.0**	**200.7**	**217.1**	**36.2**
Total adults	**107.3**	**114.0**	**126.0**	**115.8**	**9.5**
Total earthworms	**412.0**	**373.3**	**386.0**	**390.4**	**19.7**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-37: Biomass of earthworms [g/m²] in the plots treated with 3.2 kg carbendazim/ha (T3a – T3c) 188-190 DAA

Taxon	T3a	T3b	T3c	Mean	SD
Aporrectodea sp. sensu lato[a]	22.26	18.19	26.67	22.37	4.24
Allolobophora chlorotica	23.09	22.58	24.24	23.30	0.85
Aporrectodea caliginosa	8.37	13.53	15.92	12.61	3.86
Aporrectodea longa	0.00	2.71	13.01	5.24	6.87
Aporrectodea rosea	1.45	1.29	2.65	1.79	0.74
Lumbricus spp.	44.39	41.01	30.49	38.63	7.25
Lumbricus castaneus	0.00	0.31	0.00	0.10	0.18
Lumbricus terrestris	25.09	29.25	19.87	24.74	4.70
Octolasion spp.	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.00	0.00	0.70	0.23	0.40
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	9.49	16.84	15.33	13.89	3.88
Epigeic adults	**0.00**	**0.31**	**0.00**	**0.10**	**0.18**
Endogeic adults	**32.91**	**37.40**	**43.51**	**37.94**	**5.32**
Anecic adults	**25.09**	**31.97**	**32.88**	**29.98**	**4.26**
Epilobous juveniles	**22.26**	**18.19**	**26.67**	**22.37**	**4.24**
Epilobous adults	**32.91**	**40.11**	**56.52**	**43.18**	**12.10**
Tanylobous juveniles	**44.39**	**41.01**	**30.49**	**38.63**	**7.25**
Tanylobous adults	**25.09**	**29.56**	**19.87**	**24.84**	**4.85**
Total juveniles	**66.65**	**59.20**	**57.15**	**61.00**	**5.00**
Total adults	**58.00**	**69.67**	**76.39**	**68.02**	**9.30**
Total earthworms	**134.14**	**145.71**	**148.87**	**142.91**	**7.76**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-38: Abundance of earthworms [ind/m²] in the plots treated with 5.8 kg carbendazim/ha (T4a – T4c) 188-190 DAA

Taxon	T4a	T4b	T4c	Mean	SD
Aporrectodea sp. sensu lato[a]	119.3	152.7	166.0	146.0	24.0
Allolobophora chlorotica	78.7	80.7	78.0	79.1	1.4
Aporrectodea caliginosa	10.7	13.3	18.0	14.0	3.7
Aporrectodea longa	0.0	8.0	8.0	5.3	4.6
Aporrectodea rosea	4.0	6.0	8.7	6.2	2.3
Lumbricus spp.	48.0	41.3	48.7	46.0	4.1
Lumbricus castaneus	0.0	0.7	0.0	0.2	0.4
Lumbricus terrestris	4.0	1.3	2.0	2.4	1.4
Octolasion spp.	0.0	0.7	0.0	0.2	0.4
Octolasion cyaneum	0.7	0.0	0.7	0.4	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	28.0	30.7	37.3	32.0	4.8
Epigeic adults	**0.0**	**0.7**	**0.0**	**0.2**	**0.4**
Endogeic adults	**94.0**	**100.0**	**105.3**	**99.8**	**5.7**
Anecic adults	**4.0**	**9.3**	**10.0**	**7.8**	**3.3**
Epilobous juveniles	**119.3**	**153.3**	**166.0**	**146.2**	**24.1**
Epilobous adults	**94.0**	**108.0**	**113.3**	**105.1**	**10.0**
Tanylobous juveniles	**48.0**	**41.3**	**48.7**	**46.0**	**4.1**
Tanylobous adults	**4.0**	**2.0**	**2.0**	**2.7**	**1.2**
Total juveniles	**167.3**	**194.7**	**214.7**	**192.2**	**23.8**
Total adults	**98.0**	**110.0**	**115.3**	**107.8**	**8.9**
Total earthworms	**293.3**	**335.3**	**367.3**	**332.0**	**37.1**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-39: Biomass of earthworms [g/m²] in the plots treated with 5.8 kg carbendazim/ha (T4a – T4c) 188-190 DAA

Taxon	T4a	T4b	T4c	Mean	SD
Aporrectodea sp. sensu lato[a]	13.45	24.29	22.03	19.93	5.72
Allolobophora chlorotica	24.25	24.24	22.05	23.51	1.27
Aporrectodea caliginosa	11.92	12.86	18.79	14.52	3.72
Aporrectodea longa	0.00	14.88	16.64	10.51	9.14
Aporrectodea rosea	0.69	1.17	1.54	1.14	0.42
Lumbricus spp.	36.75	28.63	38.71	34.70	5.34
Lumbricus castaneus	0.00	0.12	0.00	0.04	0.07
Lumbricus terrestris	15.49	5.29	8.51	9.77	5.21
Octolasion spp.	0.00	0.56	0.00	0.19	0.32
Octolasion cyaneum	0.85	0.00	1.02	0.62	0.55
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	6.03	7.72	11.07	8.27	2.57
Epigeic adults	**0.00**	**0.12**	**0.00**	**0.04**	**0.07**
Endogeic adults	**37.71**	**38.27**	**43.39**	**39.79**	**3.13**
Anecic adults	**15.49**	**20.17**	**25.15**	**20.27**	**4.83**
Epilobous juveniles	**13.45**	**24.85**	**22.03**	**20.11**	**5.94**
Epilobous adults	**37.71**	**53.15**	**60.03**	**50.30**	**11.43**
Tanylobous juveniles	**36.75**	**28.63**	**38.71**	**34.70**	**5.34**
Tanylobous adults	**15.49**	**5.41**	**8.51**	**9.81**	**5.16**
Total juveniles	**50.20**	**53.49**	**60.74**	**54.81**	**5.39**
Total adults	**53.21**	**58.57**	**68.55**	**60.11**	**7.79**
Total earthworms	**109.43**	**119.77**	**140.36**	**123.19**	**15.74**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-40: Abundance of earthworms [ind/m²] in the plots treated with 10.5 kg carbendazim/ha (T5a – T5f) 188-190 DAA

Taxon	T5a	T5b	T5c	T5d	T5e	T5f	Mean	SD
Aporrectodea sp. sensu lato[a]	104.0	114.7	126.7	106.7	112.7	118.0	113.8	8.2
Allolobophora chlorotica	90.7	85.3	75.3	54.0	90.0	96.7	82.0	15.5
Aporrectodea caliginosa	12.0	4.0	14.7	8.7	7.3	10.7	9.6	3.7
Aporrectodea longa	0.0	19.3	1.3	2.7	11.3	2.7	6.2	7.6
Aporrectodea rosea	3.3	10.0	12.7	6.7	3.3	7.3	7.2	3.7
Lumbricus spp.	55.3	24.0	50.0	38.7	50.7	38.0	42.8	11.5
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	5.3	3.3	5.3	5.3	0.7	2.7	3.8	1.9
Octolasion spp.	0.0	0.0	0.7	0.0	0.0	0.0	0.1	0.3
Octolasion cyaneum	0.0	0.7	0.7	0.7	0.0	0.0	0.3	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	47.3	43.3	42.0	35.3	31.3	43.3	40.4	5.9
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**106.0**	**100.0**	**103.3**	**70.0**	**100.7**	**114.7**	**99.1**	**15.2**
Anecic adults	**5.3**	**22.7**	**6.7**	**8.0**	**12.0**	**5.3**	**10.0**	**6.7**
Epilobous juveniles	**104.0**	**114.7**	**127.3**	**106.7**	**112.7**	**118.0**	**113.9**	**8.4**
Epilobous adults	**106.0**	**119.3**	**104.7**	**72.7**	**112.0**	**117.3**	**105.3**	**17.0**
Tanylobous juveniles	**55.3**	**24.0**	**50.0**	**38.7**	**50.7**	**38.0**	**42.8**	**11.5**
Tanylobous adults	**5.3**	**3.3**	**5.3**	**5.3**	**0.7**	**2.7**	**3.8**	**1.9**
Total juveniles	**159.3**	**138.7**	**177.3**	**145.3**	**163.3**	**156.0**	**156.7**	**13.6**
Total adults	**111.3**	**122.7**	**110.0**	**78.0**	**112.7**	**120.0**	**109.1**	**16.1**
Total earthworms	**318.0**	**304.7**	**329.3**	**258.7**	**307.3**	**319.3**	**306.2**	**24.9**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-41: Biomass of earthworms [g/m²] in the plots treated with 10.5 kg carbendazim/ha (T5a – T5f) 188-190 DAA

Taxon	T5a	T5b	T5c	T5d	T5e	T5f	Mean	SD
Aporrectodea sp. sensu lato[a]	14.81	20.60	19.22	13.65	21.76	16.13	17.69	3.30
Allolobophora chlorotica	23.87	24.46	22.21	14.88	26.39	31.24	23.84	5.38
Aporrectodea caliginosa	11.05	4.01	15.19	9.11	7.25	10.22	9.47	3.76
Aporrectodea longa	0.00	29.71	2.67	6.21	22.23	5.09	10.99	12.04
Aporrectodea rosea	0.59	2.05	2.47	1.24	0.60	1.28	1.37	0.76
Lumbricus spp.	45.45	17.67	35.78	32.63	36.83	30.85	33.20	9.13
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	22.81	12.95	22.45	20.75	2.61	8.46	15.00	8.37
Octolasion spp.	0.00	0.00	0.35	0.00	0.00	0.00	0.06	0.14
Octolasion cyaneum	0.00	0.27	0.40	1.67	0.00	0.00	0.39	0.65
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	12.17	10.80	9.37	9.37	6.68	8.98	9.56	1.85
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**35.52**	**30.79**	**40.27**	**26.90**	**34.25**	**42.74**	**35.08**	**5.86**
Anecic adults	**22.81**	**42.66**	**25.11**	**26.95**	**24.84**	**13.55**	**25.99**	**9.44**
Epilobous juveniles	**14.81**	**20.60**	**19.57**	**13.65**	**21.76**	**16.13**	**17.75**	**3.34**
Epilobous adults	**35.52**	**60.51**	**42.93**	**33.11**	**56.48**	**47.83**	**46.06**	**11.04**
Tanylobous juveniles	**45.45**	**17.67**	**35.78**	**32.63**	**36.83**	**30.85**	**33.20**	**9.13**
Tanylobous adults	**22.81**	**12.95**	**22.45**	**20.75**	**2.61**	**8.46**	**15.00**	**8.37**
Total juveniles	**60.25**	**38.27**	**55.35**	**46.27**	**58.59**	**46.98**	**50.95**	**8.52**
Total adults	**58.33**	**73.45**	**65.38**	**53.85**	**59.09**	**56.29**	**61.07**	**7.19**
Total earthworms	**130.76**	**122.52**	**130.09**	**109.49**	**124.36**	**112.25**	**121.58**	**8.93**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-42: Abundance of earthworms [ind/m²] in the plots treated with 31.5 kg carbendazim/ha (T6a – T6c) 188-190 DAA

Taxon	T6a	T6b	T6c	Mean	SD
Aporrectodea sp. sensu lato[a]	90.0	92.0	88.0	90.0	2.0
Allolobophora chlorotica	61.3	73.3	62.7	65.8	6.6
Aporrectodea caliginosa	14.7	21.3	10.7	15.6	5.4
Aporrectodea longa	0.7	0.7	8.7	3.3	4.6
Aporrectodea rosea	9.3	4.7	7.3	7.1	2.3
Lumbricus spp.	62.7	49.3	38.0	50.0	12.3
Lumbricus castaneus	0.0	0.0	1.3	0.4	0.8
Lumbricus terrestris	2.0	3.3	2.0	2.4	0.8
Octolasion spp.	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.7	0.0	0.0	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	32.7	50.7	50.7	44.7	10.4
Epigeic adults	**0.0**	**0.0**	**1.3**	**0.4**	**0.8**
Endogeic adults	**86.0**	**99.3**	**80.7**	**88.7**	**9.6**
Anecic adults	**2.7**	**4.0**	**10.7**	**5.8**	**4.3**
Epilobous juveniles	**90.0**	**92.0**	**88.0**	**90.0**	**2.0**
Epilobous adults	**86.7**	**100.0**	**89.3**	**92.0**	**7.1**
Tanylobous juveniles	**62.7**	**49.3**	**38.0**	**50.0**	**12.3**
Tanylobous adults	**2.0**	**3.3**	**3.3**	**2.9**	**0.8**
Total juveniles	**152.7**	**141.3**	**126.0**	**140.0**	**13.4**
Total adults	**88.7**	**103.3**	**92.7**	**94.9**	**7.6**
Total earthworms	**274.0**	**295.3**	**269.3**	**279.6**	**13.9**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-43: Biomass of earthworms [g/m²] in the plots treated with 31.5 kg carbendazim/ha (T6a – T6c) 188-190 DAA

Taxon	T6a	T6b	T6c	Mean	SD
Aporrectodea sp. sensu lato[a]	13.77	14.77	20.33	16.29	3.53
Allolobophora chlorotica	18.33	23.51	19.90	20.58	2.66
Aporrectodea caliginosa	17.33	22.56	11.71	17.20	5.42
Aporrectodea longa	1.37	1.03	18.66	7.02	10.08
Aporrectodea rosea	1.89	1.06	1.35	1.43	0.42
Lumbricus spp.	44.99	41.93	35.02	40.65	5.11
Lumbricus castaneus	0.00	0.00	0.49	0.16	0.28
Lumbricus terrestris	5.56	13.43	6.88	8.62	4.21
Octolasion spp.	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	1.91	0.00	0.00	0.64	1.10
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	7.52	16.39	12.05	11.99	4.43
Epigeic adults	**0.00**	**0.00**	**0.49**	**0.16**	**0.28**
Endogeic adults	**39.45**	**47.13**	**32.97**	**39.85**	**7.09**
Anecic adults	**6.93**	**14.45**	**25.54**	**15.64**	**9.36**
Epilobous juveniles	**13.77**	**14.77**	**20.33**	**16.29**	**3.53**
Epilobous adults	**40.83**	**48.16**	**51.63**	**46.87**	**5.51**
Tanylobous juveniles	**44.99**	**41.93**	**35.02**	**40.65**	**5.11**
Tanylobous adults	**5.56**	**13.43**	**7.37**	**8.79**	**4.12**
Total juveniles	**58.76**	**56.71**	**55.35**	**56.94**	**1.72**
Total adults	**46.39**	**61.59**	**59.00**	**55.66**	**8.13**
Total earthworms	**112.67**	**134.68**	**126.40**	**124.58**	**11.12**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

A.1.5 377-379 DAA: mean abundance & biomass of earthworms per plot and treatment

Table A1-44: Abundance of earthworms [ind/m²] in the control plots (Ca – Cf) 377-379 DAA

Taxon	Ca	Cb	Cc	Cd	Ce	Cf	Mean	SD
Aporrectodea sp. sensu lato[a]	363.3	78.0	206.7	235.3	238.7	301.3	237.2	96.2
Allolobophora chlorotica	42.0	17.3	28.7	47.3	40.0	53.3	38.1	13.1
Aporrectodea caliginosa	2.0	0.0	0.7	0.0	0.0	2.7	0.9	1.2
Aporrectodea longa	0.0	0.0	0.7	0.0	1.3	2.0	0.7	0.8
Aporrectodea rosea	3.3	1.3	2.7	4.0	6.0	7.3	4.1	2.2
Lumbricus spp.	12.7	12.0	9.3	16.0	25.3	15.3	15.1	5.6
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	5.3	4.7	3.3	5.3	4.0	3.3	4.3	0.9
Octolasion spp.	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.3
Octolasion cyaneum	0.0	0.0	0.0	0.0	2.0	1.3	0.6	0.9
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	35.3	7.3	20.7	31.3	16.7	52.0	27.2	15.8
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**47.3**	**18.7**	**32.0**	**51.3**	**48.0**	**64.7**	**43.7**	**16.1**
Anecic adults	**5.3**	**4.7**	**4.0**	**5.3**	**5.3**	**5.3**	**5.0**	**0.6**
Epilobous juveniles	**363.3**	**78.0**	**206.7**	**235.3**	**238.7**	**302.0**	**237.3**	**96.3**
Epilobous adults	**47.3**	**18.7**	**32.7**	**51.3**	**49.3**	**66.7**	**44.3**	**16.6**
Tanylobous juveniles	**12.7**	**12.0**	**9.3**	**16.0**	**25.3**	**15.3**	**15.1**	**5.6**
Tanylobous adults	**5.3**	**4.7**	**3.3**	**5.3**	**4.0**	**3.3**	**4.3**	**0.9**
Total juveniles	**376.0**	**90.0**	**216.0**	**251.3**	**264.0**	**317.3**	**252.4**	**97.3**
Total adults	**52.7**	**23.3**	**36.0**	**56.7**	**53.3**	**70.0**	**48.7**	**16.5**
Total earthworms	**464.0**	**120.7**	**272.7**	**339.3**	**334.0**	**439.3**	**328.3**	**124.2**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-45: Biomass of earthworms [g/m²] in the control plots (Ca – Cf) 377-379 DAA

Taxon	Ca	Cb	Cc	Cd	Ce	Cf	Mean	SD
Aporrectodea sp. sensu lato[a]	33.99	6.53	19.60	20.04	23.30	34.39	22.97	10.41
Allolobophora chlorotica	8.13	2.79	5.70	8.59	6.45	9.50	6.86	2.43
Aporrectodea caliginosa	1.18	0.00	0.51	0.00	0.00	1.97	0.61	0.81
Aporrectodea longa	0.00	0.00	0.74	0.00	1.67	2.37	0.80	1.02
Aporrectodea rosea	0.51	0.19	0.40	0.54	0.80	1.15	0.60	0.34
Lumbricus spp.	17.28	12.86	9.95	21.27	36.39	18.96	19.45	9.26
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	19.61	20.19	15.19	19.33	20.37	14.38	18.18	2.67
Octolasion spp.	0.00	0.00	0.00	0.00	0.00	0.31	0.05	0.13
Octolasion cyaneum	0.00	0.00	0.00	0.00	3.55	2.64	1.03	1.62
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	2.93	0.56	1.83	3.51	2.43	5.80	2.84	1.77
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**9.82**	**2.99**	**6.61**	**9.13**	**10.81**	**15.26**	**9.10**	**4.12**
Anecic adults	**19.61**	**20.19**	**15.93**	**19.33**	**22.05**	**16.75**	**18.98**	**2.27**
Epilobous juveniles	**33.99**	**6.53**	**19.60**	**20.04**	**23.30**	**34.70**	**23.03**	**10.48**
Epilobous adults	**9.82**	**2.99**	**7.35**	**9.13**	**12.48**	**17.63**	**9.90**	**4.93**
Tanylobous juveniles	**17.28**	**12.86**	**9.95**	**21.27**	**36.39**	**18.96**	**19.45**	**9.26**
Tanylobous adults	**19.61**	**20.19**	**15.19**	**19.33**	**20.37**	**14.38**	**18.18**	**2.67**
Total juveniles	**51.27**	**19.39**	**29.55**	**41.31**	**59.69**	**53.66**	**42.48**	**15.49**
Total adults	**29.43**	**23.18**	**22.54**	**28.46**	**32.85**	**32.01**	**28.08**	**4.36**
Total earthworms	**83.63**	**43.13**	**53.91**	**73.27**	**94.97**	**91.47**	**73.40**	**20.94**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-46: Abundance of earthworms [ind/m²] in the plots treated with 0.6 kg carbendazim/ha (T1a – T1c) 377-379 DAA

Taxon	T1a	T1b	T1c	Mean	SD
Aporrectodea sp. sensu lato[a]	203.3	176.7	78.0	152.7	66.0
Allolobophora chlorotica	42.0	37.3	21.3	33.6	10.8
Aporrectodea caliginosa	0.7	0.7	0.7	0.7	0.0
Aporrectodea longa	2.0	0.7	0.7	1.1	0.8
Aporrectodea rosea	10.0	10.7	3.3	8.0	4.1
Lumbricus spp.	12.7	15.3	22.0	16.7	4.8
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	4.7	8.0	2.7	5.1	2.7
Octolasion spp.	0.7	0.0	0.0	0.2	0.4
Octolasion cyaneum	0.7	0.0	0.0	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	26.0	28.0	15.3	23.1	6.8
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**53.3**	**48.7**	**25.3**	**42.4**	**15.0**
Anecic adults	**6.7**	**8.7**	**3.3**	**6.2**	**2.7**
Epilobous juveniles	**204.0**	**176.7**	**78.0**	**152.9**	**66.3**
Epilobous adults	**55.3**	**49.3**	**26.0**	**43.6**	**15.5**
Tanylobous juveniles	**12.7**	**15.3**	**22.0**	**16.7**	**4.8**
Tanylobous adults	**4.7**	**8.0**	**2.7**	**5.1**	**2.7**
Total juveniles	**216.7**	**192.0**	**100.0**	**169.6**	**61.5**
Total adults	**60.0**	**57.3**	**28.7**	**48.7**	**17.4**
Total earthworms	**302.7**	**277.3**	**144.0**	**241.3**	**85.2**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-47: Biomass of earthworms [g/m²] in the plots treated with 0.6 kg carbendazim/ha (T1a – T1c) 377-379 DAA

Taxon	T1a	T1b	T1c	Mean	SD
Aporrectodea sp. sensu lato[a]	21.37	18.33	8.07	15.92	6.97
Allolobophora chlorotica	7.53	6.87	3.51	5.97	2.16
Aporrectodea caliginosa	0.55	0.66	0.37	0.53	0.14
Aporrectodea longa	2.73	0.68	0.83	1.42	1.14
Aporrectodea rosea	1.67	1.86	0.52	1.35	0.72
Lumbricus spp.	25.27	29.24	30.98	28.50	2.92
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	19.97	39.98	13.33	24.43	13.87
Octolasion spp.	0.71	0.00	0.00	0.24	0.41
Octolasion cyaneum	1.37	0.00	0.00	0.46	0.79
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	3.82	5.37	2.92	4.04	1.24
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**11.11**	**9.39**	**4.40**	**8.30**	**3.48**
Anecic adults	**22.70**	**40.66**	**14.17**	**25.84**	**13.52**
Epilobous juveniles	**22.08**	**18.33**	**8.07**	**16.16**	**7.26**
Epilobous adults	**13.84**	**10.07**	**5.23**	**9.71**	**4.31**
Tanylobous juveniles	**25.27**	**29.24**	**30.98**	**28.50**	**2.92**
Tanylobous adults	**19.97**	**39.98**	**13.33**	**24.43**	**13.87**
Total juveniles	**47.35**	**47.57**	**39.05**	**44.66**	**4.86**
Total adults	**33.81**	**50.05**	**18.57**	**34.14**	**15.74**
Total earthworms	**84.98**	**102.99**	**60.53**	**82.83**	**21.31**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-48: Abundance of earthworms [ind/m²] in the plots treated with 1.8 kg carbendazim/ha (T2a – T2f) 377-379 DAA

Taxon	T2a	T2b	T2c	T2d	T2e	T2f	Mean	SD
Aporrectodea sp. sensu lato[a]	150.0	101.3	168.0	155.3	110.7	206.0	148.6	38.4
Allolobophora chlorotica	42.0	18.0	46.0	34.7	33.3	42.0	36.0	10.0
Aporrectodea caliginosa	2.7	0.0	2.7	1.3	0.7	2.7	1.7	1.2
Aporrectodea longa	0.0	0.7	0.0	0.7	7.3	2.0	1.8	2.8
Aporrectodea rosea	8.0	4.7	16.0	3.3	11.3	10.0	8.9	4.6
Lumbricus spp.	31.3	14.7	14.0	23.3	7.3	8.0	16.4	9.3
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	3.3	4.0	3.3	6.0	0.7	2.7	3.3	1.7
Octolasion spp.	0.0	0.0	0.7	0.7	0.0	0.0	0.2	0.3
Octolasion cyaneum	1.3	0.0	0.0	0.7	0.0	4.0	1.0	1.6
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	27.3	14.0	26.0	23.3	29.3	38.7	26.4	8.0
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**54.0**	**22.7**	**64.7**	**40.0**	**45.3**	**58.7**	**47.6**	**15.1**
Anecic adults	**3.3**	**4.7**	**3.3**	**6.7**	**8.0**	**4.7**	**5.1**	**1.9**
Epilobous juveniles	**150.0**	**101.3**	**168.7**	**156.0**	**110.7**	**206.0**	**148.8**	**38.5**
Epilobous adults	**54.0**	**23.3**	**64.7**	**40.7**	**52.7**	**60.7**	**49.3**	**15.1**
Tanylobous juveniles	**31.3**	**14.7**	**14.0**	**23.3**	**7.3**	**8.0**	**16.4**	**9.3**
Tanylobous adults	**3.3**	**4.0**	**3.3**	**6.0**	**0.7**	**2.7**	**3.3**	**1.7**
Total juveniles	**181.3**	**116.0**	**182.7**	**179.3**	**118.0**	**214.0**	**165.2**	**39.5**
Total adults	**57.3**	**27.3**	**68.0**	**46.7**	**53.3**	**63.3**	**52.7**	**14.5**
Total earthworms	**266.0**	**157.3**	**276.7**	**249.3**	**200.7**	**316.0**	**244.3**	**56.8**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-49: Biomass of earthworms [g/m²] in the plots treated with 1.8 kg carbendazim/ha (T2a – T2f) 377-379 DAA

Taxon	T2a	T2b	T2c	T2d	T2e	T2f	Mean	SD
Aporrectodea sp. sensu lato[a]	16.26	10.25	22.13	18.57	17.35	26.94	18.58	5.63
Allolobophora chlorotica	8.05	3.15	8.54	5.71	6.23	8.57	6.71	2.12
Aporrectodea caliginosa	2.13	0.00	1.96	0.89	0.63	2.77	1.40	1.05
Aporrectodea longa	0.00	1.17	0.00	1.19	12.79	3.95	3.18	4.92
Aporrectodea rosea	1.77	0.67	2.33	0.61	2.22	1.44	1.51	0.74
Lumbricus spp.	58.51	23.47	19.29	31.27	15.33	13.73	26.94	16.70
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	13.08	16.99	17.05	33.05	2.14	13.88	16.03	9.98
Octolasion spp.	0.00	0.00	0.14	0.14	0.00	0.00	0.05	0.07
Octolasion cyaneum	2.14	0.00	0.00	0.91	0.00	7.67	1.79	3.00
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	4.39	1.12	3.15	4.66	5.88	4.21	3.90	1.62
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**14.09**	**3.82**	**12.83**	**8.11**	**9.09**	**20.44**	**11.40**	**5.74**
Anecic adults	**13.08**	**18.16**	**17.05**	**34.24**	**14.93**	**17.83**	**19.22**	**7.61**
Epilobous juveniles	**16.26**	**10.25**	**22.27**	**18.71**	**17.35**	**26.94**	**18.63**	**5.65**
Epilobous adults	**14.09**	**4.99**	**12.83**	**9.30**	**21.88**	**24.39**	**14.58**	**7.38**
Tanylobous juveniles	**58.51**	**23.47**	**19.29**	**31.27**	**15.33**	**13.73**	**26.94**	**16.70**
Tanylobous adults	**13.08**	**16.99**	**17.05**	**33.05**	**2.14**	**13.88**	**16.03**	**9.98**
Total juveniles	**74.77**	**33.73**	**41.57**	**49.98**	**32.68**	**40.67**	**45.57**	**15.61**
Total adults	**27.17**	**21.98**	**29.88**	**42.35**	**24.02**	**38.27**	**30.61**	**8.09**
Total earthworms	**106.33**	**56.83**	**74.59**	**96.99**	**62.58**	**83.15**	**80.08**	**19.30**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-50: **Abundance of earthworms [ind/m²] in the plots treated with 3.2 kg carbendazim/ha (T3a – T3c) 377-379 DAA**

Taxon	T3a	T3b	T3c	Mean	SD
Aporrectodea sp. sensu lato[a]	92.7	107.3	106.7	102.2	8.3
Allolobophora chlorotica	35.3	44.7	52.0	44.0	8.4
Aporrectodea caliginosa	0.7	1.3	1.3	1.1	0.4
Aporrectodea longa	0.0	1.3	4.7	2.0	2.4
Aporrectodea rosea	3.3	10.7	8.0	7.3	3.7
Lumbricus spp.	14.7	9.3	6.0	10.0	4.4
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	4.0	5.3	2.0	3.8	1.7
Octolasion spp.	0.0	0.0	2.0	0.7	1.2
Octolasion cyaneum	0.0	0.0	0.7	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	8.0	18.7	14.0	13.6	5.3
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**39.3**	**56.7**	**62.0**	**52.7**	**11.9**
Anecic adults	**4.0**	**6.7**	**6.7**	**5.8**	**1.5**
Epilobous juveniles	**92.7**	**107.3**	**108.7**	**102.9**	**8.9**
Epilobous adults	**39.3**	**58.0**	**66.7**	**54.7**	**14.0**
Tanylobous juveniles	**14.7**	**9.3**	**6.0**	**10.0**	**4.4**
Tanylobous adults	**4.0**	**5.3**	**2.0**	**3.8**	**1.7**
Total juveniles	**107.3**	**116.7**	**114.7**	**112.9**	**4.9**
Total adults	**43.3**	**63.3**	**68.7**	**58.4**	**13.4**
Total earthworms	**158.7**	**198.7**	**197.3**	**184.9**	**22.7**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-51: Biomass of earthworms [g/m²] in the plots treated with 3.2 kg carbendazim/ha (T3a – T3c) 377-379 DAA

Taxon	T3a	T3b	T3c	Mean	SD
Aporrectodea sp. sensu lato[a]	9.35	13.33	12.64	11.77	2.12
Allolobophora chlorotica	6.41	8.80	9.34	8.18	1.56
Aporrectodea caliginosa	0.42	1.48	1.28	1.06	0.56
Aporrectodea longa	0.00	2.45	6.46	2.97	3.26
Aporrectodea rosea	0.51	1.82	1.45	1.26	0.68
Lumbricus spp.	24.83	15.43	8.65	16.30	8.12
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	18.70	20.93	7.49	15.71	7.20
Octolasion spp.	0.00	0.00	0.67	0.22	0.39
Octolasion cyaneum	0.00	0.00	1.29	0.43	0.74
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	0.90	3.75	2.35	2.33	1.42
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**7.33**	**12.10**	**13.35**	**10.93**	**3.18**
Anecic adults	**18.70**	**23.39**	**13.95**	**18.68**	**4.72**
Epilobous juveniles	**9.35**	**13.33**	**13.31**	**12.00**	**2.29**
Epilobous adults	**7.33**	**14.55**	**19.81**	**13.90**	**6.27**
Tanylobous juveniles	**24.83**	**15.43**	**8.65**	**16.30**	**8.12**
Tanylobous adults	**18.70**	**20.93**	**7.49**	**15.71**	**7.20**
Total juveniles	**34.18**	**28.75**	**21.97**	**28.30**	**6.12**
Total adults	**26.03**	**35.49**	**27.31**	**29.61**	**5.13**
Total earthworms	**61.11**	**67.99**	**51.62**	**60.24**	**8.22**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-52: Abundance of earthworms [ind/m²] in the plots treated with 5.8 kg carbendazim/ha (T4a – T4c) 377-379 DAA

Taxon	T4a	T4b	T4c	Mean	SD
Aporrectodea sp. sensu lato[a]	70.0	86.7	141.3	99.3	37.3
Allolobophora chlorotica	37.3	31.3	36.0	34.9	3.2
Aporrectodea caliginosa	0.0	0.7	4.0	1.6	2.1
Aporrectodea longa	0.0	0.0	4.0	1.3	2.3
Aporrectodea rosea	8.7	5.3	6.7	6.9	1.7
Lumbricus spp.	14.0	4.7	14.0	10.9	5.4
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	4.0	3.3	14.0	7.1	6.0
Octolasion spp.	0.0	0.7	0.0	0.2	0.4
Octolasion cyaneum	0.0	0.0	0.0	0.0	0.0
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	11.3	21.3	21.3	18.0	5.8
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**46.0**	**37.3**	**46.7**	**43.3**	**5.2**
Anecic adults	**4.0**	**3.3**	**18.0**	**8.4**	**8.3**
Epilobous juveniles	**70.0**	**87.3**	**141.3**	**99.6**	**37.2**
Epilobous adults	**46.0**	**37.3**	**50.7**	**44.7**	**6.8**
Tanylobous juveniles	**14.0**	**4.7**	**14.0**	**10.9**	**5.4**
Tanylobous adults	**4.0**	**3.3**	**14.0**	**7.1**	**6.0**
Total juveniles	**84.0**	**92.0**	**155.3**	**110.4**	**39.1**
Total adults	**50.0**	**40.7**	**64.7**	**51.8**	**12.1**
Total earthworms	**145.3**	**154.0**	**241.3**	**180.2**	**53.1**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-53: Biomass of earthworms [g/m²] in the plots treated with 5.8 kg carbendazim/ha (T4a – T4c) 377-379 DAA

Taxon	T4a	T4b	T4c	Mean	SD
Aporrectodea sp. sensu lato[a]	6.04	9.59	20.45	12.03	7.51
Allolobophora chlorotica	7.27	5.98	8.16	7.14	1.10
Aporrectodea caliginosa	0.00	0.37	3.32	1.23	1.82
Aporrectodea longa	0.00	0.00	5.68	1.89	3.28
Aporrectodea rosea	1.53	0.90	1.51	1.31	0.36
Lumbricus spp.	22.12	8.14	22.49	17.58	8.18
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	15.27	14.07	45.21	24.85	17.64
Octolasion spp.	0.00	0.09	0.00	0.03	0.05
Octolasion cyaneum	0.00	0.00	0.00	0.00	0.00
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	0.72	2.15	4.95	2.61	2.15
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**8.79**	**7.26**	**12.99**	**9.68**	**2.97**
Anecic adults	**15.27**	**14.07**	**50.89**	**26.75**	**20.92**
Epilobous juveniles	**6.04**	**9.68**	**20.45**	**12.06**	**7.50**
Epilobous adults	**8.79**	**7.26**	**18.67**	**11.57**	**6.19**
Tanylobous juveniles	**22.12**	**8.14**	**22.49**	**17.58**	**8.18**
Tanylobous adults	**15.27**	**14.07**	**45.21**	**24.85**	**17.64**
Total juveniles	**28.16**	**17.82**	**42.95**	**29.64**	**12.63**
Total adults	**24.07**	**21.33**	**63.88**	**36.43**	**23.82**
Total earthworms	**52.95**	**41.30**	**111.77**	**68.67**	**37.78**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-54: Abundance of earthworms [ind/m²] in the plots treated with 10.5 kg carbendazim/ha (T5a – T5f) 377-379 DAA

Taxon	T5a	T5b	T5c	T5d	T5e	T5f	Mean	SD
Aporrectodea sp. sensu lato[a]	79.3	88.7	88.0	115.3	63.3	74.7	84.9	17.6
Allolobophora chlorotica	46.7	25.3	30.0	34.0	31.3	48.7	36.0	9.5
Aporrectodea caliginosa	2.7	1.3	2.0	2.7	0.0	4.0	2.1	1.4
Aporrectodea longa	2.7	4.0	0.0	5.3	1.3	0.0	2.2	2.2
Aporrectodea rosea	8.7	7.3	7.3	13.3	3.3	1.3	6.9	4.2
Lumbricus spp.	15.3	10.7	8.7	12.7	8.7	14.0	11.7	2.8
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	9.3	6.7	7.3	8.0	8.7	8.0	8.0	0.9
Octolasion spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Undetermined	16.7	11.3	15.3	37.3	10.0	16.7	17.9	9.9
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**58.0**	**34.0**	**39.3**	**50.0**	**34.7**	**54.0**	**45.0**	**10.3**
Anecic adults	**12.0**	**10.7**	**7.3**	**13.3**	**10.0**	**8.0**	**10.2**	**2.3**
Epilobous juveniles	**79.3**	**88.7**	**88.0**	**115.3**	**63.3**	**74.7**	**84.9**	**17.6**
Epilobous adults	**60.7**	**38.0**	**39.3**	**55.3**	**36.0**	**54.0**	**47.2**	**10.6**
Tanylobous juveniles	**15.3**	**10.7**	**8.7**	**12.7**	**8.7**	**14.0**	**11.7**	**2.8**
Tanylobous adults	**9.3**	**6.7**	**7.3**	**8.0**	**8.7**	**8.0**	**8.0**	**0.9**
Total juveniles	**94.7**	**99.3**	**96.7**	**128.0**	**72.0**	**88.7**	**96.6**	**18.2**
Total adults	**70.0**	**44.7**	**46.7**	**63.3**	**44.7**	**62.0**	**55.2**	**11.2**
Total earthworms	**181.3**	**155.3**	**158.7**	**228.7**	**126.7**	**167.3**	**169.7**	**34.1**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-55: Biomass of earthworms [g/m²] in the plots treated with 10.5 kg carbendazim/ha (T5a – T5f) 377-379 DAA

Taxon	T5a	T5b	T5c	T5d	T5e	T5f	Mean	SD
Aporrectodea sp. sensu lato[a]	11.71	9.95	9.13	17.11	8.95	7.93	10.80	3.34
Allolobophora chlorotica	9.45	4.95	5.73	7.55	6.16	10.00	7.31	2.06
Aporrectodea caliginosa	2.56	1.26	1.87	2.21	0.00	4.09	2.00	1.36
Aporrectodea longa	3.04	7.21	0.00	8.63	1.53	0.00	3.40	3.71
Aporrectodea rosea	1.44	1.39	1.19	2.46	0.47	0.23	1.20	0.79
Lumbricus spp.	24.08	18.63	12.17	19.59	12.61	20.99	18.01	4.73
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	27.25	24.59	21.41	30.49	28.17	24.83	26.12	3.19
Octolasion spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Undetermined	3.40	1.05	2.81	5.67	0.99	2.78	2.78	1.73
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**13.45**	**7.61**	**8.79**	**12.23**	**6.63**	**14.33**	**10.50**	**3.24**
Anecic adults	**30.29**	**31.80**	**21.41**	**39.13**	**29.69**	**24.83**	**29.53**	**6.10**
Epilobous juveniles	**11.71**	**9.95**	**9.13**	**17.11**	**8.95**	**7.93**	**10.80**	**3.34**
Epilobous adults	**16.49**	**14.82**	**8.79**	**20.86**	**8.15**	**14.33**	**13.91**	**4.80**
Tanylobous juveniles	**24.08**	**18.63**	**12.17**	**19.59**	**12.61**	**20.99**	**18.01**	**4.73**
Tanylobous adults	**27.25**	**24.59**	**21.41**	**30.49**	**28.17**	**24.83**	**26.12**	**3.19**
Total juveniles	**35.79**	**28.57**	**21.31**	**36.70**	**21.57**	**28.93**	**28.81**	**6.63**
Total adults	**43.74**	**39.41**	**30.20**	**51.35**	**36.32**	**39.16**	**40.03**	**7.12**
Total earthworms	**82.93**	**69.03**	**54.31**	**93.73**	**58.87**	**70.87**	**71.62**	**14.74**

[a]Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

Table A1-56: Abundance of earthworms [ind/m²] in the plots treated with 31.5 kg carbendazim/ha (T6a – T6c) 377-379 DAA

Taxon	T6a	T6b	T6c	Mean	SD
Aporrectodea sp. sensu lato[a]	57.3	51.3	34.0	47.6	12.1
Allolobophora chlorotica	40.7	35.3	24.7	33.6	8.1
Aporrectodea caliginosa	3.3	4.0	0.7	2.7	1.8
Aporrectodea longa	0.0	0.0	2.0	0.7	1.2
Aporrectodea rosea	16.0	14.0	7.3	12.4	4.5
Lumbricus spp.	12.7	6.7	10.7	10.0	3.1
Lumbricus castaneus	0.0	0.0	0.0	0.0	0.0
Lumbricus terrestris	4.7	6.0	7.3	6.0	1.3
Octolasion spp.	0.0	0.0	0.0	0.0	0.0
Octolasion cyaneum	0.7	0.0	0.0	0.2	0.4
Octolasion tyrtaeum	0.0	0.0	0.0	0.0	0.0
Proctodrilus antipae	0.0	0.0	0.0	0.0	0.0
Undetermined	10.7	8.0	12.7	10.4	2.3
Epigeic adults	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Endogeic adults	**60.7**	**53.3**	**32.7**	**48.9**	**14.5**
Anecic adults	**4.7**	**6.0**	**9.3**	**6.7**	**2.4**
Epilobous juveniles	**57.3**	**51.3**	**34.0**	**47.6**	**12.1**
Epilobous adults	**60.7**	**53.3**	**34.7**	**49.6**	**13.4**
Tanylobous juveniles	**12.7**	**6.7**	**10.7**	**10.0**	**3.1**
Tanylobous adults	**4.7**	**6.0**	**7.3**	**6.0**	**1.3**
Total juveniles	**70.0**	**58.0**	**44.7**	**57.6**	**12.7**
Total adults	**65.3**	**59.3**	**42.0**	**55.6**	**12.1**
Total earthworms	**146.0**	**125.3**	**99.3**	**123.6**	**23.4**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

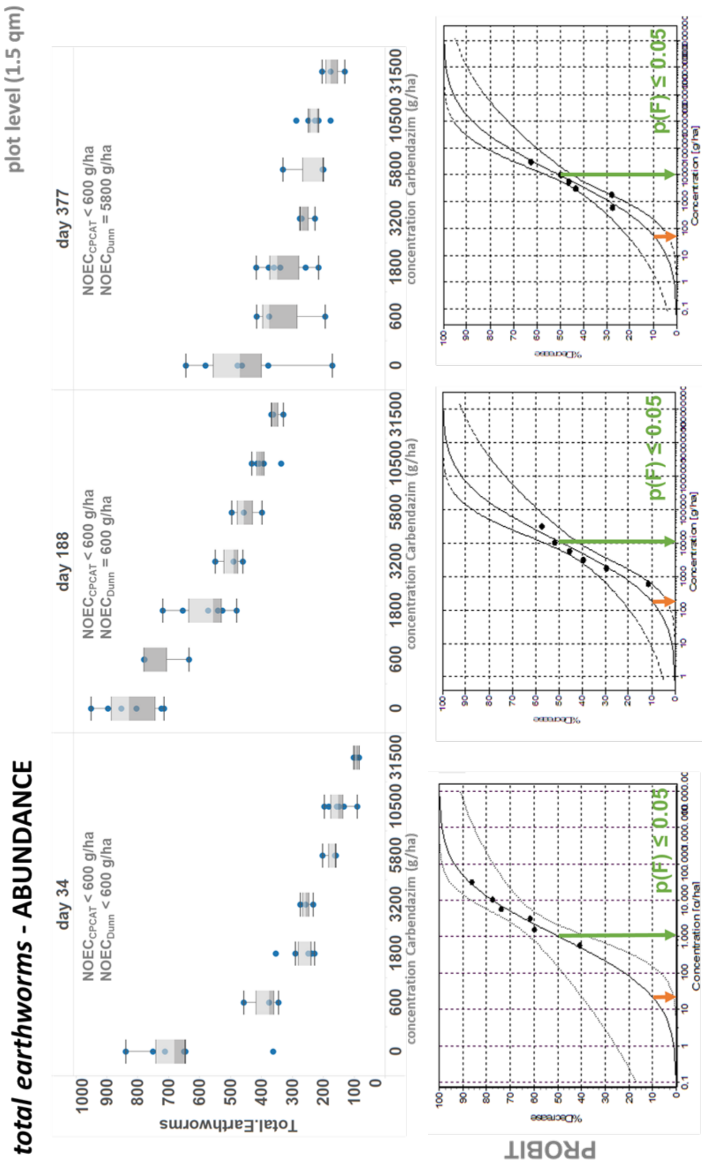
Table A1-57: Biomass of earthworms [g/m²] in the plots treated with 31.5 kg carbendazim/ha (T6a – T6c) 377-379 DAA

Taxon	T6a	T6b	T6c	Mean	SD
Aporrectodea sp. sensu lato[a]	6.35	8.49	4.61	6.48	1.94
Allolobophora chlorotica	8.09	7.16	5.61	6.96	1.25
Aporrectodea caliginosa	2.81	4.55	0.45	2.60	2.05
Aporrectodea longa	0.00	0.00	2.40	0.80	1.39
Aporrectodea rosea	2.63	2.37	1.70	2.23	0.48
Lumbricus spp.	18.71	12.06	19.19	16.65	3.98
Lumbricus castaneus	0.00	0.00	0.00	0.00	0.00
Lumbricus terrestris	17.84	21.59	21.89	20.44	2.25
Octolasion spp.	0.00	0.00	0.00	0.00	0.00
Octolasion cyaneum	1.76	0.00	0.00	0.59	1.02
Octolasion tyrtaeum	0.00	0.00	0.00	0.00	0.00
Proctodrilus antipae	0.00	0.00	0.00	0.00	0.00
Undetermined	1.13	0.67	3.04	1.61	1.26
Epigeic adults	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Endogeic adults	**15.29**	**14.08**	**7.77**	**12.38**	**4.04**
Anecic adults	**17.84**	**21.59**	**24.29**	**21.24**	**3.24**
Epilobous juveniles	**6.35**	**8.49**	**4.61**	**6.48**	**1.94**
Epilobous adults	**15.29**	**14.08**	**10.17**	**13.18**	**2.68**
Tanylobous juveniles	**18.71**	**12.06**	**19.19**	**16.65**	**3.98**
Tanylobous adults	**17.84**	**21.59**	**21.89**	**20.44**	**2.25**
Total juveniles	**25.05**	**20.55**	**23.80**	**23.13**	**2.33**
Total adults	**33.13**	**35.67**	**32.05**	**33.62**	**1.86**
Total earthworms	**59.31**	**56.88**	**58.89**	**58.36**	**1.30**

[a] Not differentiated between the closely related genera *Allolobophora* and *Aporrectodea*. DAA = days after application, SD = standard deviation.

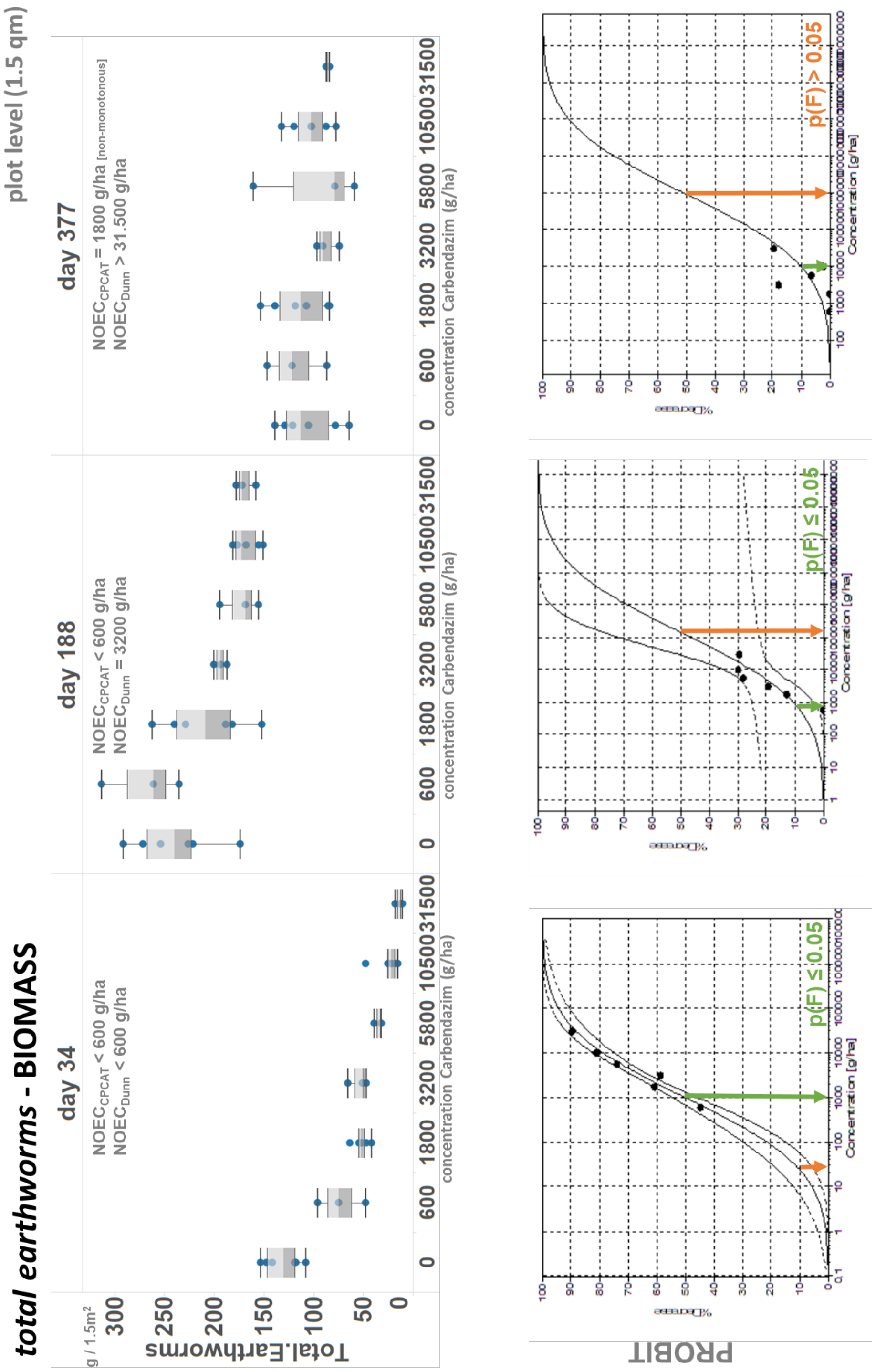
A.2 Pilot study: statistical fact sheets for earthworm species, ecological and taxonomical groups

Figure A2-1: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "total earthworms". Top row shows Box-Whisker-plots of the data distribution on plot level for all treatments and sampling days after application (from left to right 34 daa, 188 daa, 377 daa). The respective NOEC values are presented for CPCAT and Dunnett procedure. Bottom row shows the respective dose-response curves for all sampling days after application using a two-parametric Probit regression. Derived EC_{10} and EC_{50} values are marked by arrows. Green marks indicate determined ECx-values by interpolating between measured concentration level. Orange arrows for values beyond tested concentrations. F-test statistic was calculated for all regression curves, indicating a significant relationship between applied concentrations and measured effect (p≤0.05).



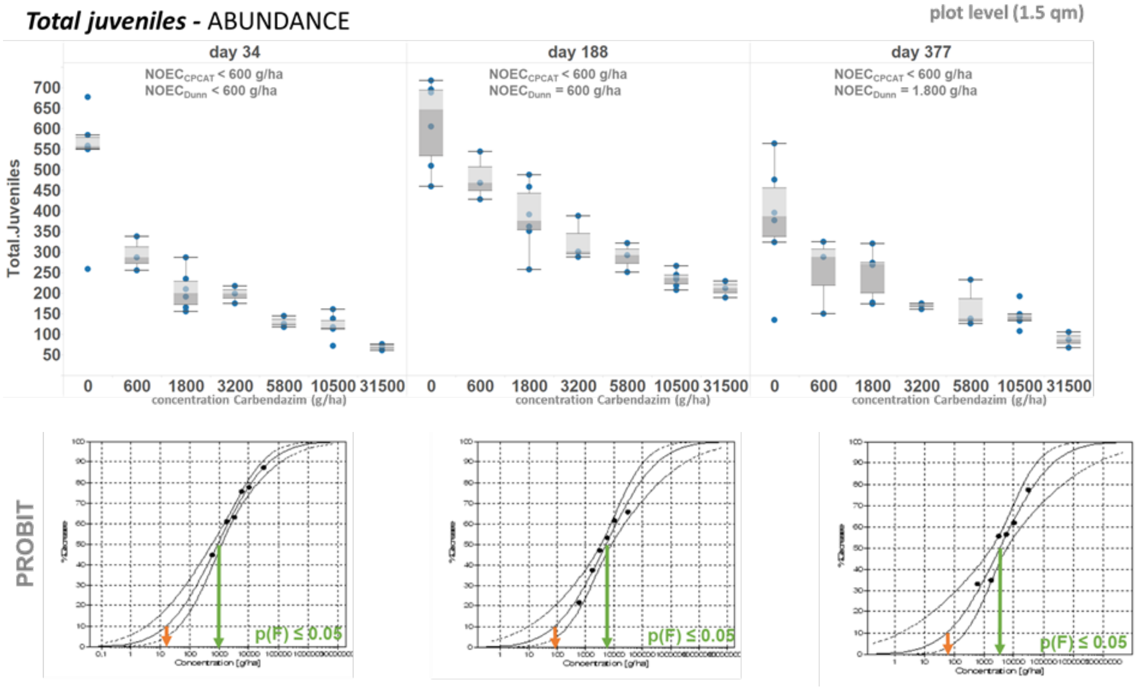
Source: RWTH Aachen University

Figure A2-2: **Earthworm pilot field study: Statistical fact sheet of biomass data for the group "total earthworms".**



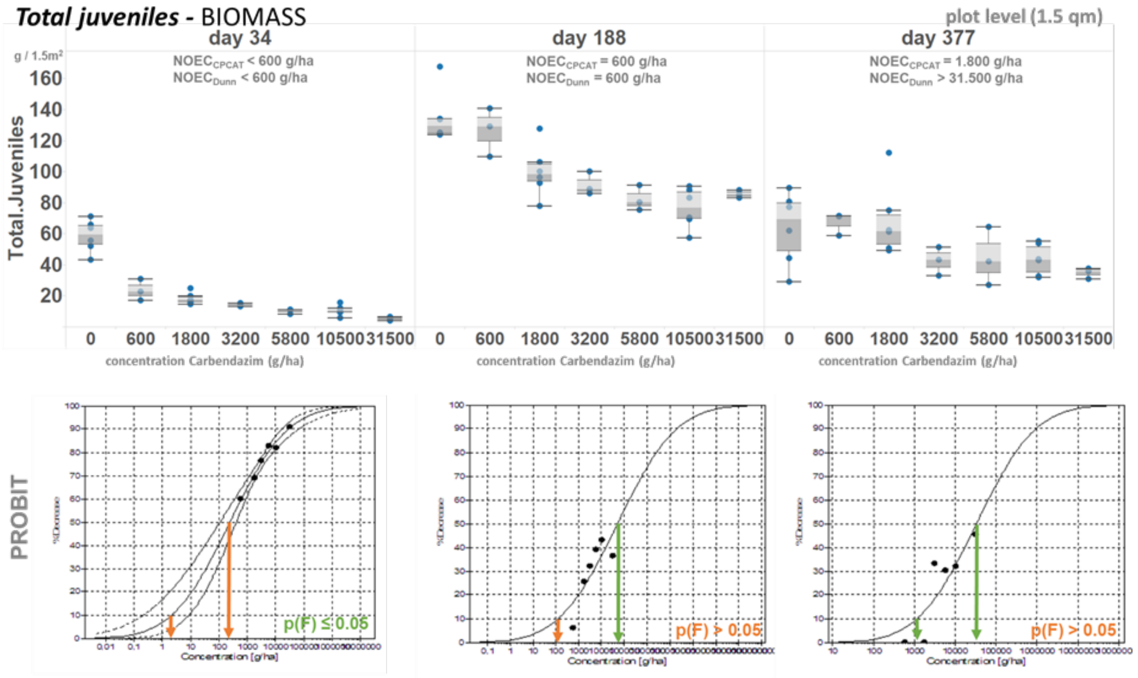
Source: RWTH Aachen University

Figure A2-3: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “total juveniles”.



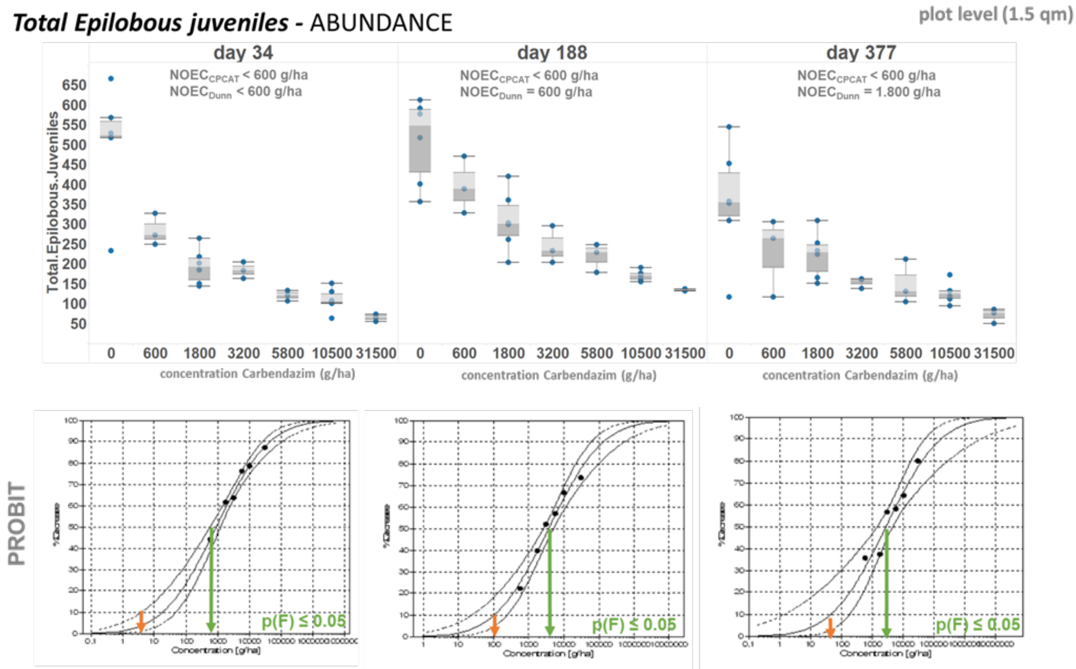
Source: RWTH Aachen University

Figure A2-4: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “total juveniles”.



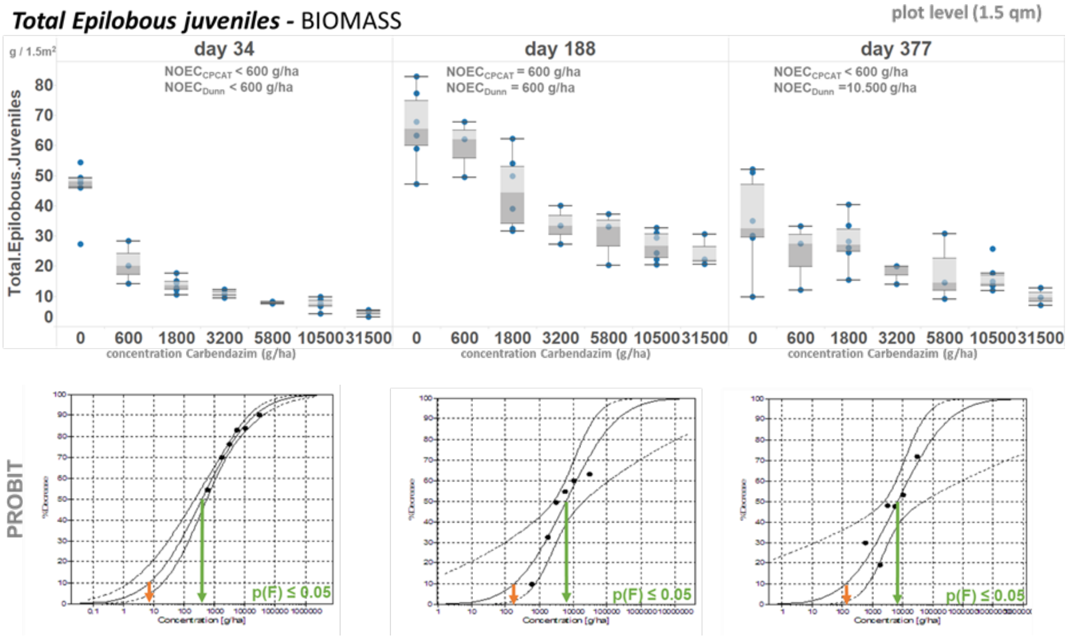
Source: RWTH Aachen University

Figure A2-5: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “total Epilobous juveniles”.



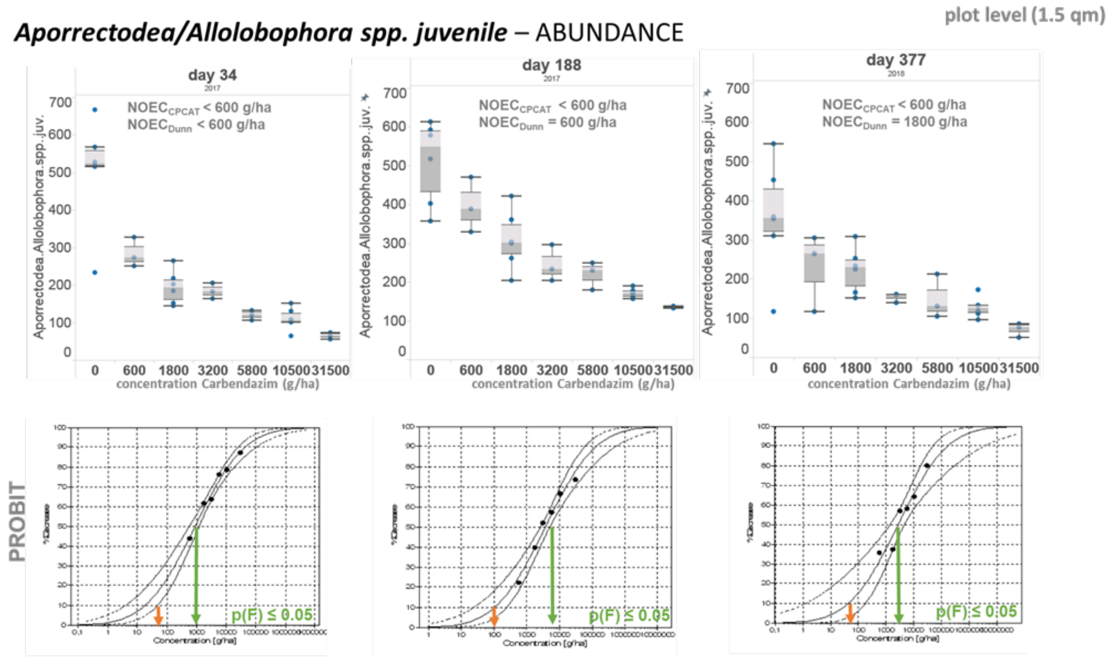
Source: RWTH Aachen University

Figure A2-6: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “total Epilobous juveniles”.



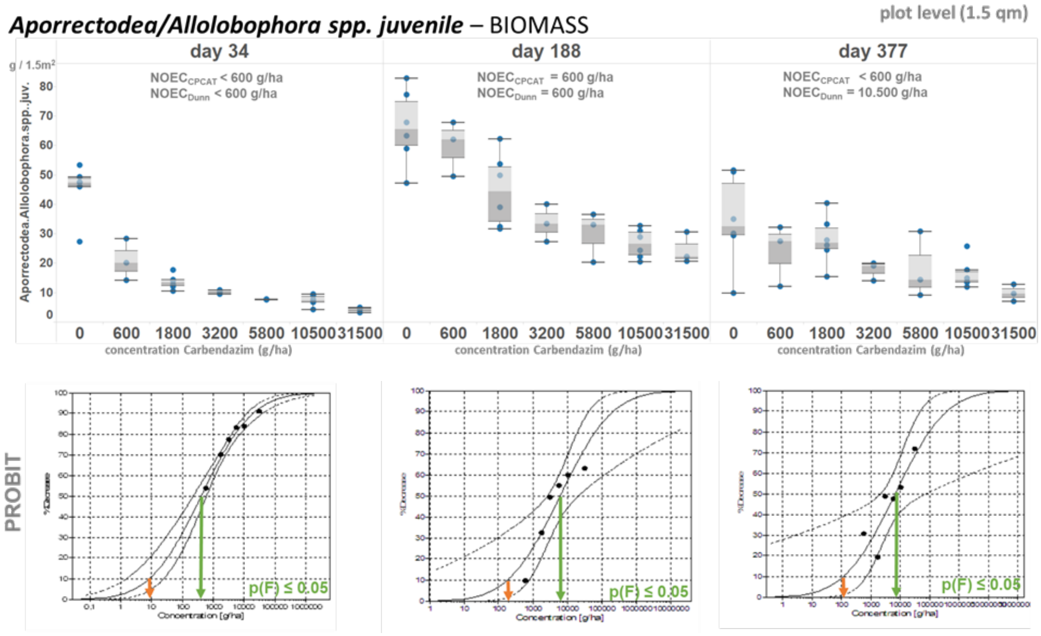
Source: RWTH Aachen University

Figure A2-7: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “Aporrectodea/Allolobophora spp. juvenile”.



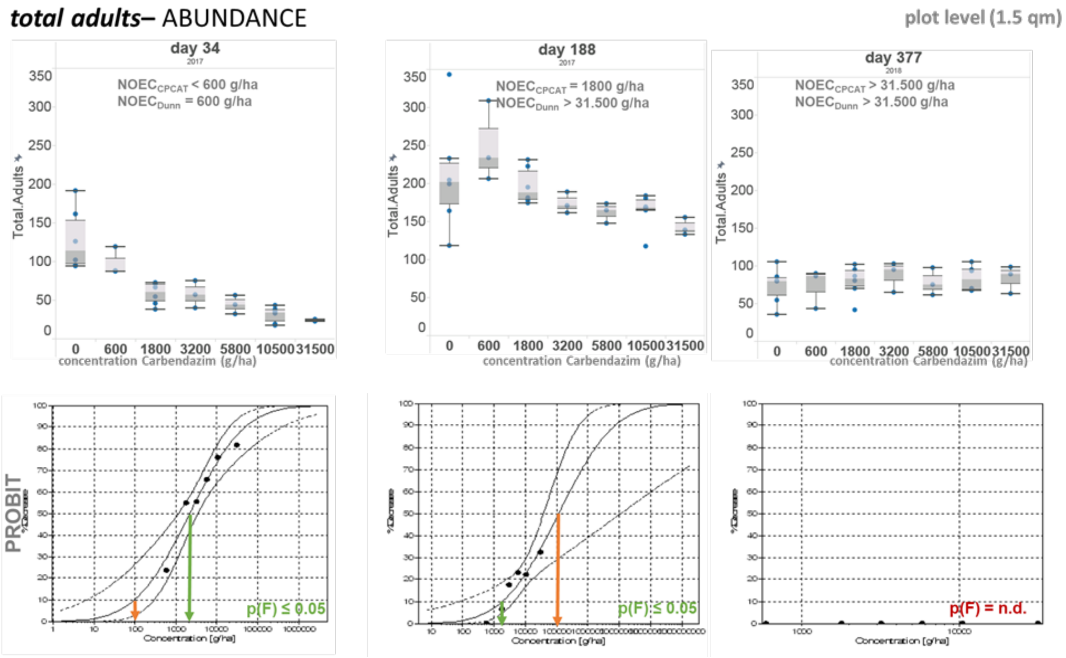
Source: RWTH Aachen University

Figure A2-8: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “Aporrectodea/Allolobophora spp. juvenile”.



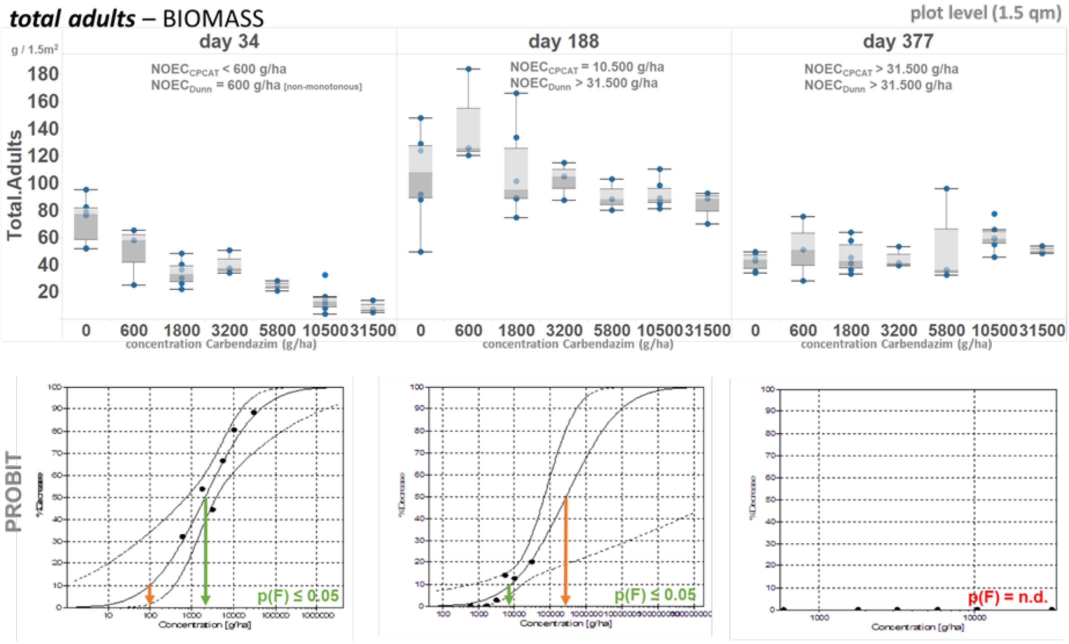
Source: RWTH Aachen University

Figure A2-9: **Earthworm pilot field study: Statistical fact sheet of abundance data for the group “total adults”.**



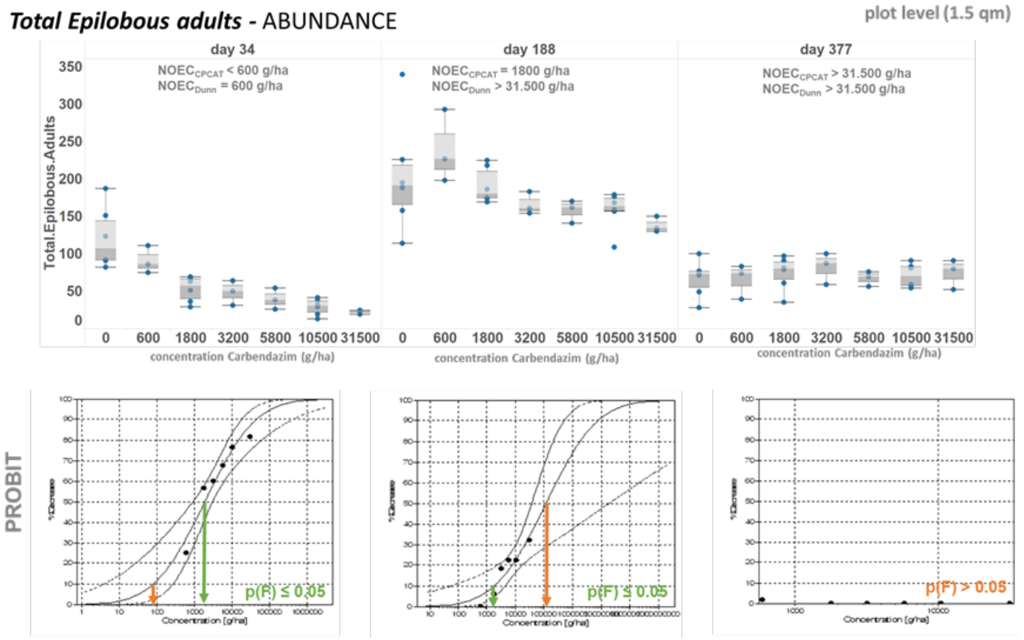
Source: RWTH Aachen University

Figure A2-10: **Earthworm pilot field study: Statistical fact sheet of biomass data for the group “total adults”.**



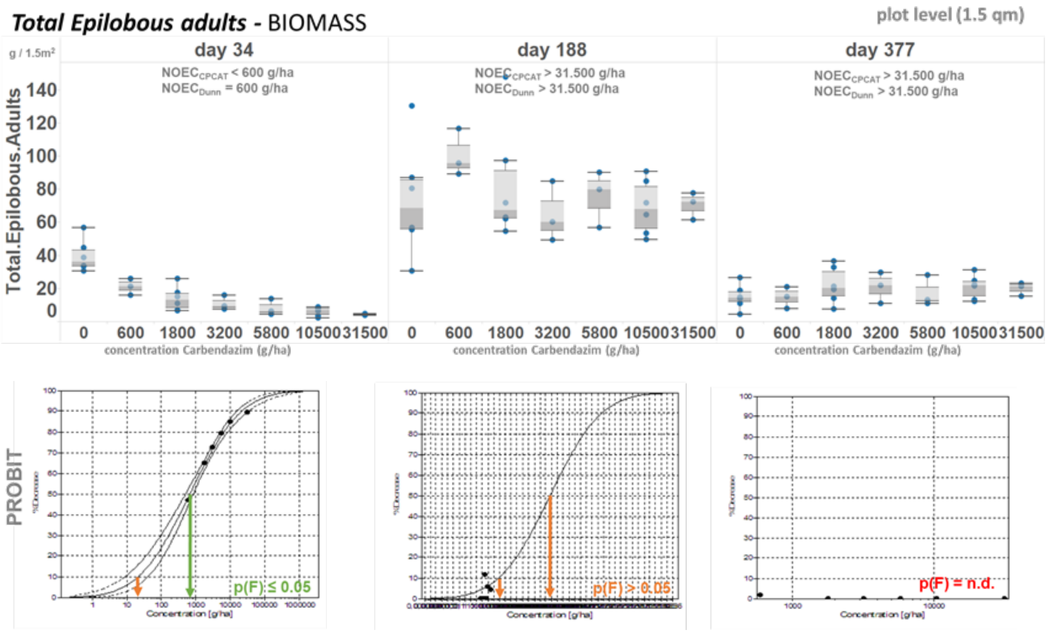
Source: RWTH Aachen University

Figure A2-11: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “total Epilobous adults”.



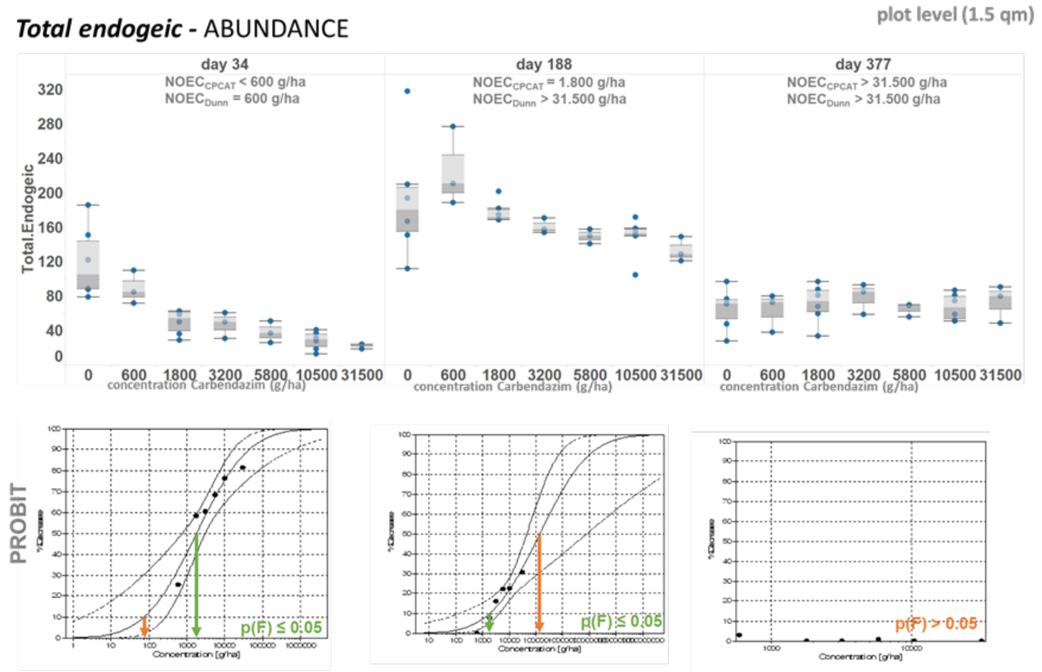
Source: RWTH Aachen University

Figure A2-12: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “total Epilobous adults”.



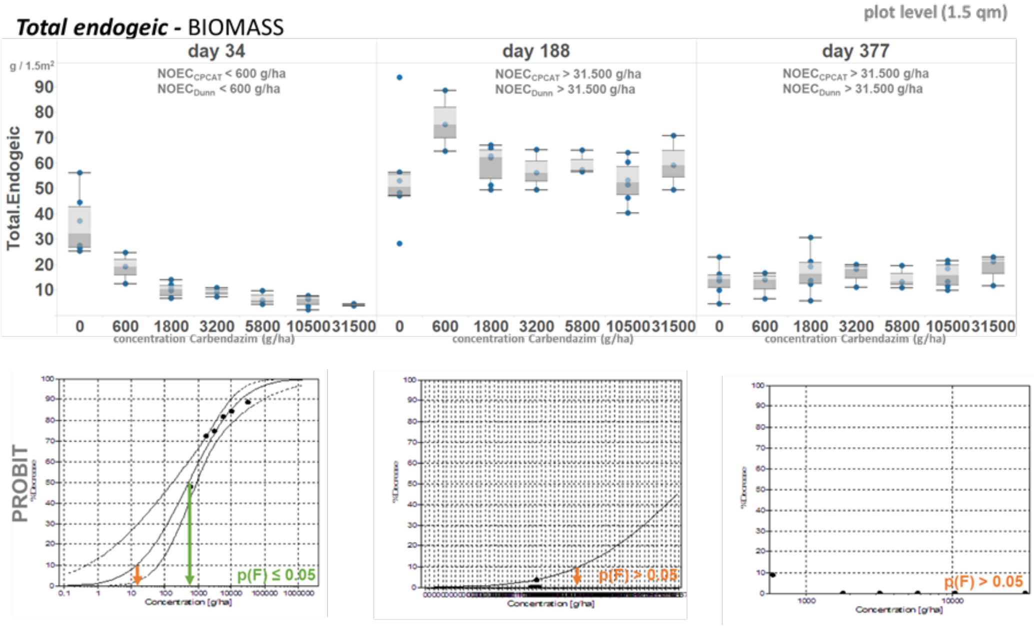
Source: RWTH Aachen University

Figure A2-13: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “total endogeic”.



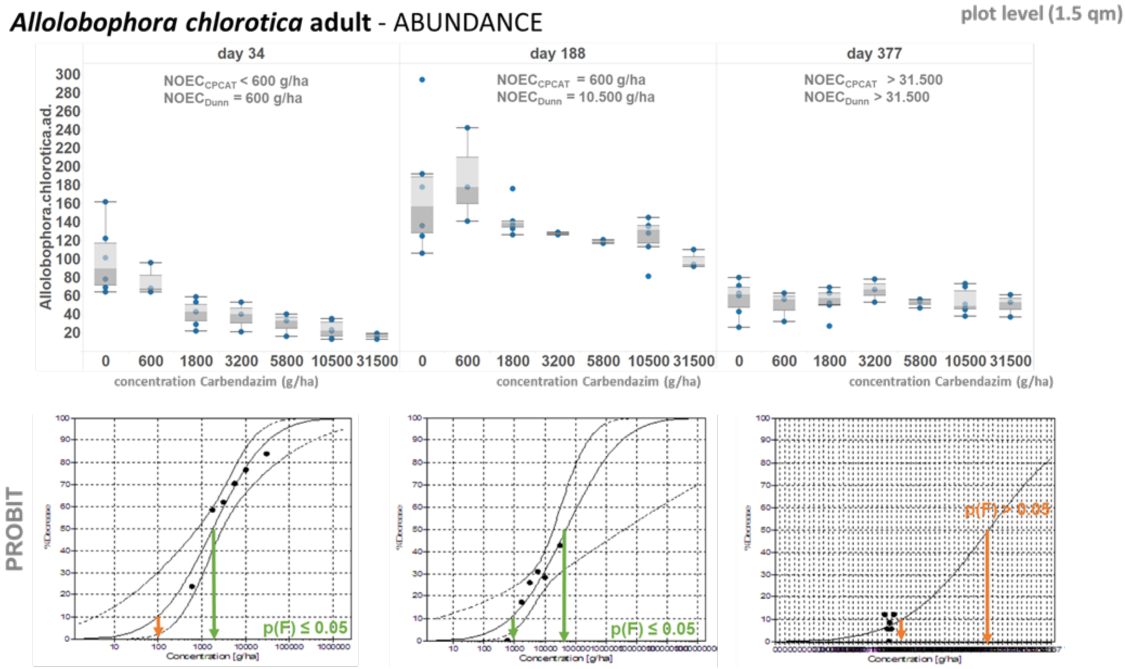
Source: RWTH Aachen University

Figure A2-14: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “total endogeic”.



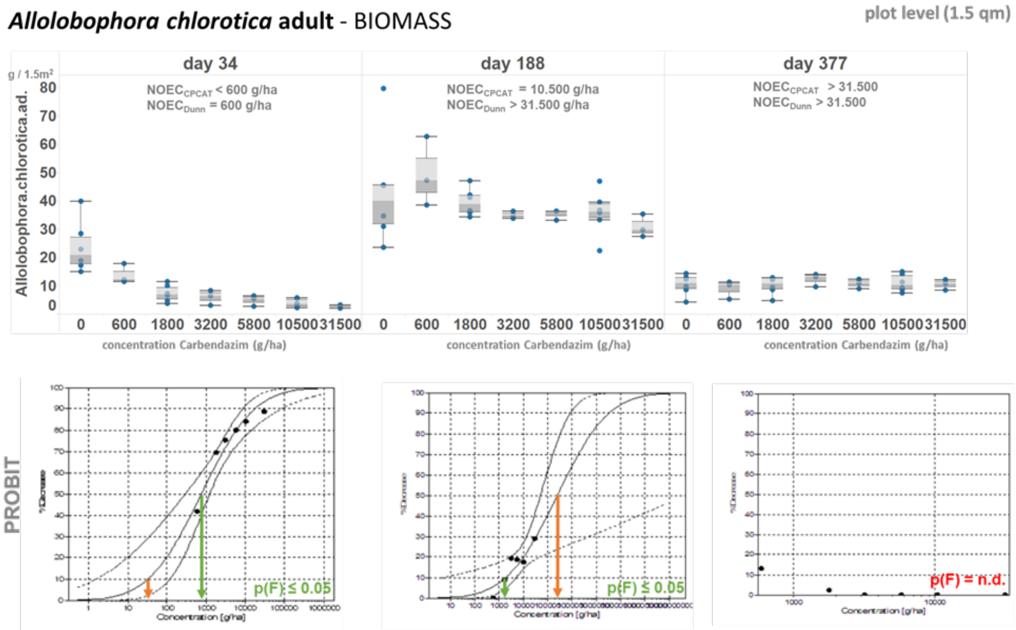
Source: RWTH Aachen University

Figure A2-15: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "*Allolobophora chlorotica* adult".



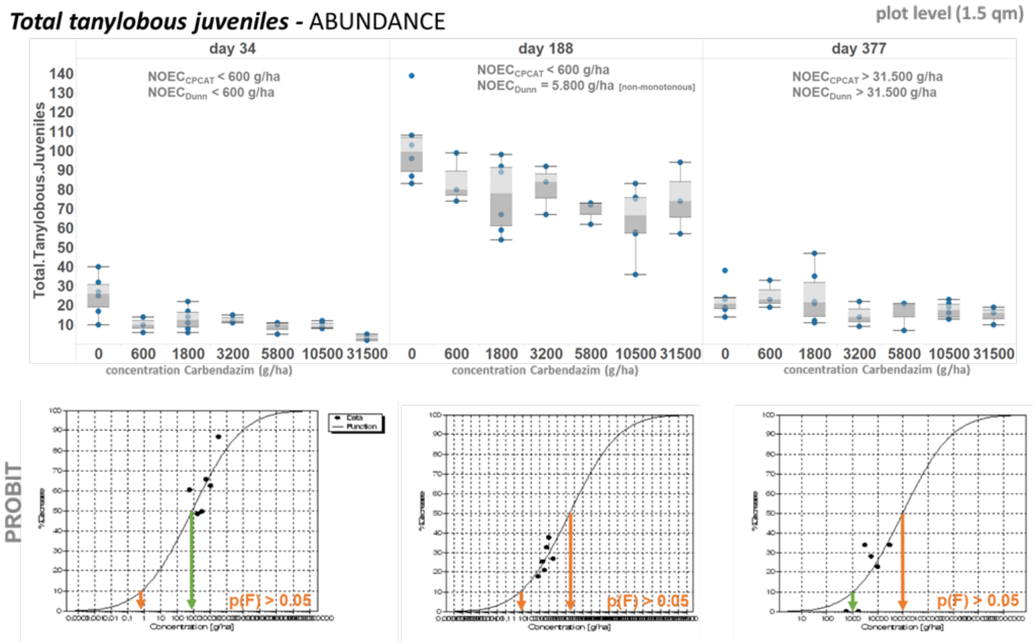
Source: RWTH Aachen University

Figure A2-16: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "*Allolobophora chlorotica* adult".



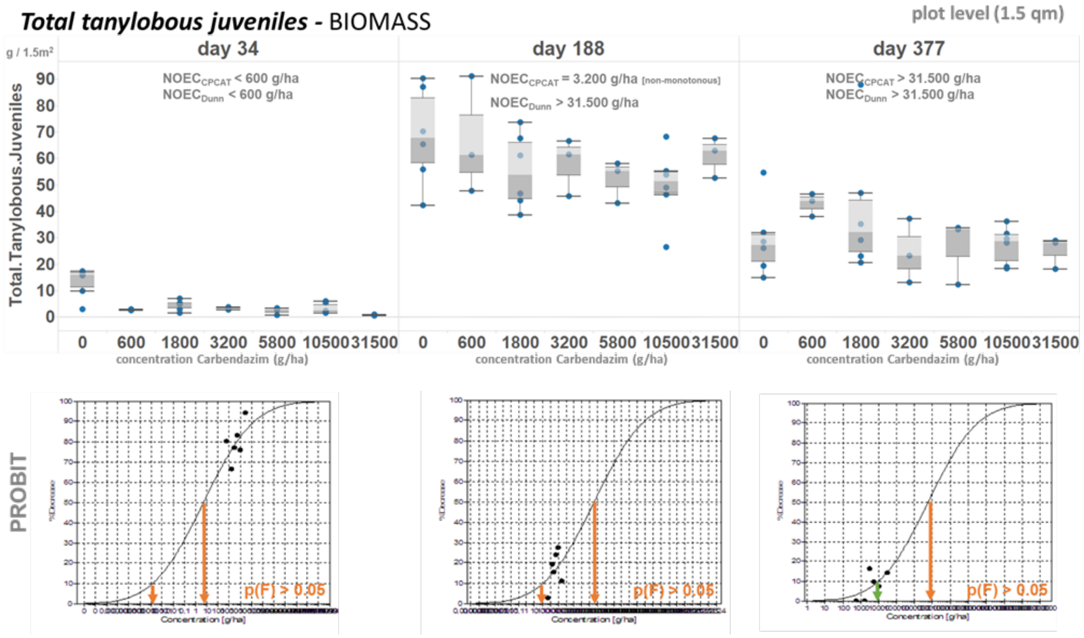
Source: RWTH Aachen University

Figure A2-17: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “total tanylobous juveniles”.



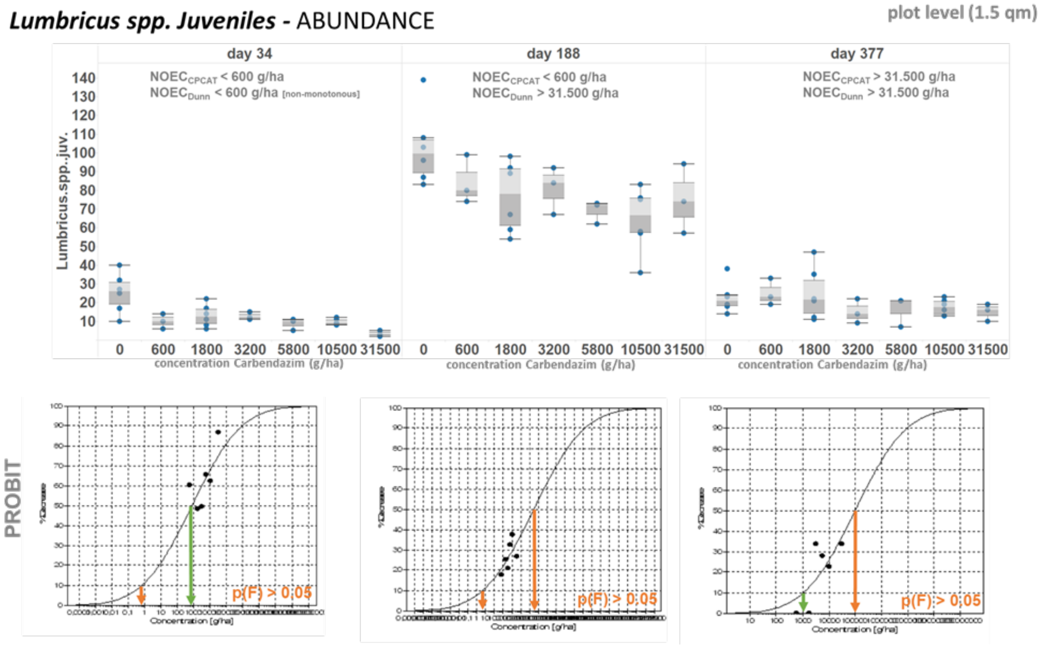
Source: RWTH Aachen University

Figure A2-18: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “total tanylobous juveniles”.



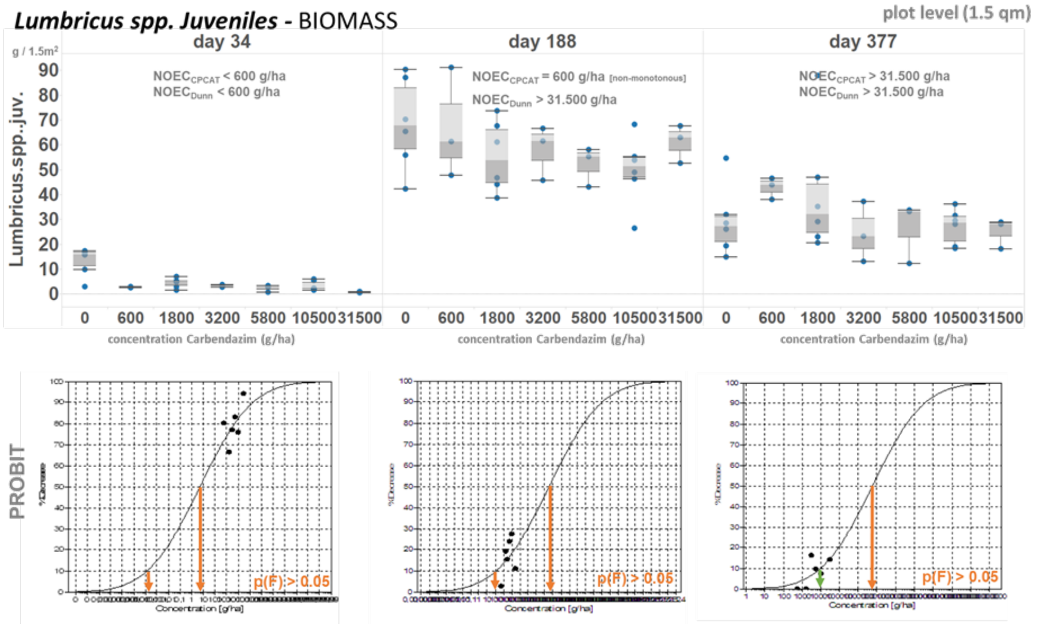
Source: RWTH Aachen University

Figure A2-19: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "*Lumbricus spp.* juveniles".



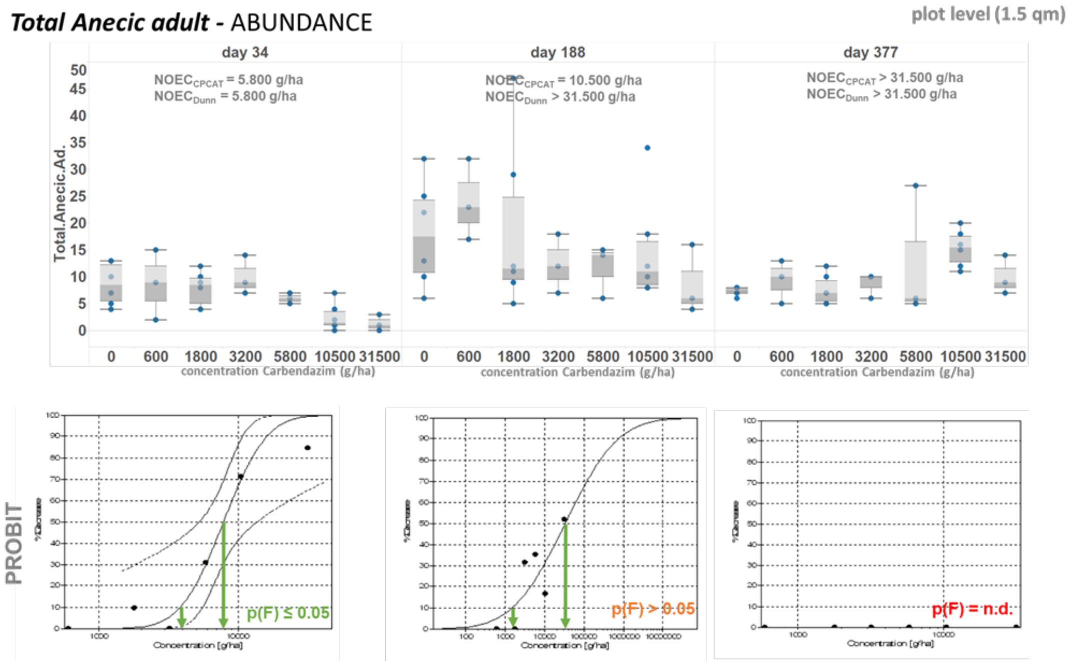
Source: RWTH Aachen University

Figure A2-20: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "*Lumbricus spp.* juveniles".



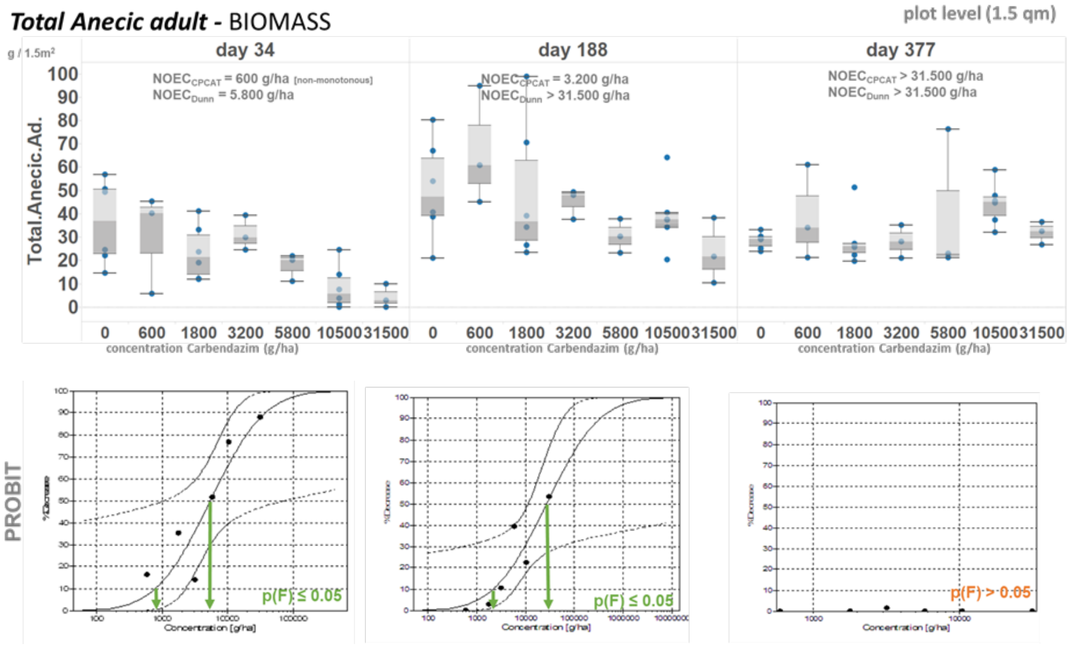
Source: RWTH Aachen University

Figure A2-21: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "total anecic adult".



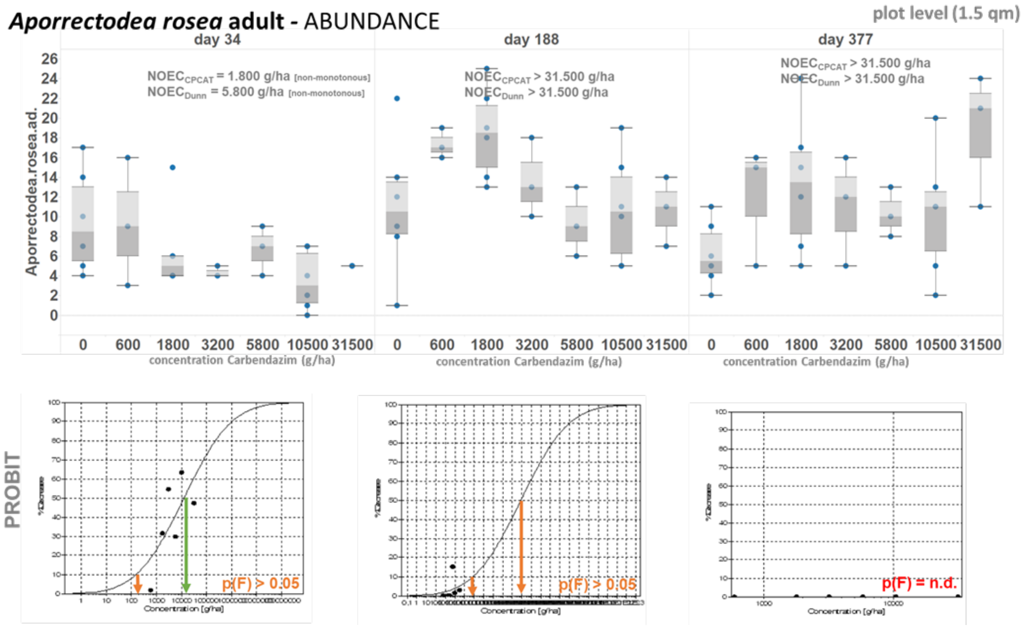
Source: RWTH Aachen University

Figure A2-22: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "total anecic adult".



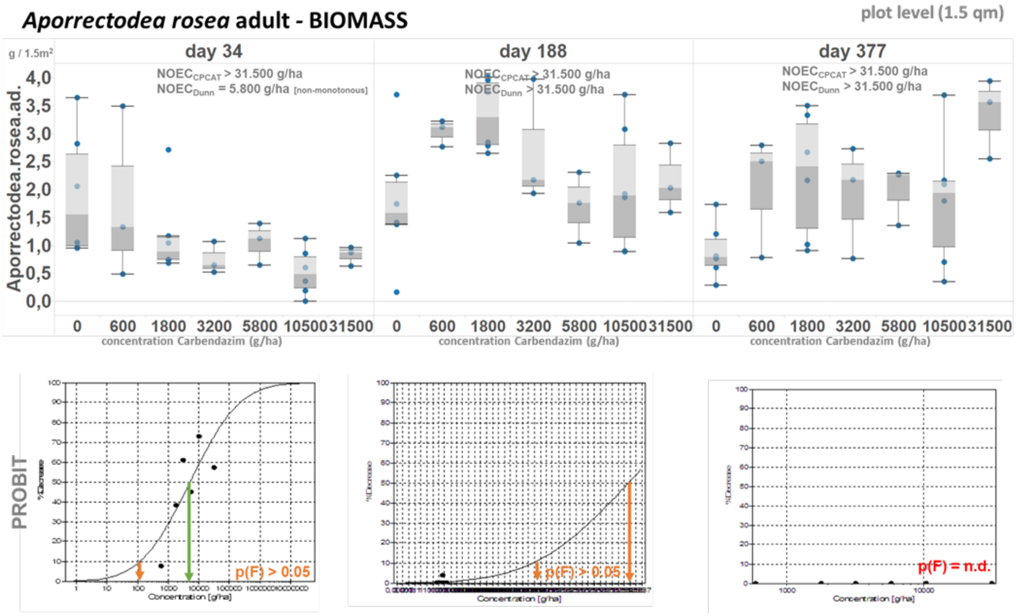
Source: RWTH Aachen University

Figure A2-23: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “*Aporrectodea rosea* adult”.



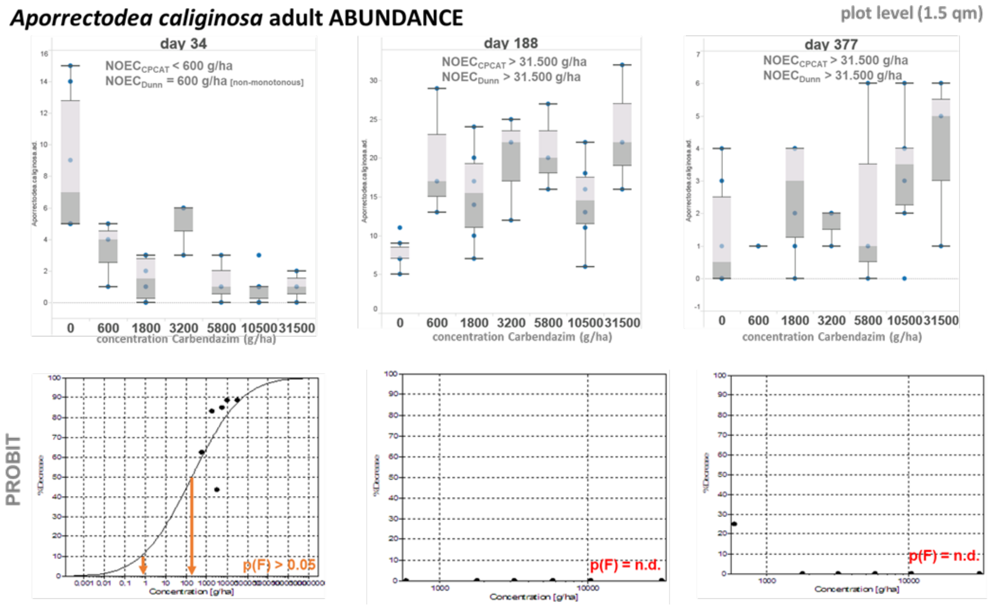
Source: RWTH Aachen University

Figure A2-24: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “*Aporrectodea rosea* adult”.



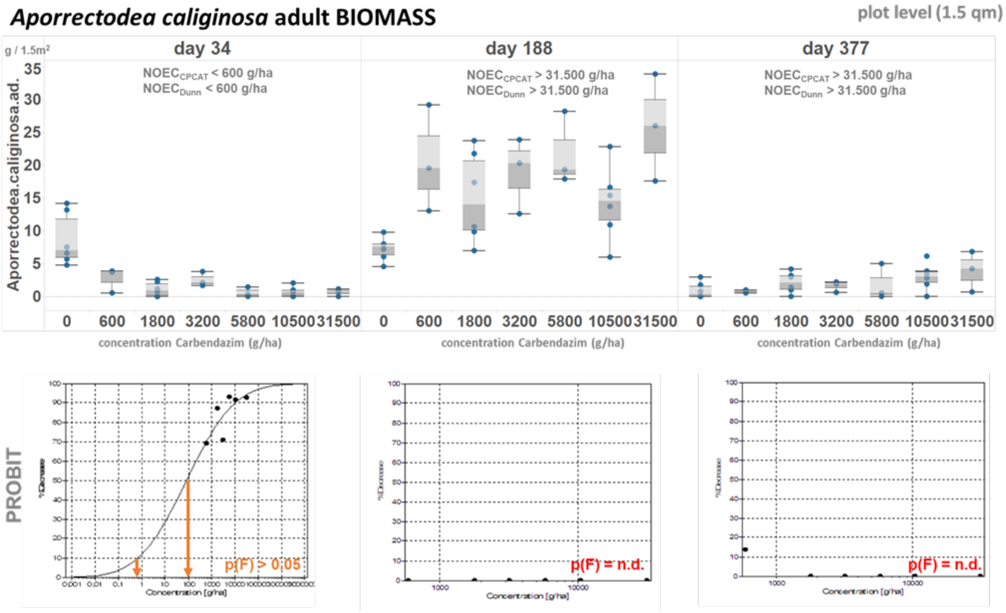
Source: RWTH Aachen University

Figure A2-25: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "*Aporrectodea caliginosa* adult".



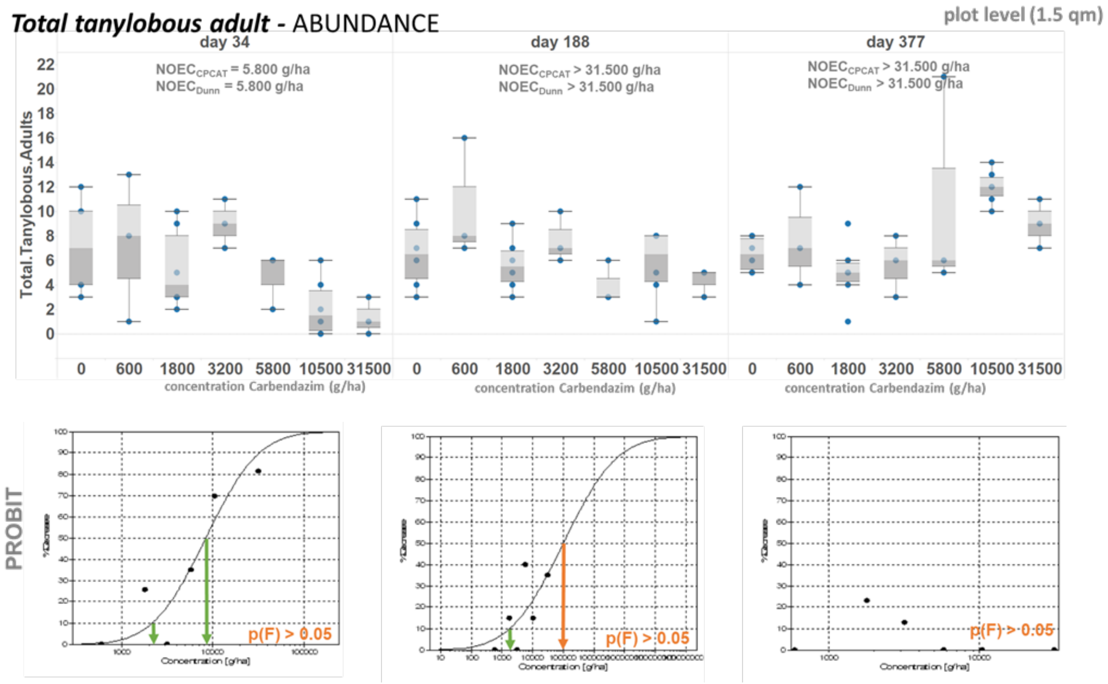
Source: RWTH Aachen University

Figure A2-26: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "*Aporrectodea caliginosa* adult".



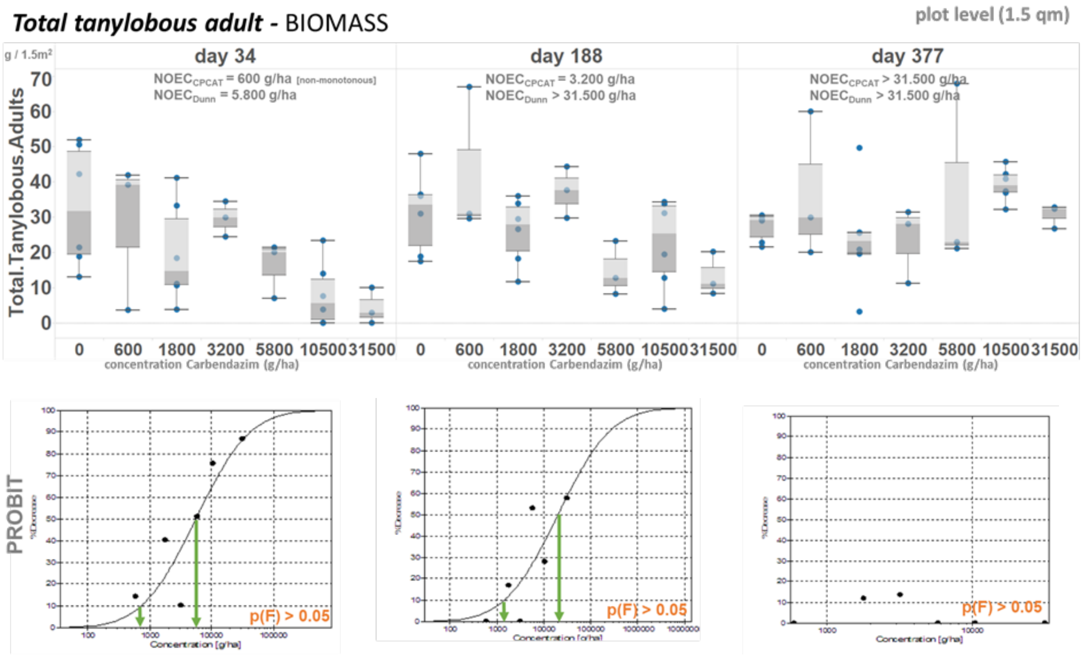
Source: RWTH Aachen University

Figure A2-27: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "total tanylobous adult".



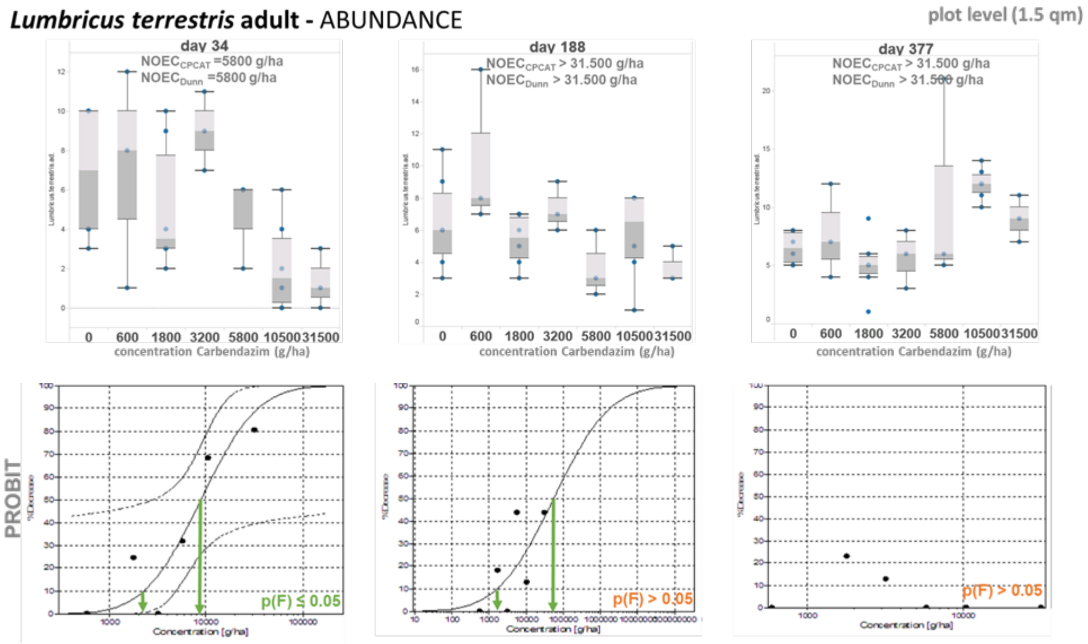
Source: RWTH Aachen University

Figure A2-28: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "total tanylobous adult".



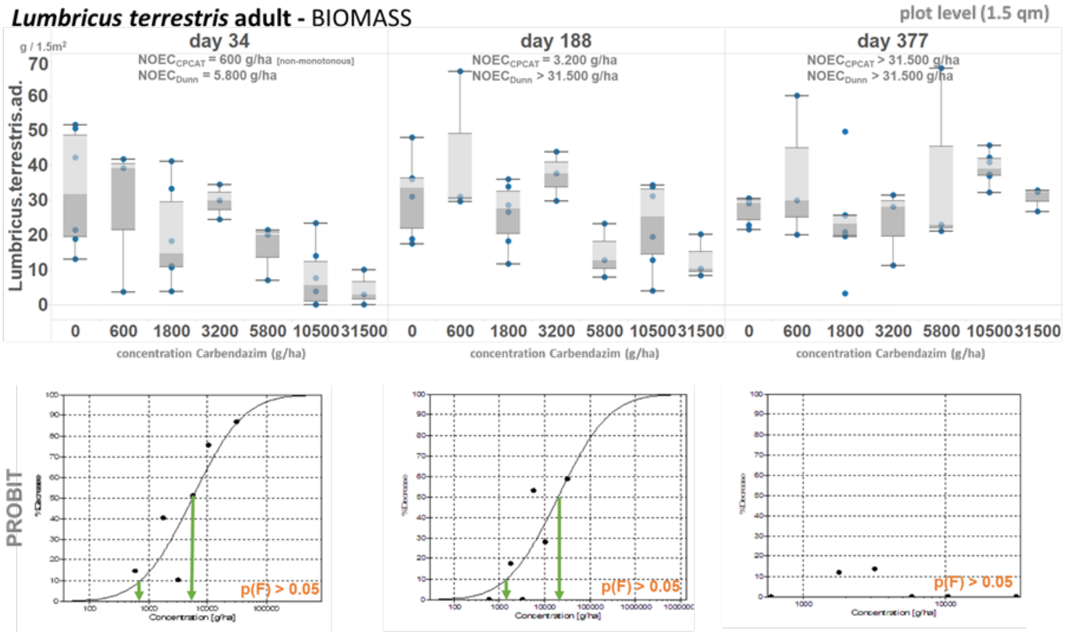
Source: RWTH Aachen University

Figure A2-29: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “*Lumbricus terrestris* adult”.



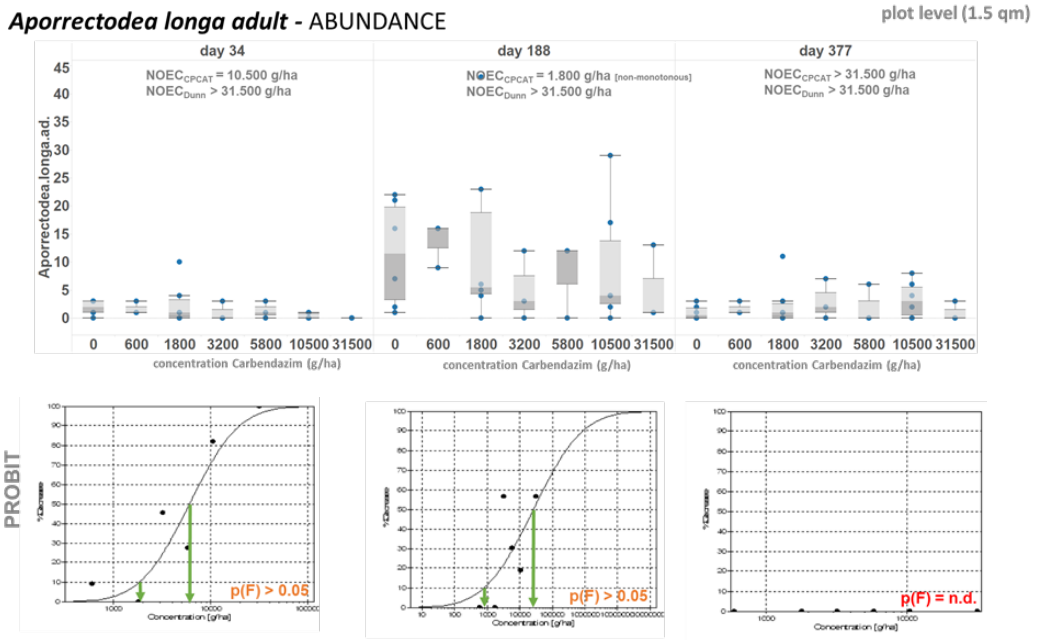
Source: RWTH Aachen University

Figure A2-30: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “*Lumbricus terrestris* adult”.



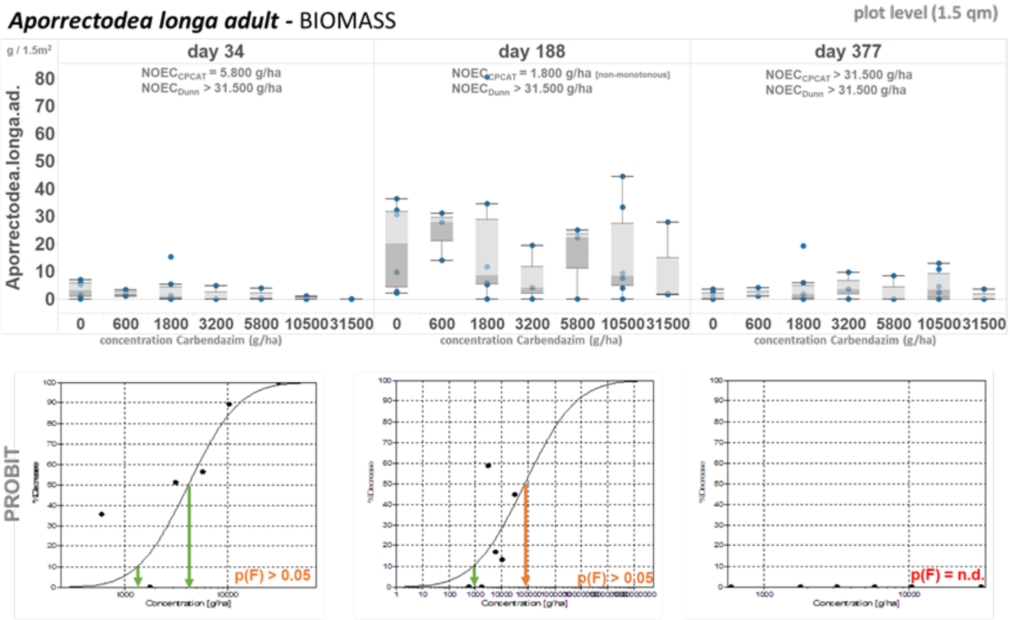
Source: RWTH Aachen University

Figure A2-31: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "*Aporrectodea longa* adult".



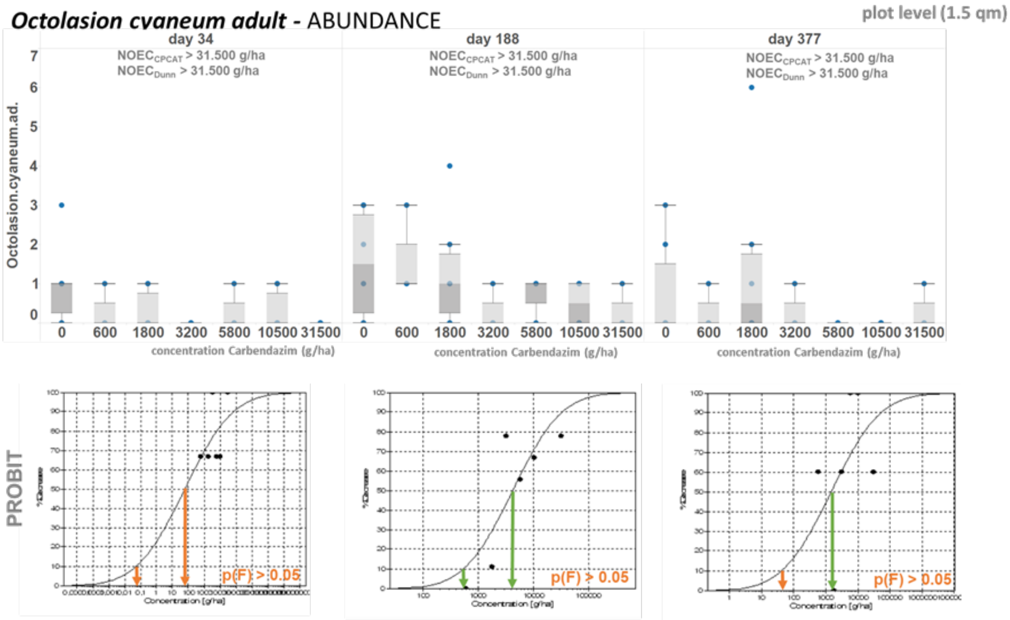
Source: RWTH Aachen University

Figure A2-32: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "*Aporrectodea longa* adult".



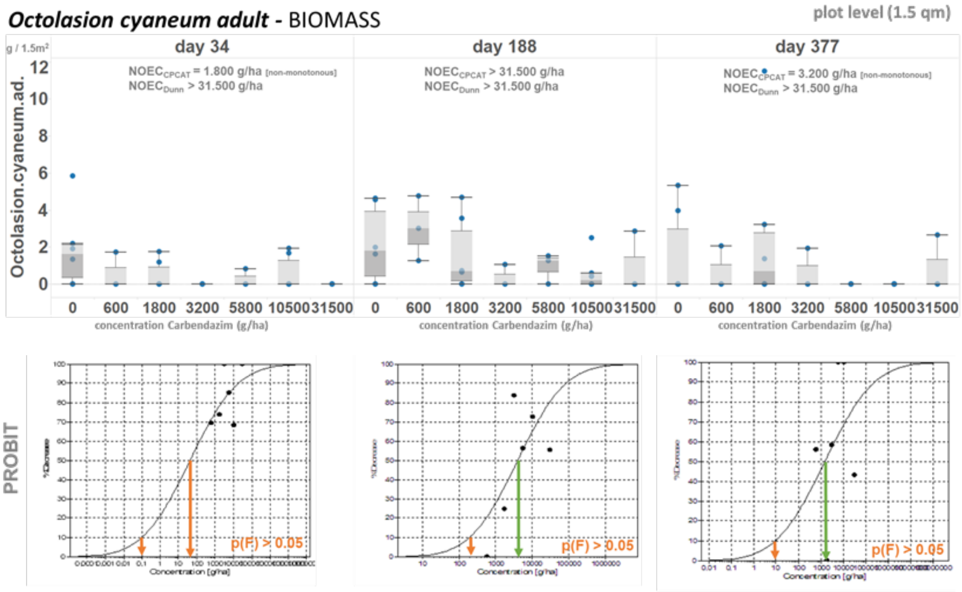
Source: RWTH Aachen University

Figure A2-33: Earthworm pilot field study: Statistical fact sheet of abundance data for the group “*Octolasion cyaneum* adult”.



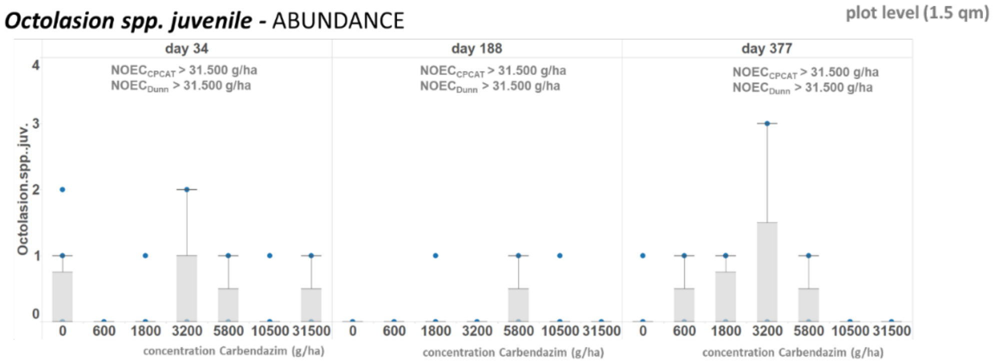
Source: RWTH Aachen University

Figure A2-34: Earthworm pilot field study: Statistical fact sheet of biomass data for the group “*Octolasion cyaneum* adult”.



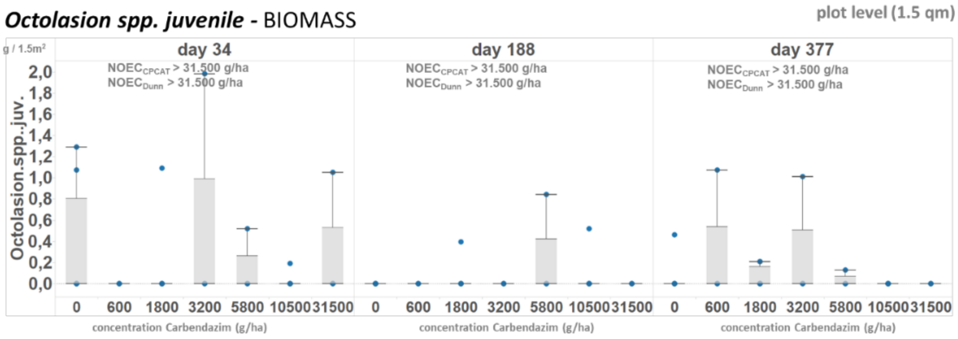
Source: RWTH Aachen University

Figure A2-35: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "*Octolasion spp*. juvenile".



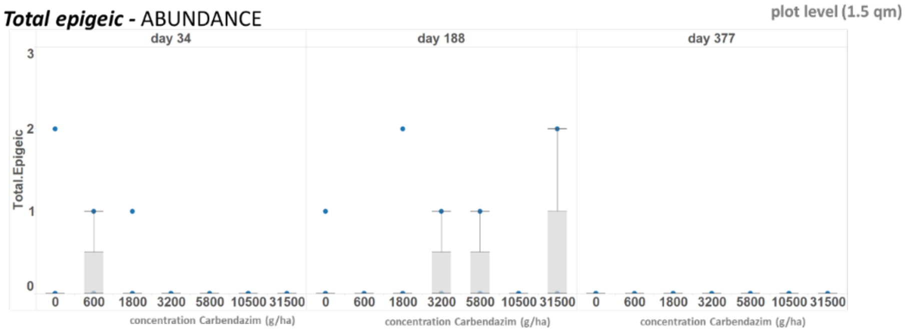
Source: RWTH Aachen University

Figure A2-36: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "*Octolasion spp*. juvenile".



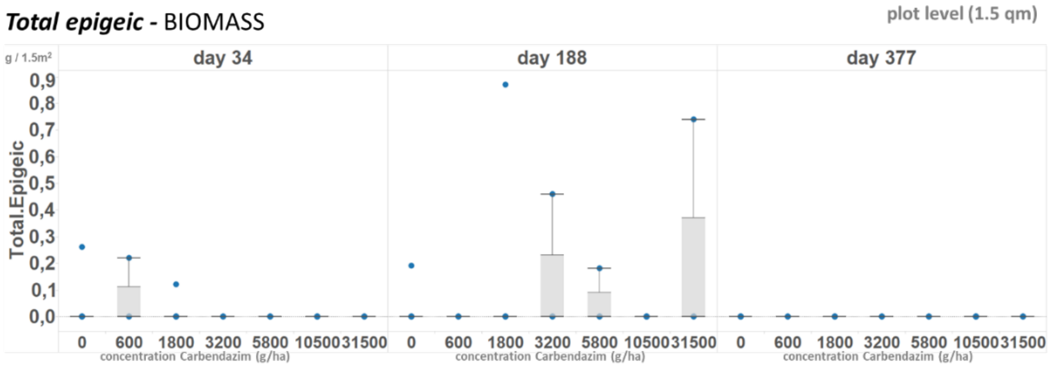
Source: RWTH Aachen University

Figure A2-37: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "*Total epigeic*".



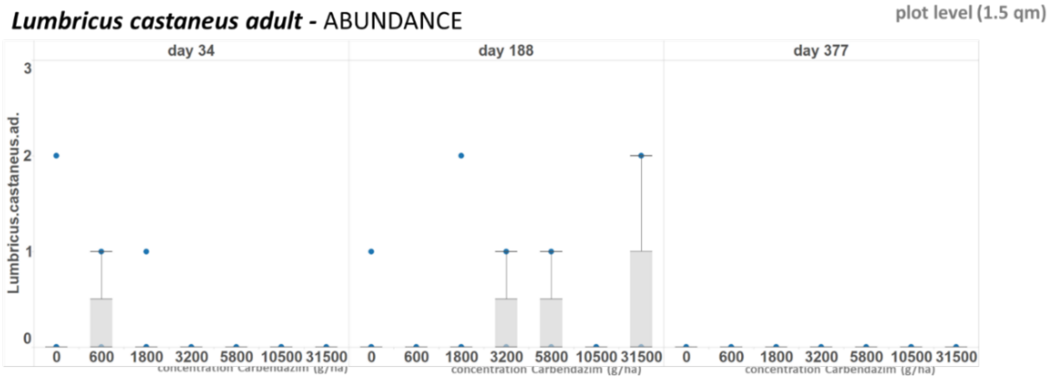
Source: RWTH Aachen University

Figure A2-38: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "*Total epigeic*".



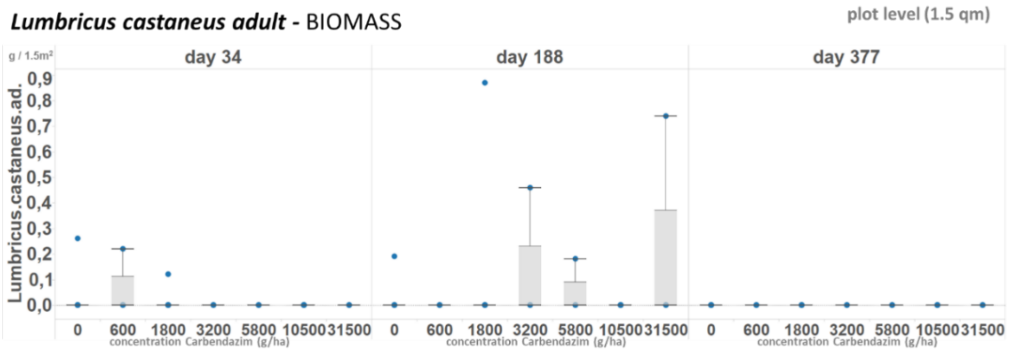
Source: RWTH Aachen University

Figure A2-39: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "*Lumbricus castaneus* adult".



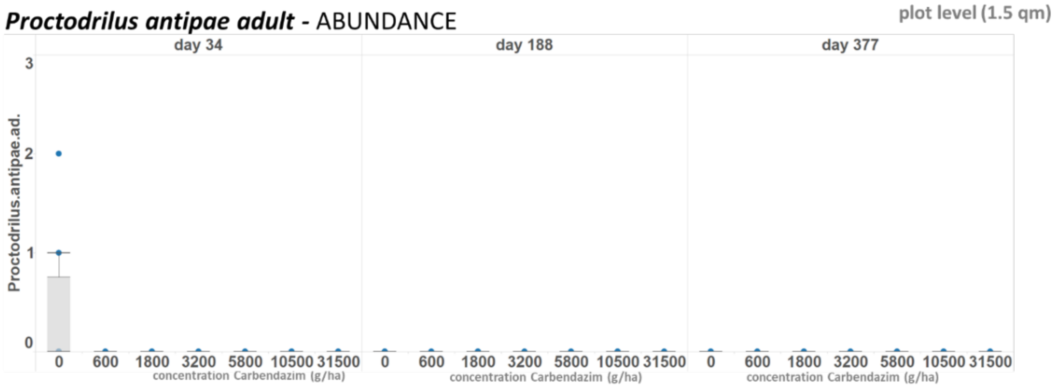
Source: RWTH Aachen University

Figure A2-40: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "*Lumbricus castaneus* adult".



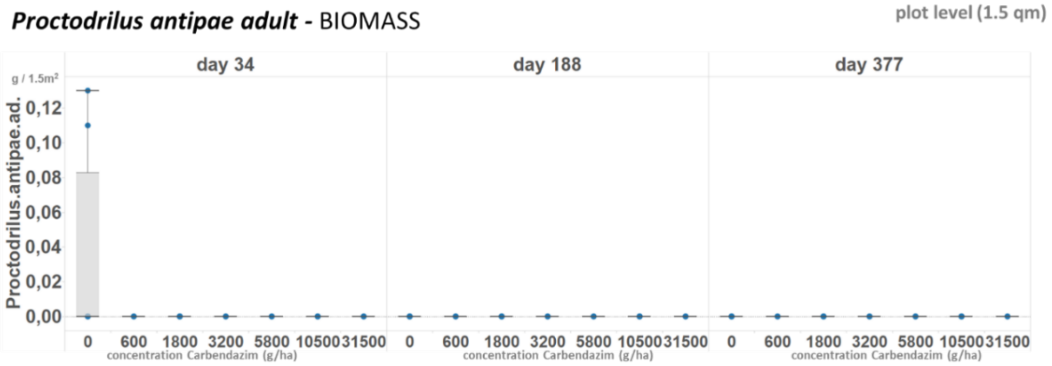
Source: RWTH Aachen University

Figure A2-41: Earthworm pilot field study: Statistical fact sheet of abundance data for the group "*Proctodrilus antipae* adult".



Source: RWTH Aachen University

Figure A2-42: Earthworm pilot field study: Statistical fact sheet of biomass data for the group "*Proctodrilus antipae* adult".



Source: RWTH Aachen University

A.3 Test power: Distribution of Minimum detectable differences (MDD %) and coefficients of variation for control treatments (%)

Table A3-1: Database field studies: Table of mean, median and standard deviation for minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application).

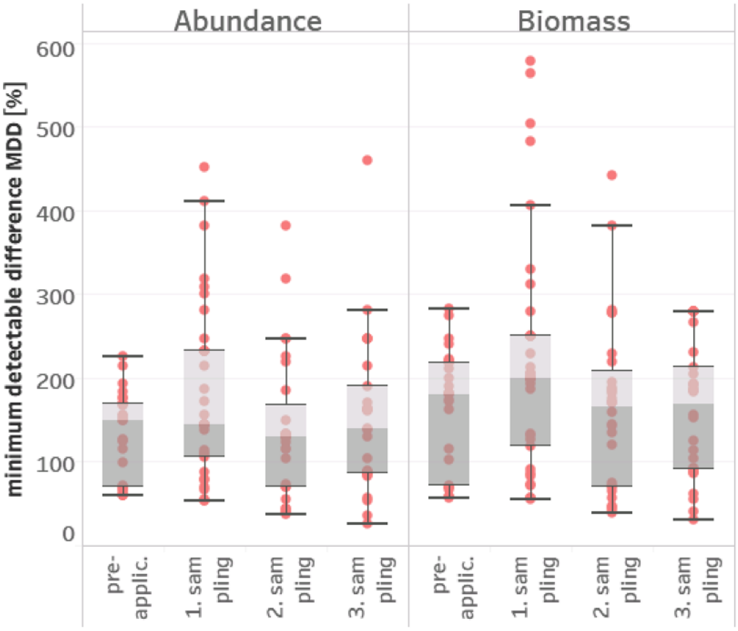
Minimum detectable difference MDD - backtransformed data [%] DATABASE earthworm field studies																								
endpoint measure	**Abundance data - database**												**biomass data - database**											
sampling point	pre- application			~ 1 month after application			~ 6 month after application			~ 12 month after application			pre- application			~ 1 month after application			~ 6 month after application			~ 12 month after application		
	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev
Allolobophora chlorotica adults	132.2	150.0	52.1	181.0	145.5	107.9	136.8	129.4	86.7	150.6	139.4	94.3	160.0	180.6	73.7	221.9	199.6	145.2	164.1	165.5	102.5	160.4	169.8	79.9
Aporrectodea caliginosa adults	59.8	53.8	29.4	89.0	77.0	47.3	68.4	54.7	40.5	76.3	70.9	27.5	64.5	56.2	32.4	97.7	81.2	55.8	71.7	56.0	44.0	82.4	79.0	31.3
Aporrectodea rosea adults	83.3	76.2	31.8	85.6	83.2	31.0	93.6	99.4	32.7	100.0	93.3	45.2	92.6	86.1	43.0	100.6	99.4	40.8	112.7	108.0	48.3	129.7	102.5	78.8
Lumbricus castaneus adults	123.6	115.2	41.4	128.1	121.5	44.6	182.6	140.8	114.1	143.2	124.7	58.9	153.4	142.4	54.8	176.9	171.6	61.1	303.0	159.0	344.0	188.2	169.3	100.7
Lumbricus terrestris adults	115.3	75.3	91.2	99.6	80.3	49.5	103.9	80.6	61.8	89.6	80.2	43.6	102.8	70.6	70.2	94.9	87.1	40.6	96.1	80.5	48.3	85.0	76.2	37.8
total adults	46.2	45.5	15.8	56.9	54.1	19.0	48.3	47.6	16.1	55.5	53.6	20.5	54.7	51.3	18.4	57.3	51.6	17.7	52.1	47.5	20.2	59.4	56.3	22.0
total anecic adults	102.1	67.7	90.3	94.9	68.0	49.8	102.0	79.3	62.7	85.1	76.6	44.1	95.1	68.2	67.0	91.3	76.6	41.2	94.2	78.8	49.2	82.6	76.9	39.0
total earthworms	44.2	41.4	17.2	47.2	44.4	16.8	43.2	40.4	16.2	42.9	39.4	17.1	48.2	47.8	17.4	50.3	49.0	20.5	44.7	44.9	18.7	47.5	47.8	16.5
total endogeic	51.1	49.0	18.6	68.4	64.6	30.1	58.7	49.2	32.7	64.7	58.5	26.8	54.9	52.7	19.3	76.7	68.1	42.5	64.0	55.3	38.5	68.4	62.0	28.1
total epigeic	115.4	112.4	43.8	111.3	100.4	43.4	174.1	140.8	119.3	145.8	118.9	72.0	134.9	126.3	56.8	149.6	145.4	67.9	286.6	151.1	345.4	183.3	140.8	136.2

Minimum detectable difference MDD - backtransformed data [%] DATABASE earthworm field studies

total epilobous adults	50.1	48.4	17.8	66.8	63.9	30.1	57.1	48.0	32.2	62.0	56.2	25.5	57.9	54.2	24.2	76.0	68.1	42.1	62.2	52.2	38.6	67.1	61.9	30.5
total epilobous juveniles	56.9	51.1	24.1	56.0	52.3	19.6	55.8	52.6	19.5	52.2	47.3	22.9	58.5	50.0	31.6	66.9	65.5	26.8	61.6	60.1	25.2	56.9	52.3	31.6
total juveniles	51.4	47.3	21.8	52.2	47.8	18.6	48.6	45.6	17.1	46.1	42.4	18.0	58.3	56.9	22.4	63.0	58.2	22.5	53.9	54.5	22.5	51.6	47.7	20.5
total tanylobous adults	72.4	66.6	25.2	82.7	76.9	33.4	90.7	72.0	53.5	76.8	72.5	28.6	75.7	68.0	27.4	81.7	78.0	30.7	91.9	80.6	47.6	76.1	72.8	27.9
total tanylobous juveniles	71.1	62.4	31.1	72.3	67.1	28.7	58.8	52.0	21.7	65.4	64.4	22.6	80.9	68.7	32.2	85.1	82.9	30.4	68.3	63.1	27.7	73.5	71.6	31.8

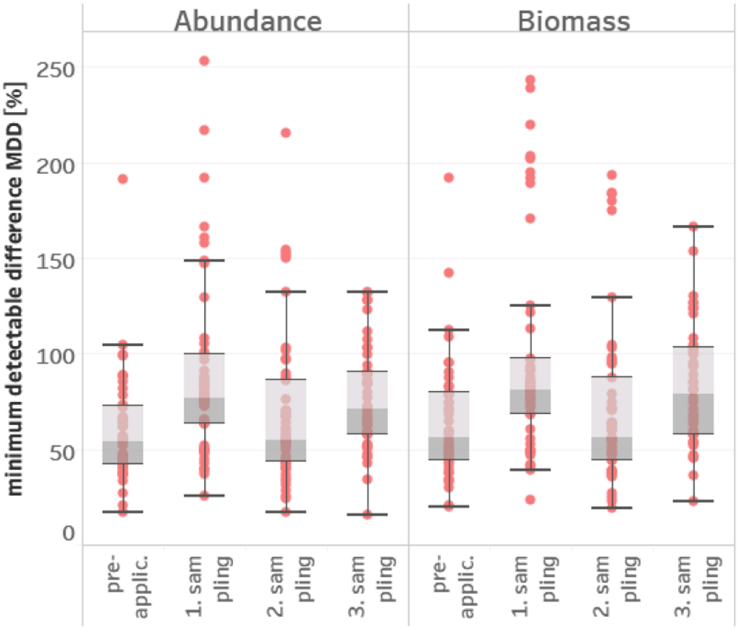
Figure A3-1: Database field studies: Boxplots of minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application).

***Allolobophora chlorotica* adults**



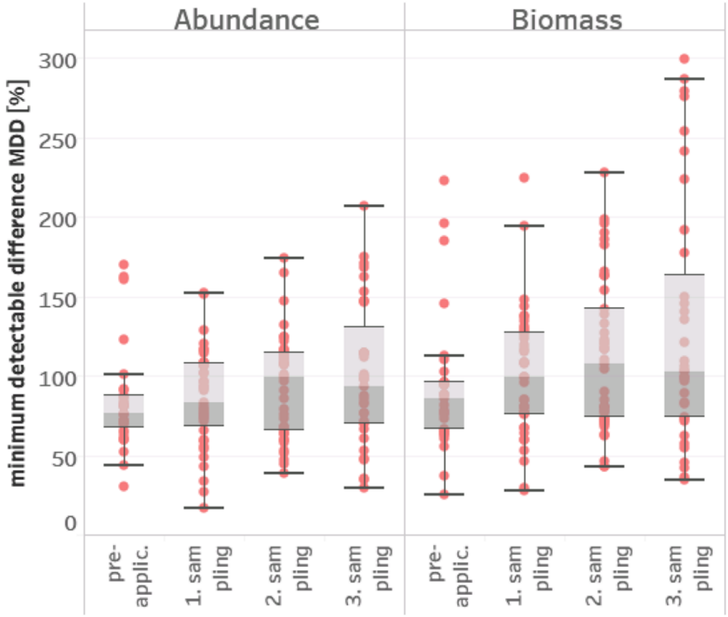
Source: RWTH Aachen University

***Aporrectodea caliginosa* adults**



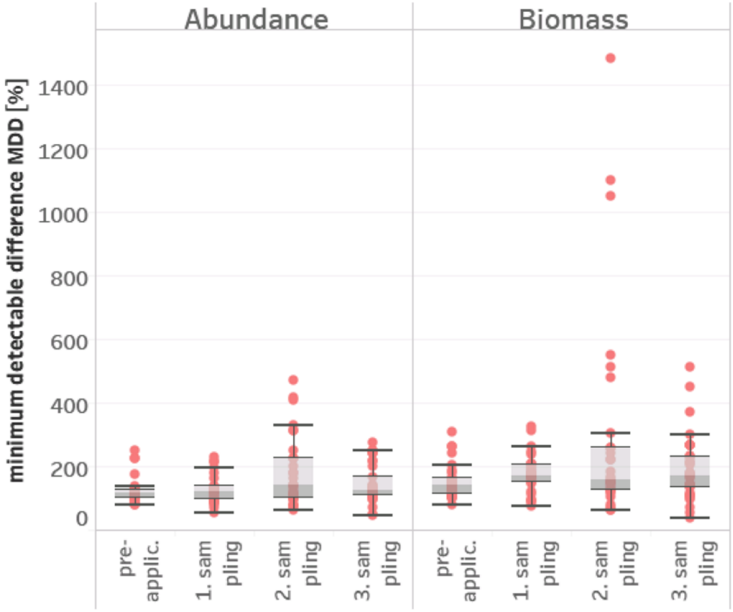
Source: RWTH Aachen University

Aporrectodea rosea **adults**



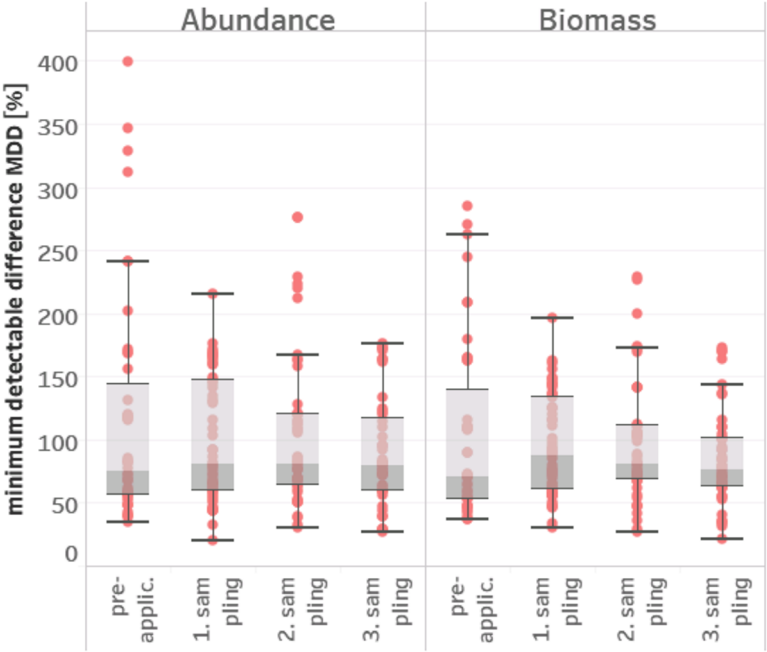
Source: RWTH Aachen University

Lumbricus castaneus **adults**



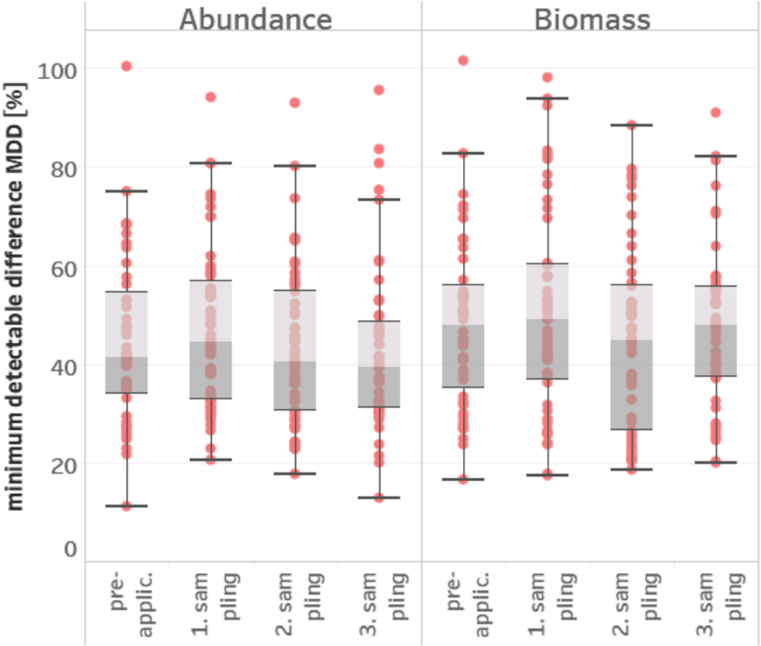
Source: RWTH Aachen University

Lumbricus terrestris **adults**



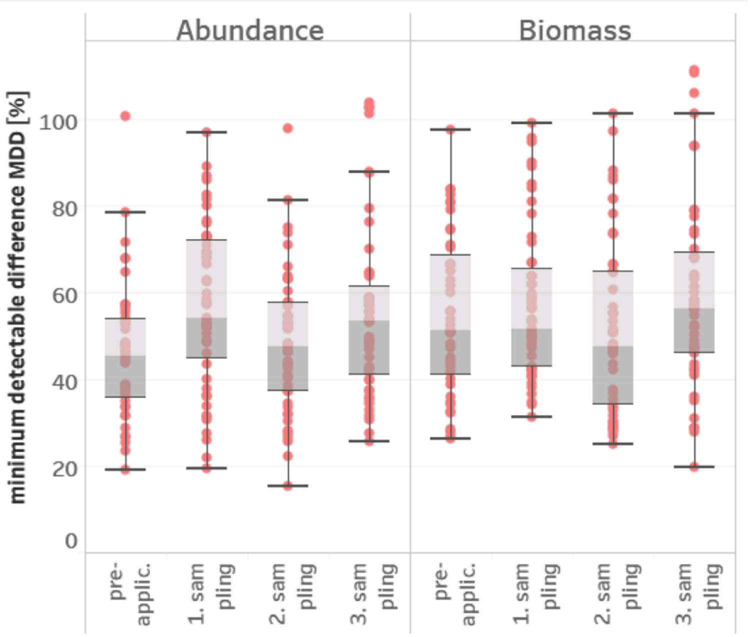
Source: RWTH Aachen University

total earthworms



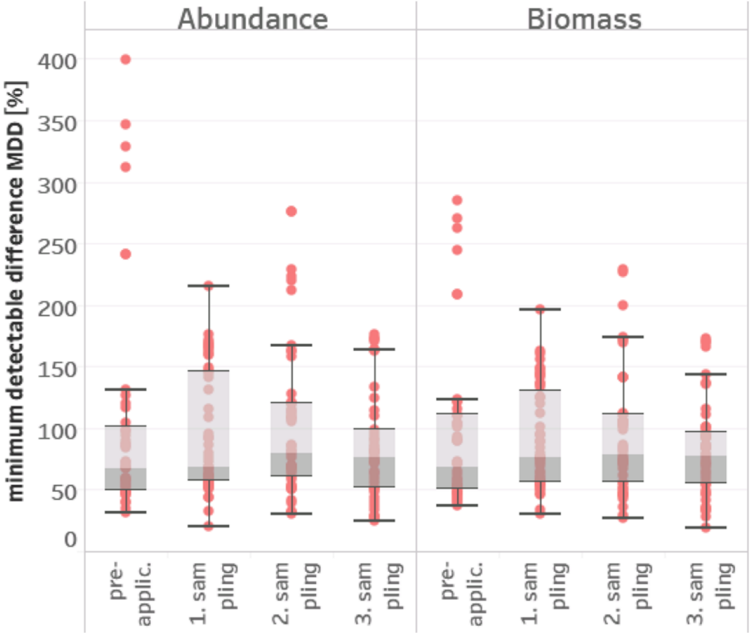
Source: RWTH Aachen University

total adults



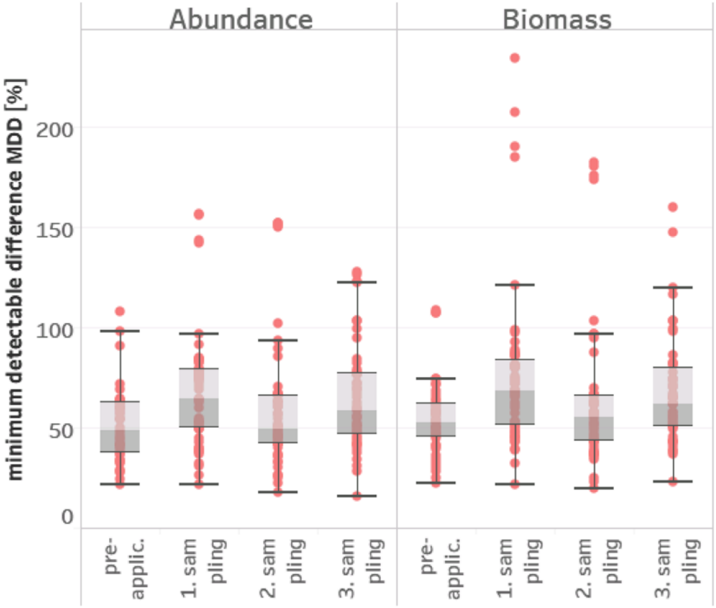
Source: RWTH Aachen University

total anecic adults



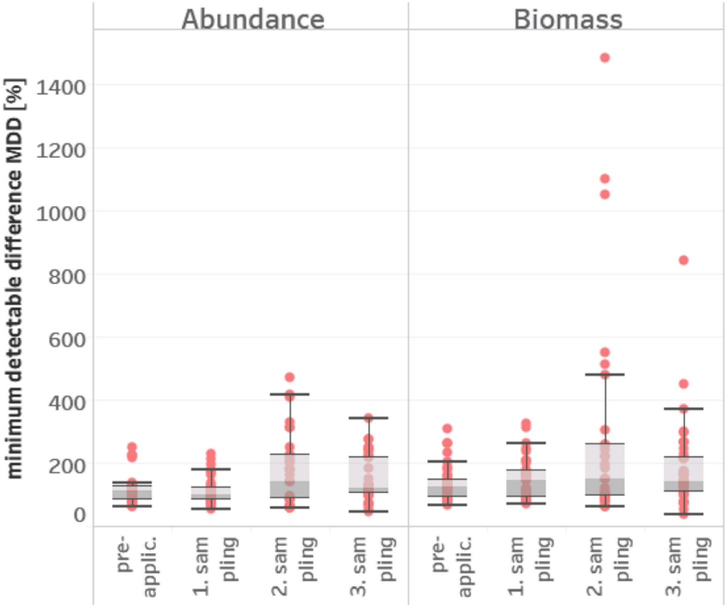
Source: RWTH Aachen University

total endogeic



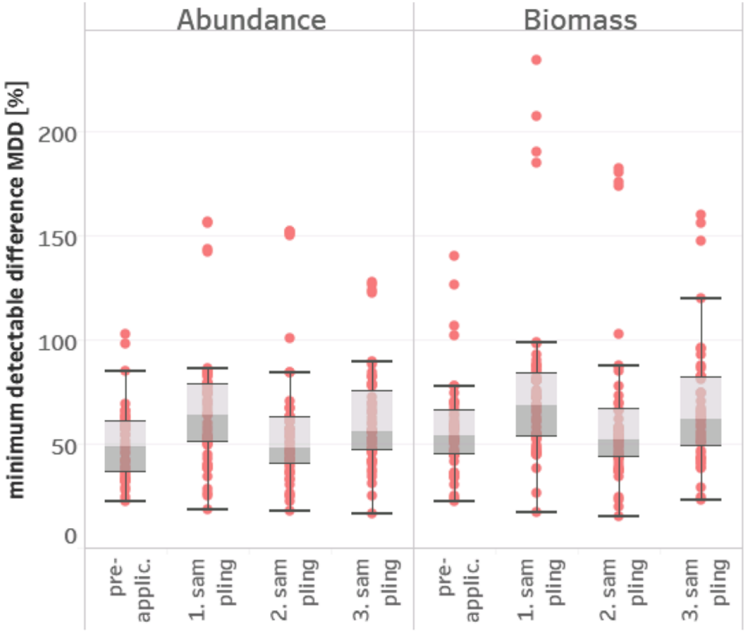
Source: RWTH Aachen University

total epigeic



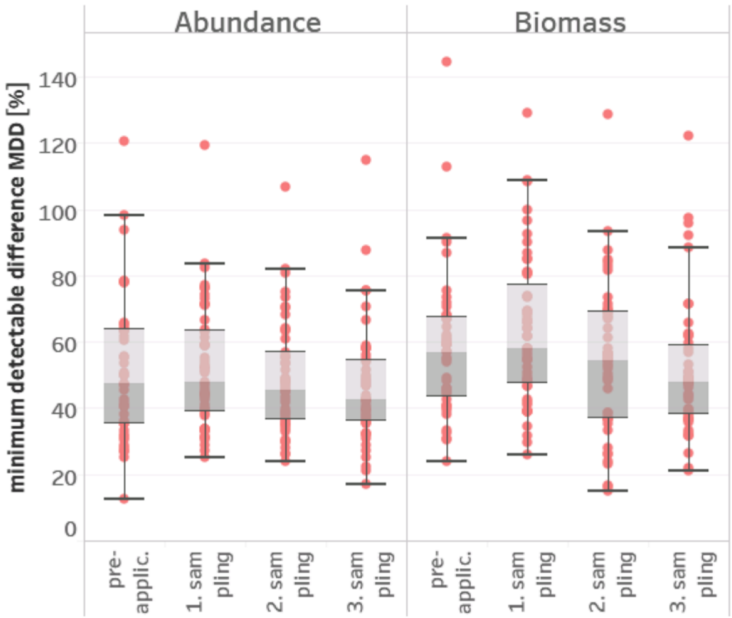
Source: RWTH Aachen University

total epilobous adults



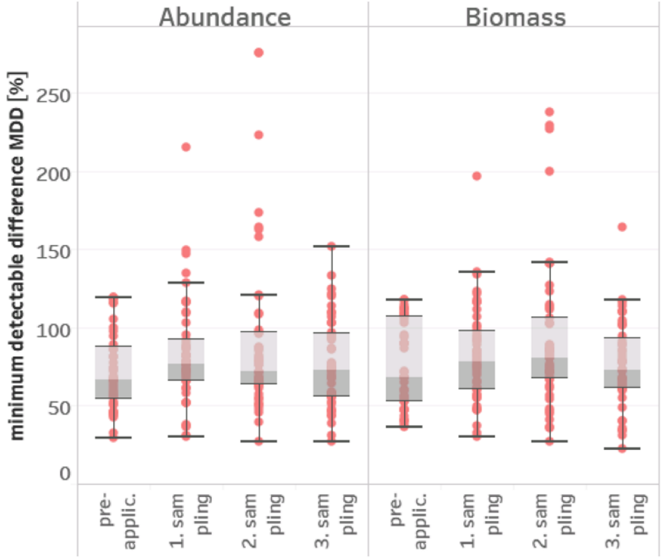
Source: RWTH Aachen University

total juveniles



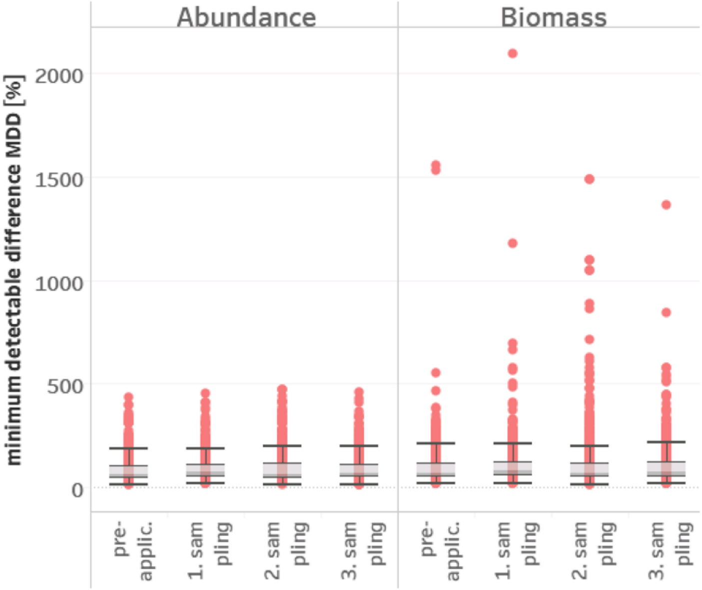
Source: RWTH Aachen University

total tanylobous adults



Source: RWTH Aachen University

All groups



Source: RWTH Aachen University

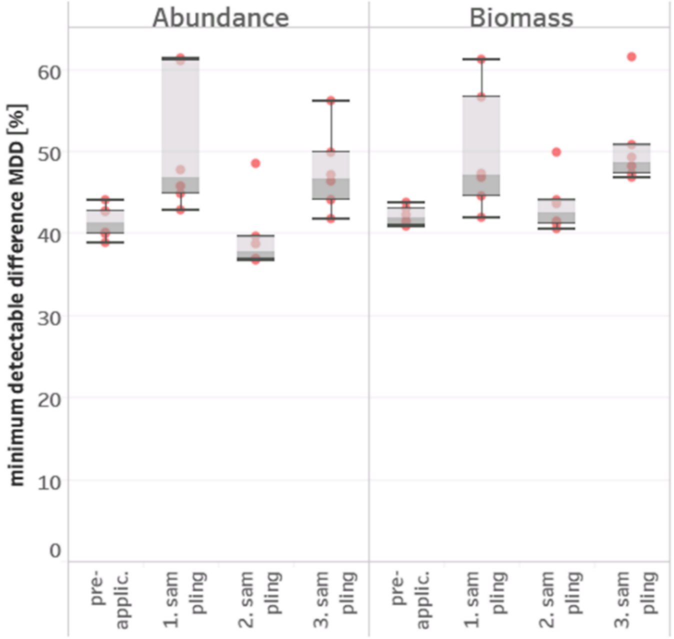
Table A3-2: **Pilot field study: Table of mean, median and standard deviation for minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application).**

Minimum detectable difference MDD - backtransformed data [%] Pilot field study																								
endpoint measure	Abundance data – pilot study												biomass data – pilot study											
sampling point	pre- application			~ 1 month after application			~ 6 month after application			~ 12 month after application			pre- application			~ 1 month after application			~ 6 month after application			~ 12 month after application		
	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev
Allolobophora chlorotica adults	41.4	41.4	2.0	50.6	46.8	8.3	39.5	37.8	4.6	47.5	46.7	5.0	42.1	41.9	1.1	49.7	47.1	7.5	43.4	42.5	3.4	50.6	48.7	5.5
Aporrectodea caliginosa adults	58.7	58.6	14.4	72.3	72.7	8.5	53.0	52.1	5.2	154.9	151.2	20.7	61.0	58.0	13.2	65.3	63.6	8.8	52.0	52.5	4.8	175.1	166.1	28.6
Aporrectodea rosea adults	62.3	61.1	15.8	66.0	63.9	12.8	75.6	76.5	2.6	73.4	73.5	7.9	71.9	65.4	21.6	73.0	67.9	15.7	80.2	82.0	6.4	98.2	96.0	17.8
Lumbricus castaneus adults	NaN	NA	NA	275.0	256.3	40.7	432.9	464.5	144.5	NaN	NA	NA	NaN	NA	NA	337.7	294.1	105.9	800.0	776.4	494.1	NaN	NA	NA
Lumbricus terrestris adults	66.6	64.4	5.9	77.2	77.0	14.1	61.1	61.6	8.3	56.0	59.5	20.2	53.9	54.3	5.0	78.4	79.4	14.8	58.6	61.8	8.5	51.7	64.2	22.5
total adults	36.2	35.9	2.8	41.0	41.0	6.1	38.2	37.3	2.8	48.2	47.5	5.8	30.0	28.5	5.6	48.2	48.2	13.4	43.1	42.8	2.9	38.8	30.3	19.7
total anecic adults	62.1	62.6	8.8	69.1	66.6	15.4	69.9	70.2	5.4	51.3	46.5	21.4	50.0	49.0	10.0	73.6	71.9	16.1	57.1	55.5	8.9	43.6	36.6	19.9
total earthworms	32.2	30.8	5.6	34.9	34.5	2.1	19.3	19.7	2.6	51.0	49.3	6.1	22.7	22.1	5.0	33.0	33.3	10.1	24.6	24.3	3.5	41.7	37.0	11.5
total endogeic	38.4	37.3	4.0	46.1	45.6	5.5	37.5	36.7	2.9	50.9	50.3	5.8	36.9	35.3	4.2	44.9	44.0	7.0	41.0	40.7	2.2	61.6	61.8	4.8
total epigeic	NaN	NA	NA	275.0	256.3	40.7	432.9	464.5	144.5	NaN	NA	NA	NaN	NA	NA	337.7	294.1	105.9	800.0	776.4	494.1	NaN	NA	NA
total epilobous adults	38.4	37.8	3.1	46.1	45.5	6.4	38.9	38.4	2.5	51.4	51.5	5.4	38.8	37.4	2.8	46.3	47.3	11.6	50.8	50.5	2.8	65.4	67.4	6.4

Minimum detectable difference MDD - backtransformed data [%] Pilot field study																								
total epilobous juveniles	40.6	38.8	7.8	40.4	40.3	1.3	31.0	33.5	5.0	55.7	53.6	7.3	29.0	28.5	4.1	35.5	33.1	8.0	34.9	33.9	6.1	62.1	58.5	8.1
total juveniles	38.4	37.3	7.1	38.2	37.9	1.2	26.8	27.2	3.2	52.4	50.5	6.2	27.1	26.0	3.2	31.9	32.0	6.8	19.8	20.6	3.2	48.0	45.5	6.8
total tanylobous adults	66.4	64.6	6.0	78.0	77.6	13.6	60.7	59.4	5.9	56.0	59.5	20.2	53.8	54.3	5.0	78.5	79.4	14.8	58.5	61.6	8.2	51.7	64.2	22.5
total tanylobous juveniles	51.3	49.8	6.4	55.8	57.9	6.5	31.7	31.0	4.9	54.1	53.5	10.4	71.5	72.4	5.7	63.5	61.5	7.0	39.4	38.0	5.2	56.3	54.6	10.3

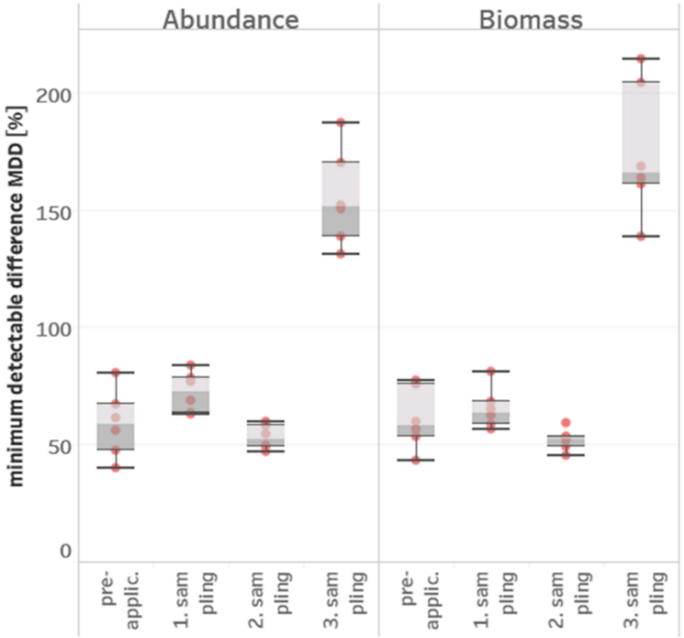
Figure A3-2: Pilot field study: Boxplots of minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application).

Allolobophora chlorotica **adults**



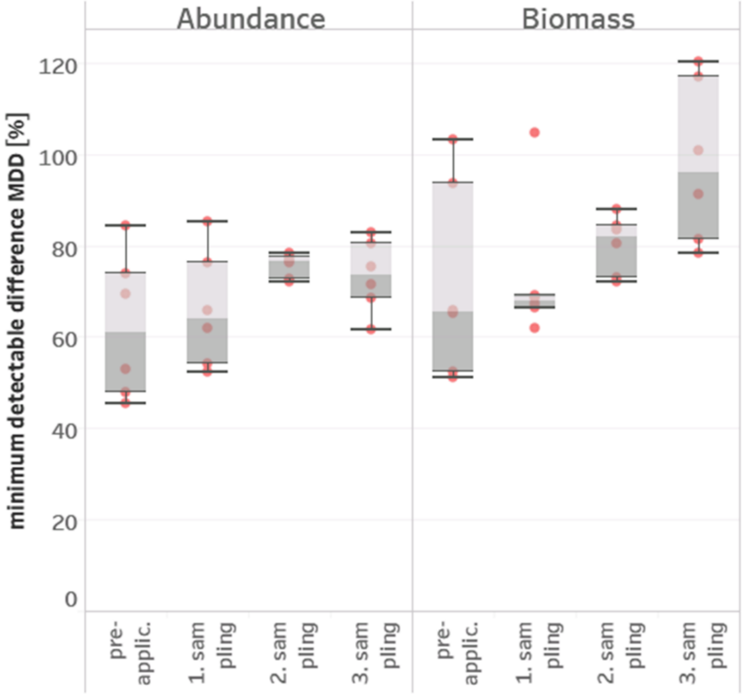
Source: RWTH Aachen University

Aporrectodea caliginosa **adults**



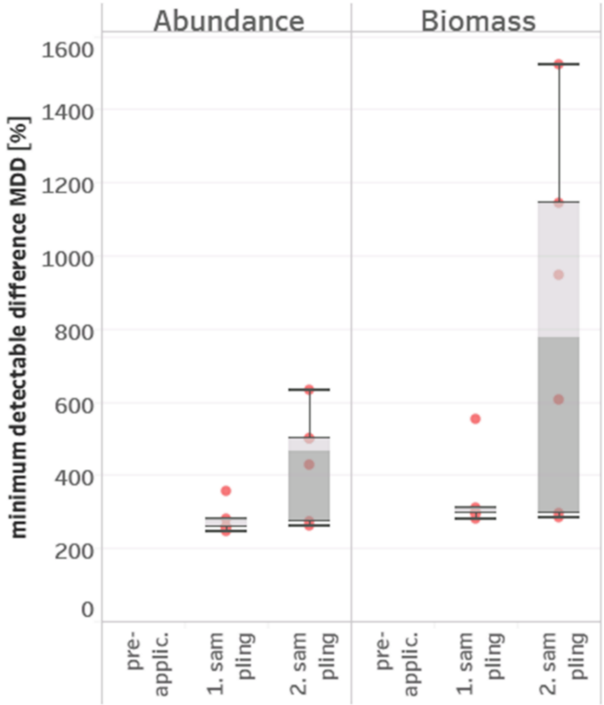
Source: RWTH Aachen University

Aporrectodea rosea **adults**



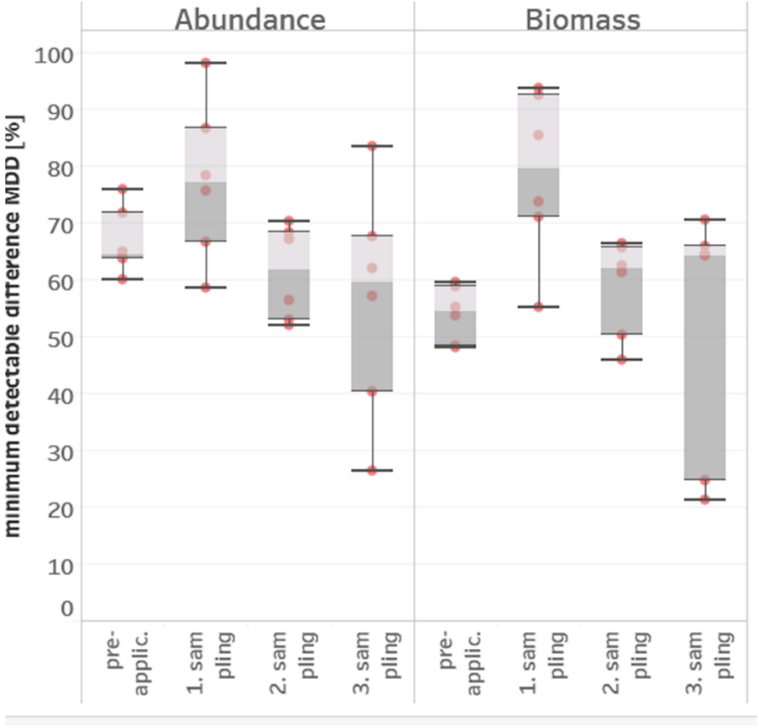
Source: RWTH Aachen University

Lumbricus castaneus **adults**



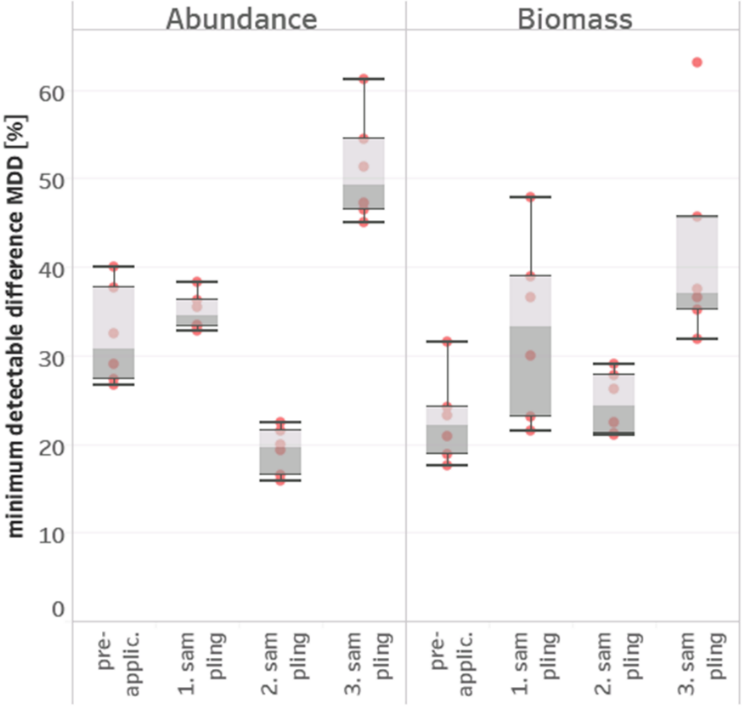
Source: RWTH Aachen University

Lumbricus terrestris adults



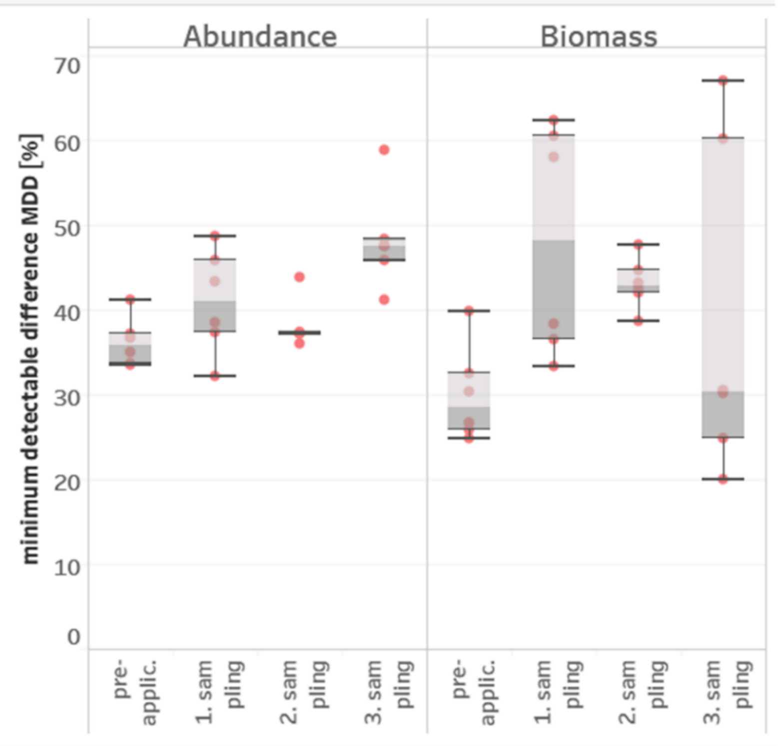
Source: RWTH Aachen University

total earthworms



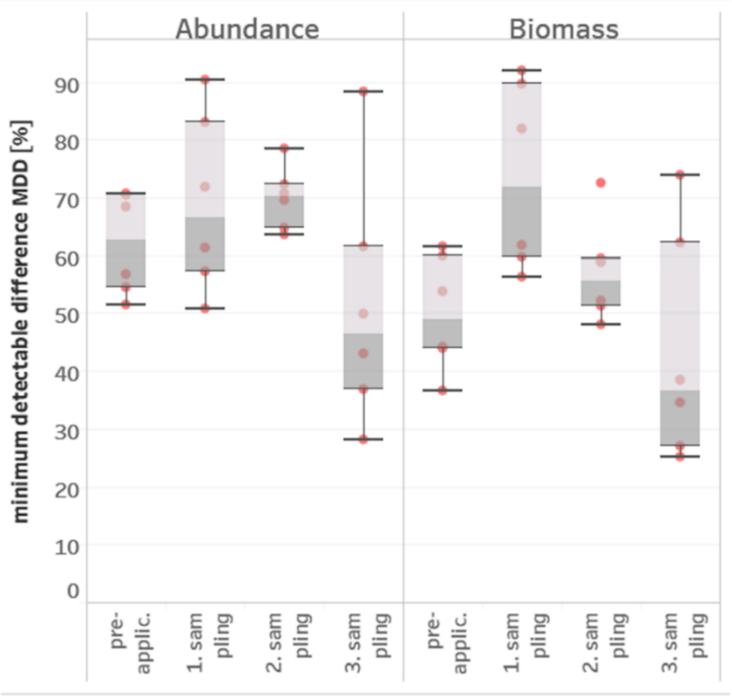
Source: RWTH Aachen University

total adults



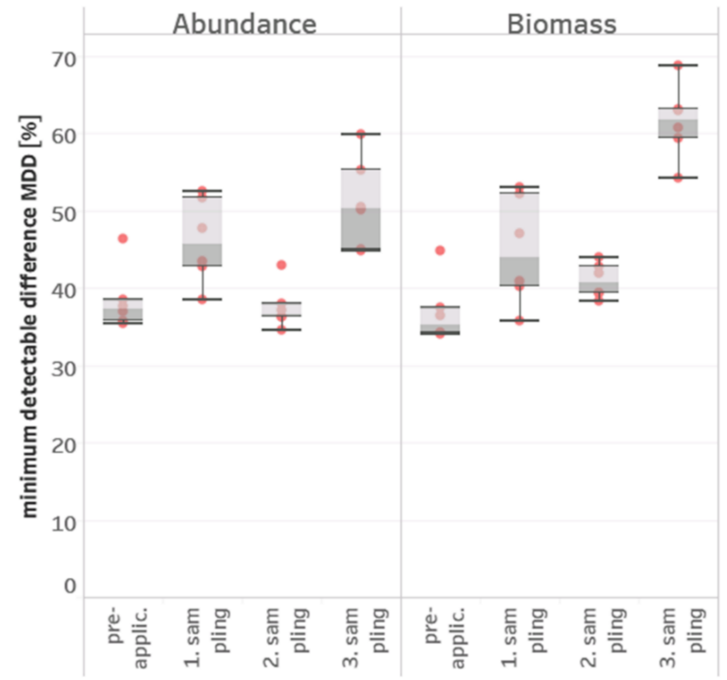
Source: RWTH Aachen University

total anecic adults



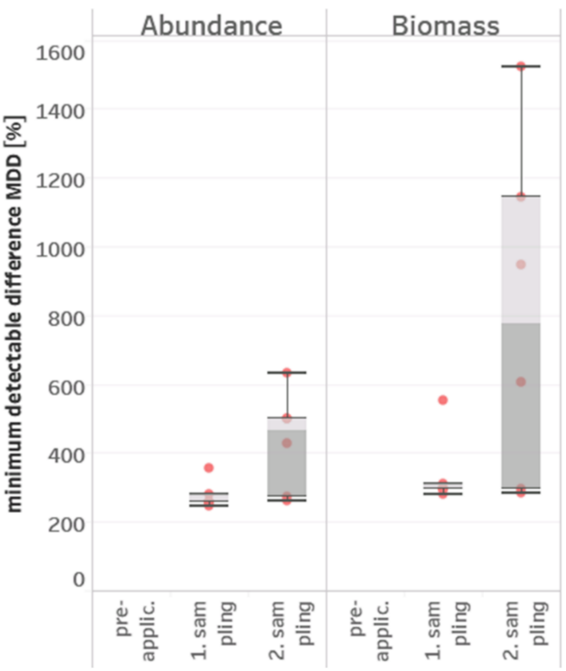
Source: RWTH Aachen University

total endogeic



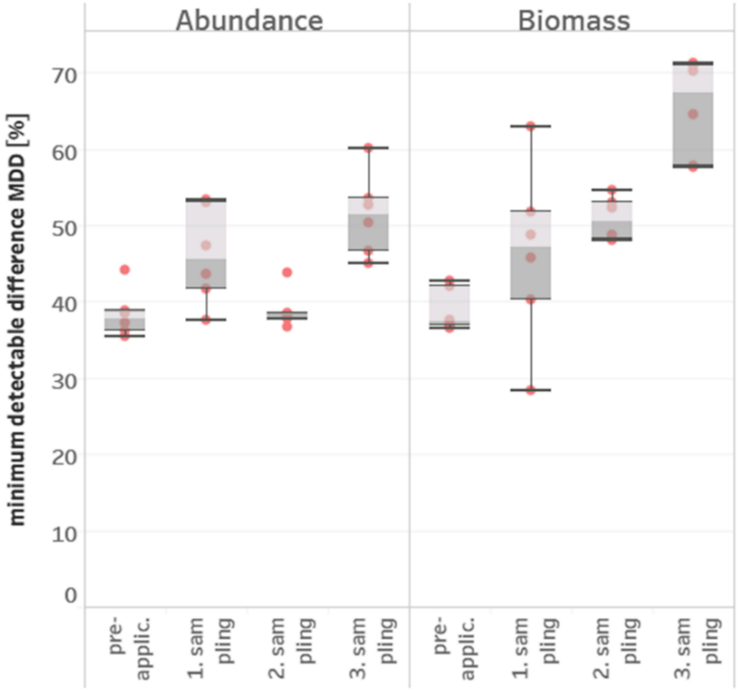
Source: RWTH Aachen University

total epigeic



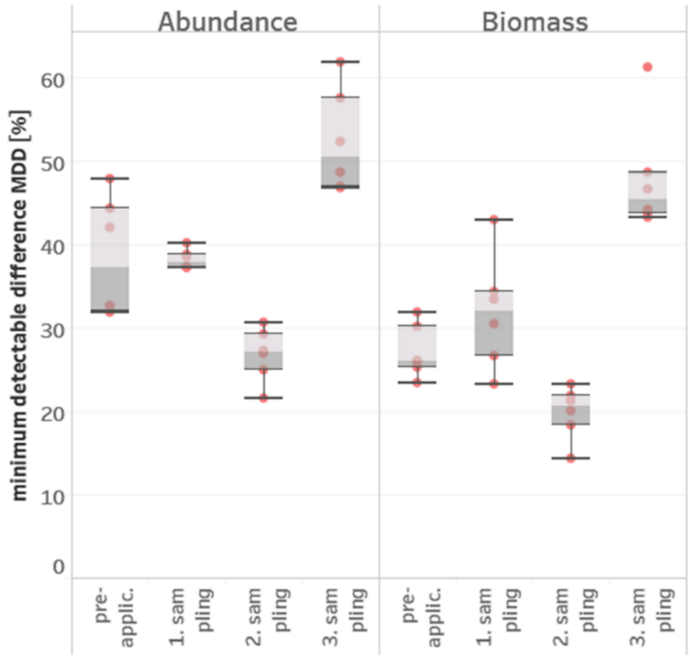
Source: RWTH Aachen University

total epilobous adults



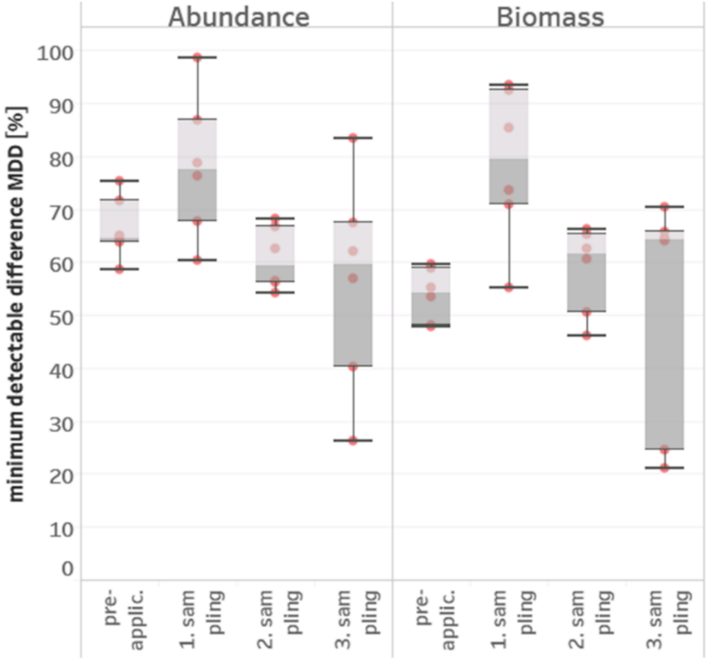
Source: RWTH Aachen University

total juveniles



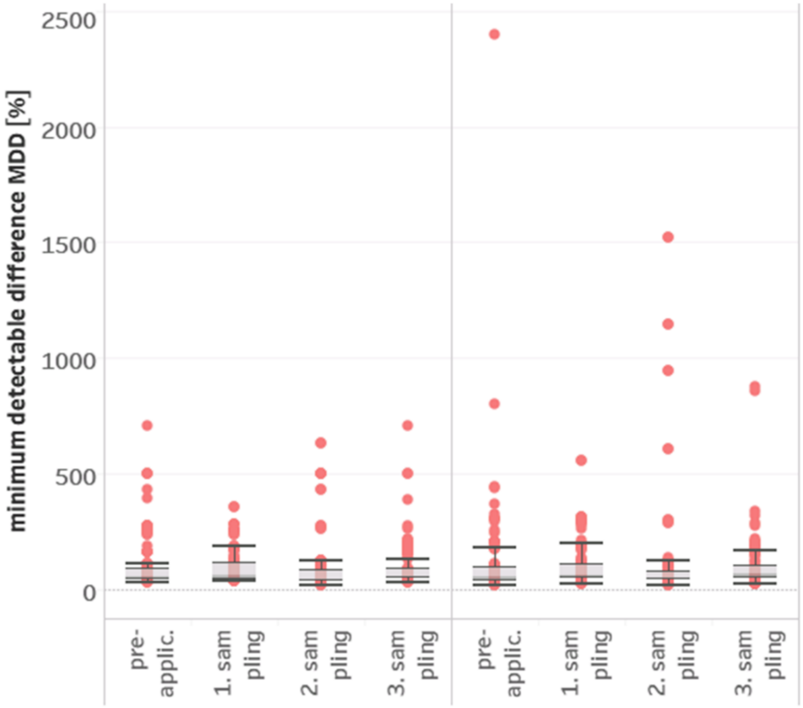
Source: RWTH Aachen University

total tanylobous adults



Source: RWTH Aachen University

All groups



Source: RWTH Aachen University

Table A3-3: Differences between database studies and pilot study: Table of differences in mean, median and standard deviation for minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application). Positive values indicate the percentage improvement of the pilot study-MDD compared to the database studies, negative values indicate the percentage increase of MDD.

Minimum detectable difference MDD - backtransformed data [% improvement]																								
endpoint measure	**Abundance data – comparison (database – pilot study)**												**biomass data – comparison (database – pilot study)**											
sampling point	pre- application			~ 1 month after application			~ 6 month after application			~ 12 month after application			pre- application			~ 1 month after application			~ 6 month after application			~ 12 month after application		
	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev
Allolobophora chlorotica adults	90.8	108.7	50.0	130.4	98.8	99.5	97.3	91.6	82.2	103.1	92.7	89.3	118.0	138.8	72.6	172.2	152.6	137.7	120.7	123.0	99.0	109.8	121.1	74.4
Aporrectodea caliginosa adults	1.2	-4.8	15.0	16.7	4.3	38.8	15.4	2.6	35.3	-78.6	-80.3	6.7	3.5	-1.8	19.2	32.4	17.6	47.0	19.7	3.5	39.3	-92.7	-87.1	2.7
Aporrectodea rosea adults	21.0	15.1	16.0	19.6	19.3	18.2	18.0	23.0	30.1	26.6	19.8	37.3	20.7	20.7	21.3	27.6	31.5	25.2	32.5	26.1	41.8	31.5	6.5	61.0
Lumbricus castaneus adults	NA	NA	NA	-147.0	-134.9	3.9	-250.3	-323.7	-30.4	NA	NA	NA	NA	NA	NA	-160.8	-122.6	-44.8	-497.1	-617.4	-150.0	NA	NA	NA
Lumbricus terrestris adults	48.7	11.0	85.4	22.4	3.3	35.4	42.8	19.0	53.6	33.6	20.7	23.4	48.9	16.3	65.2	16.5	7.6	25.8	37.5	18.7	39.8	33.3	12.0	15.3
total adults	10.0	9.7	13.0	15.9	13.2	12.9	10.1	10.3	13.3	7.3	6.1	14.6	24.7	22.8	12.8	9.1	3.4	4.2	9.0	4.7	17.3	20.6	26.0	2.3
total anecic adults	40.1	5.1	81.5	25.8	1.5	34.4	32.2	9.1	57.2	33.8	30.2	22.7	45.0	19.2	57.0	17.7	4.7	25.0	37.1	23.3	40.4	39.0	40.4	19.1
total earthworms	12.0	10.6	11.6	12.3	9.9	14.6	24.0	20.8	13.5	12.0	-9.9	11.0	25.5	25.8	12.4	17.3	15.8	10.4	20.1	20.6	15.2	5.9	10.8	5.1
total endogeic	12.7	11.7	14.6	22.3	19.0	24.6	21.2	12.6	29.8	13.8	8.2	21.0	18.1	17.4	15.1	31.9	24.1	35.5	23.0	14.7	36.3	6.9	0.2	23.3

Minimum detectable difference MDD - backtransformed data [% improvement]																								
total epigeic	NA	NA	NA	-163.7	-155.9	2.7	-258.8	-323.7	-25.1	NA	NA	NA	NA	NA	NA	-188.2	-148.7	-38.1	-513.4	-625.3	-148.6	NA	NA	NA
total epilobous adults	11.7	10.6	14.7	20.7	18.4	23.7	18.2	9.7	29.7	10.6	4.8	20.1	19.0	16.9	21.4	29.8	20.9	30.5	11.3	1.7	35.7	1.6	-5.5	24.0
total epilobous juveniles	16.3	12.4	16.4	15.6	12.0	18.4	24.9	19.2	14.5	-3.5	-6.3	15.6	29.6	21.6	27.6	31.5	32.5	18.8	26.8	26.2	19.1	-5.2	-6.2	23.5
total juveniles	13.0	10.0	14.6	14.0	9.9	17.4	21.9	18.4	13.8	-6.3	-8.1	11.8	31.2	30.9	19.2	31.2	26.3	15.7	34.1	33.9	19.3	3.6	2.3	13.7
total tanylobous adults	6.0	2.1	19.2	4.7	-0.6	19.7	30.0	12.6	47.6	20.8	13.0	8.3	21.9	13.7	22.4	3.3	-1.5	15.9	33.4	19.1	39.3	24.4	8.6	5.4
total tanylobous juveniles	19.8	12.6	24.7	16.5	9.2	22.2	27.2	21.0	16.7	11.4	10.9	12.2	9.4	-3.6	26.5	21.6	21.5	23.4	29.0	25.1	22.4	17.3	17.1	21.4

Table A3-4: Database studies: Differences between statistical evaluation on plot and subplot level: Table of differences in mean minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application). Positive values indicate the percentage improvement of the MDD at subplot level compared to the plot level, negative values indicate the percentage increase of MDD.

Minimum detectable difference MDD - backtransformed data [% improvement] Database studies								
endpoint measure	**Abundance data – comparison (mean MDD plot level – mean MDD subplot level)**				**biomass data – comparison (mean MDD plot level – mean MDD subplot level)**			
sampling point	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application
Allolobophora chlorotica adults	0.5	2.1	9.9	-4.2	9.5	27.0	23.0	6.9
Aporrectodea caliginosa adults	1.8	-3.6	1.1	-1.3	2.7	-1.1	1.5	-0.1
Aporrectodea rosea adults	9.4	1.2	4.7	2.4	14.5	8.2	12.4	16.5
Lumbricus castaneus adults	-7.5	-17.2	-30.0	0.9	1.4	-1.6	2.3	9.4
Lumbricus terrestris adults	0.8	-7.4	0.0	-0.4	-3.4	-10.7	-2.4	-3.5
total adults	6.8	7.2	6.9	5.2	3.8	2.7	4.9	4.1
total anecic adults	2.9	-6.4	1.0	-0.5	-0.7	-9.4	-1.4	-2.9
total e-arthworms	6.9	8.0	7.8	12.0	5.8	6.4	6.2	6.9
total endogeic	7.3	8.0	6.1	4.6	6.5	8.5	7.0	3.6
total epigeic	-5.4	-15.7	-25.3	6.9	1.6	-3.2	6.0	3.8
total epilobous adults	7.3	7.7	6.0	4.2	6.6	8.5	6.4	2.3
total epilobous juveniles	7.3	7.3	9.7	6.5	6.6	11.5	9.9	6.9
total juveniles	7.0	8.1	8.5	6.1	7.6	10.5	6.7	6.5
total tanylobous adults	2.1	-3.7	1.6	1.5	-2.6	-8.2	-0.4	-1.4
total tanylobous juveniles	6.8	5.7	3.4	8.4	7.5	6.8	3.4	7.8

Table A3-5: Pilot study: Differences between statistical evaluation on plot and subplot level: Table of differences in mean minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application). Positive values indicate the percentage improvement of the MDD at subplot level compared to the plot level, negative values indicate the percentage increase of MDD.

Minimum detectable difference MDD - backtransformed data [% improvement] pilot study								
endpoint measure	**Abundance data – comparison (mean MDD plot level – mean MDD subplot level)**				**biomass data – comparison (mean MDD plot level – mean MDD subplot level)**			
sampling point	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application
Allolobophora chlorotica adults	13.8	16.5	8.5	12.9	13.8	21.4	10.1	12.9
Aporrectodea caliginosa adults	5.6	15.2	-19.3	-19.6	1.2	10.2	-23.9	-30.2
Aporrectodea rosea adults	1.9	3.3	9.9	-7.6	4.3	12.7	6.5	-15.3
Lumbricus castaneus adults	NA	55.6	-81.0	NA	NA	81.9	-47.4	NA
Lumbricus terrestris adults	8.3	8.6	-10.8	-11.0	-10.3	6.3	-16.0	-17.4
total adults	11.4	10.6	10.7	17.0	-0.1	5.8	12.1	-4.6
total anecic adults	12.6	5.8	16.7	-12.2	-5.2	5.0	-1.2	-22.3
total earthworms	14.5	10.7	-1.0	21.9	1.9	2.8	3.2	9.6
total endogeic	12.1	13.6	8.8	17.0	8.3	13.2	9.6	20.8
total epigeic	NA	55.6	-81.0	NA	NA	81.9	-47.4	NA
total epilobous adults	12.4	13.5	10.5	17.4	9.1	13.1	17.4	21.7
total epilobous juveniles	18.3	11.6	5.7	22.1	10.9	14.8	10.4	25.9
total juveniles	17.1	11.1	3.8	21.4	8.4	11.1	-2.9	12.5
total tanylobous adults	8.2	9.9	-9.6	-11.0	-10.2	6.4	-15.8	-17.4
total tanylobous juveniles	8.1	9.8	1.5	6.3	19.8	18.9	7.4	2.7

Table A3-6: Pilot study: Differences between statistical evaluation with six replicates (T2, T5) and three replicates (T1, T3, T4, T6) on plot level: Table of differences in mean minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application). Positive values indicate the percentage improvement of the MDD using six replicates compared to the treatments with three replicates, negative values indicate the percentage increase of MDD.

Minimum detectable difference MDD - backtransformed data [% improvement] pilot study PLOT Level								
endpoint measure	Abundance data – comparison (mean MDD 3 replicates – mean MDD 6 repl.)				biomass data – comparison (mean MDD 3 replicates – mean MDD 6 repl.)			
sampling point	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application
Allolobophora chlorotica adults	0.1	5.7	2.1	3.0	0.1	5.7	1.6	2.4
Aporrectodea caliginosa adults	10.4	9.2	5.3	5.6	18.8	8.9	2.2	13.6
Aporrectodea rosea adults	18.0	-4.7	2.1	2.2	19.2	7.6	3.7	3.3
Lumbricus castaneus adults	NA	18.0	131.9	NA	NA	63.4	278.7	NA
Lumbricus terrestris adults	7.2	7.2	2.5	21.6	4.4	0.5	0.6	12.4
total adults	0.5	0.9	2.3	7.1	2.8	1.4	2.2	16.6
total anecic adults	12.0	6.8	2.0	28.2	9.0	4.1	3.0	20.5
total earthworms	7.8	-1.3	2.0	12.0	1.8	4.2	0.3	8.0
total endogeic	1.1	1.3	2.5	5.2	2.3	1.8	3.1	4.4
total epigeic	NA	18.0	131.9	NA	NA	63.4	278.7	NA
total epilobous adults	0.9	0.9	2.1	5.5	2.4	-1.5	0.9	6.4
total epilobous juveniles	9.9	-1.5	3.4	8.4	2.5	4.7	6.2	9.9
total juveniles	9.8	-1.7	2.1	6.8	3.6	2.0	-2.6	3.8
total tanylobous adults	7.6	7.3	0.2	21.6	4.5	0.5	0.3	12.4
total tanylobous juveniles	2.3	7.0	-1.7	10.1	10.1	0.4	1.7	6.3

Table A3-7: Pilot study: Differences between statistical evaluation with 36 replicates (T2, T5) and 18 replicates (T1, T3, T4, T6) on subplot (=sample) level: Table of differences in mean minimum detectable differences (% MDD) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application). Positive values indicate the percentage improvement of the MDD using 36 subplot replicates compared to the treatments with 18 subplot replicates, negative values indicate the percentage increase of MDD.

Minimum detectable difference MDD - backtransformed data [% improvement] pilot study SUBPLOT Level								
endpoint measure	**Abundance data – comparison (mean MDD 18 replicates – mean MDD 36 repl.)**				**biomass data – comparison (mean MDD 18 replicates – mean MDD 36 repl.)**			
sampling point	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application
Allolobophora chlorotica adults	3.4	6.1	4.6	3.8	3.2	4.2	5.6	6.3
Aporrectodea caliginosa adults	5.7	9.8	10.6	24.5	9.3	5.8	13.1	30.1
Aporrectodea rosea adults	9.9	10.2	5.0	10.4	10.3	12.4	7.5	25.9
Lumbricus castaneus adults	NA	12.4	190.8	NA	NA	53.6	302.8	NA
Lumbricus terrestris adults	12.7	12.9	10.6	15.6	11.0	8.7	8.1	12.2
total adults	2.7	5.0	3.6	4.7	5.6	9.1	1.6	11.9
total anecic adults	8.2	11.1	1.6	13.8	8.3	8.5	3.9	12.0
total earthworms	2.1	2.1	4.1	12.0	3.0	7.3	3.0	10.1
total endogeic	2.8	4.4	3.7	4.2	2.7	4.5	5.6	5.3
total epigeic	NA	12.4	190.8	NA	NA	53.6	302.8	NA
total epilobous adults	2.5	4.3	3.8	4.5	1.9	4.2	3.8	6.8
total epilobous juveniles	3.1	2.6	3.8	5.7	1.5	2.5	2.9	6.0
total juveniles	3.0	2.5	3.9	4.4	1.7	1.3	3.1	8.1
total tanylobous adults	13.0	12.6	11.1	15.6	11.1	8.7	8.1	12.2
total tanylobous juveniles	4.3	3.6	0.4	8.0	6.9	-1.0	0.5	8.6

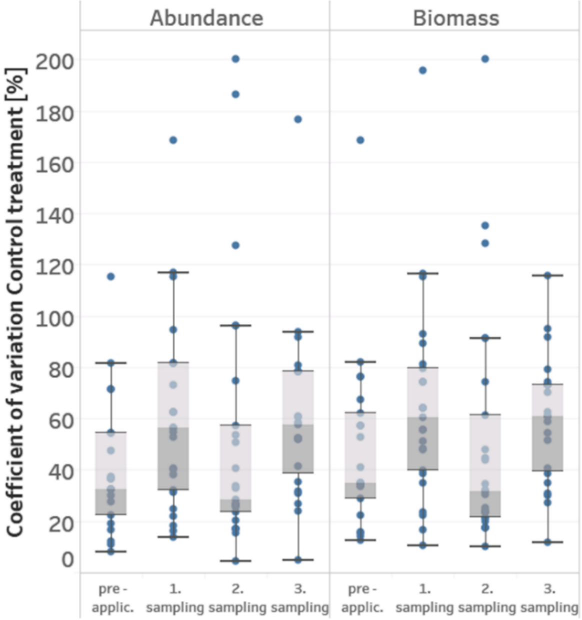
Table A3-8: Database field studies: Table of mean, median and standard deviation for coefficients of variation in control treatments (%) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application).

coefficients of variation for control treatments [%] DATABASE earthworm field studies																								
endpoint measure	Abundance data - database												biomass data - database											
sampling point	pre- application			~ 1 month after application			~ 6 month after application			~ 12 month after application			pre- application			~ 1 month after application			~ 6 month after application			~ 12 month after application		
	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev	mean	median	st.dev
Allolobophora chlorotica adults	117.7	115.5	57.8	139.3	153.9	62.2	117.6	127.7	71.9	114.9	115.5	59.5	113.8	115.6	60.0	138.1	151.5	60.3	119.9	94.5	70.4	112.0	115.5	60.7
Aporrectodea caliginosa adults	40.6	32.6	26.7	60.0	56.6	36.7	53.0	28.6	51.6	60.8	57.7	34.3	48.7	34.9	35.0	64.6	60.3	38.9	50.7	31.6	46.2	59.5	60.8	24.5
Aporrectodea rosea adults	63.1	71.7	35.9	69.7	69.3	35.0	75.0	74.8	41.4	80.3	64.7	53.6	62.0	60.6	38.0	62.4	63.0	27.7	76.7	72.8	39.0	78.6	58.8	49.5
Lumbricus castaneus adults	104.6	91.6	37.1	122.8	116.0	59.6	114.9	111.7	61.1	114.5	101.7	56.1	108.5	103.3	35.2	127.7	141.0	54.0	117.9	108.1	58.6	113.7	100.7	56.1
Lumbricus terrestris adults	69.7	45.5	62.9	67.4	46.2	51.5	65.9	51.9	48.7	61.7	60.9	46.7	70.0	43.8	63.5	67.7	42.3	47.5	69.8	54.2	51.5	64.8	61.1	43.0
total adults	28.5	26.0	20.3	40.1	38.8	22.5	29.8	28.9	12.8	38.1	32.7	19.8	34.1	34.0	19.5	40.0	32.1	25.9	32.7	32.5	12.0	39.8	35.2	25.5
total anecic adults	59.7	38.4	55.6	67.1	46.2	52.5	67.6	51.9	47.8	57.5	38.7	48.8	64.2	43.8	56.9	68.1	47.1	48.5	70.9	53.5	50.7	63.6	59.1	44.3
total earthworms	34.0	25.8	34.9	37.9	32.9	26.7	32.7	24.4	30.4	31.9	23.6	31.6	34.9	26.8	28.4	34.8	28.0	25.3	31.3	28.9	22.9	33.6	27.5	19.9
total endogeic	31.7	24.7	24.6	49.1	42.8	27.5	37.9	28.2	34.8	49.7	39.7	35.6	35.2	34.5	18.7	51.7	48.8	24.6	39.3	31.9	26.6	47.6	50.1	20.3
total epigeic	94.5	86.1	45.3	101.5	84.7	55.7	108.2	115.5	64.2	120.0	101.7	57.8	100.6	80.8	42.2	104.6	84.2	52.2	113.2	115.6	59.5	119.5	102.7	57.3

coefficients of variation for control treatments [%] DATABASE earthworm field studies																								
total epilobous adults	30.2	21.5	21.7	48.4	42.8	27.5	37.6	31.0	34.9	46.8	34.7	33.7	34.1	34.5	16.8	51.3	52.6	26.9	37.2	31.6	25.2	46.5	40.0	24.2
total epilobous juveniles	45.5	31.4	41.0	46.4	38.4	36.7	46.0	36.0	36.7	39.2	31.2	39.9	46.9	32.6	42.0	51.7	44.9	37.0	55.1	40.2	48.2	39.1	32.2	38.7
total juveniles	40.6	28.6	40.5	44.0	32.9	36.4	38.9	29.8	34.9	36.2	26.4	37.9	46.2	41.8	39.2	50.9	43.0	35.8	42.4	30.0	38.5	43.2	32.6	37.5
total tanylobous adults	48.6	44.9	27.9	67.6	61.3	38.3	62.8	49.8	43.6	61.5	46.7	42.4	51.0	43.9	33.3	59.0	43.8	37.0	68.1	54.3	48.2	61.7	59.0	41.8
total tanylobous juveniles	53.7	40.5	45.7	55.0	43.3	43.2	39.7	32.7	28.7	49.0	35.0	34.5	62.3	49.4	46.8	66.5	48.3	36.0	47.5	31.9	34.3	60.7	50.6	39.7

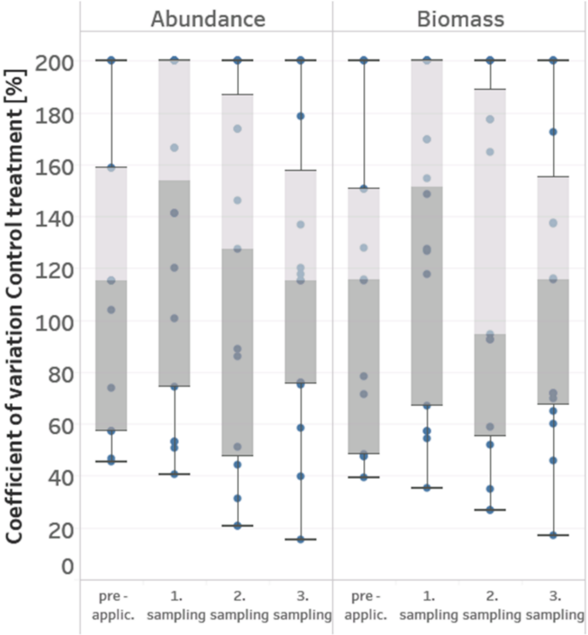
Figure A3-3: Database field studies: Boxplots of mean coefficients of variation in control treatments (%) per field study for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application).

***Aporrectodea caliginosa* adults**



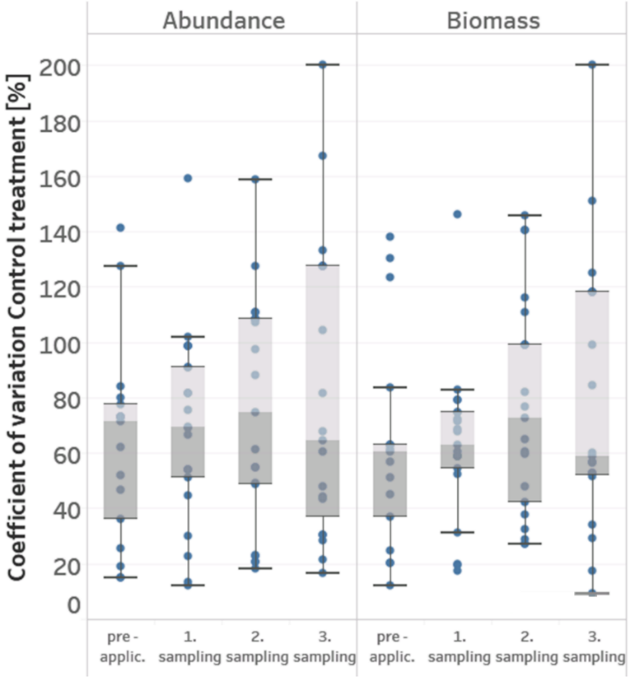
Source: RWTH Aachen University

***Allolobophora chlorotica* adults**



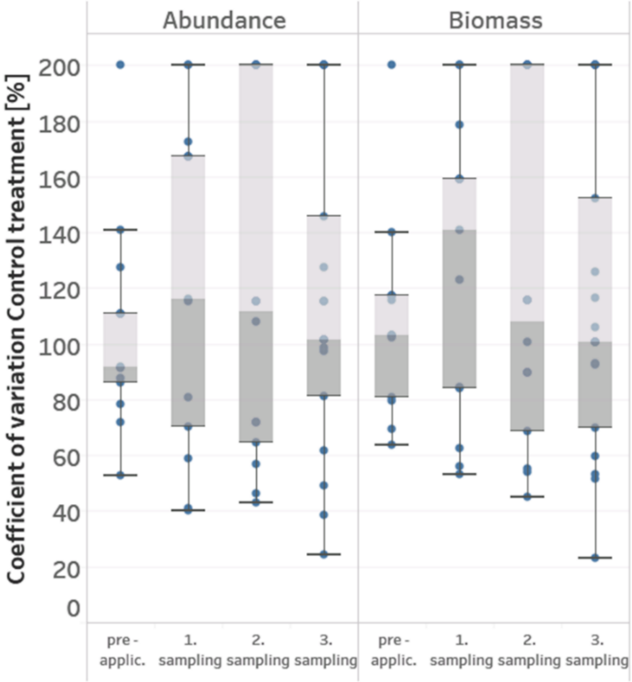
Source: RWTH Aachen University

Aporrectodea rosea **adults**



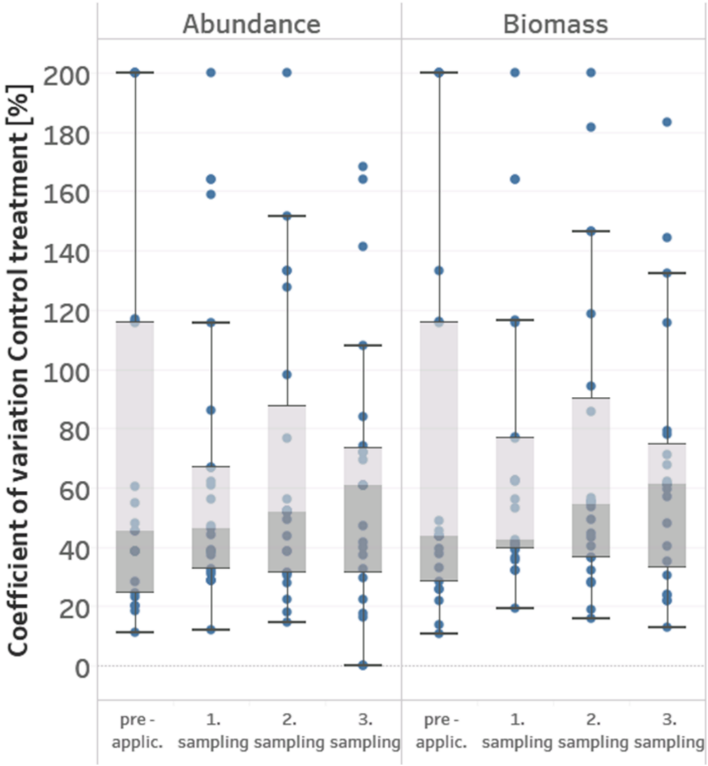
Source: RWTH Aachen University

Lumbricus castaneus **adults**



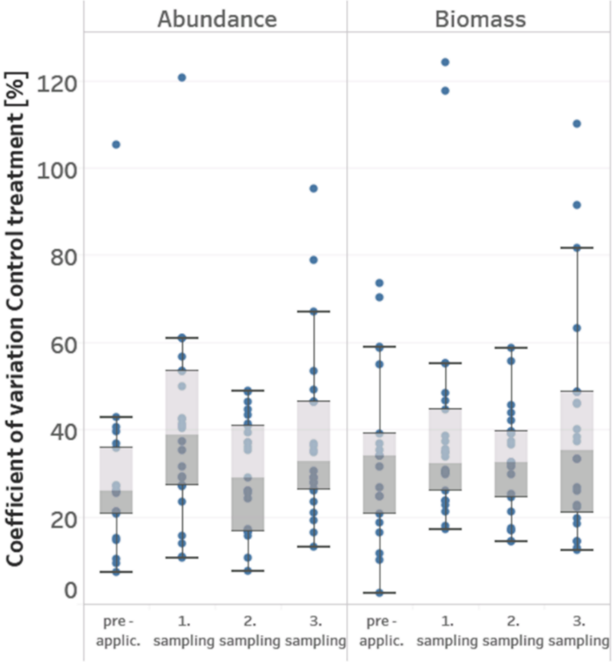
Source: RWTH Aachen University

Lumbricus terrestris adults



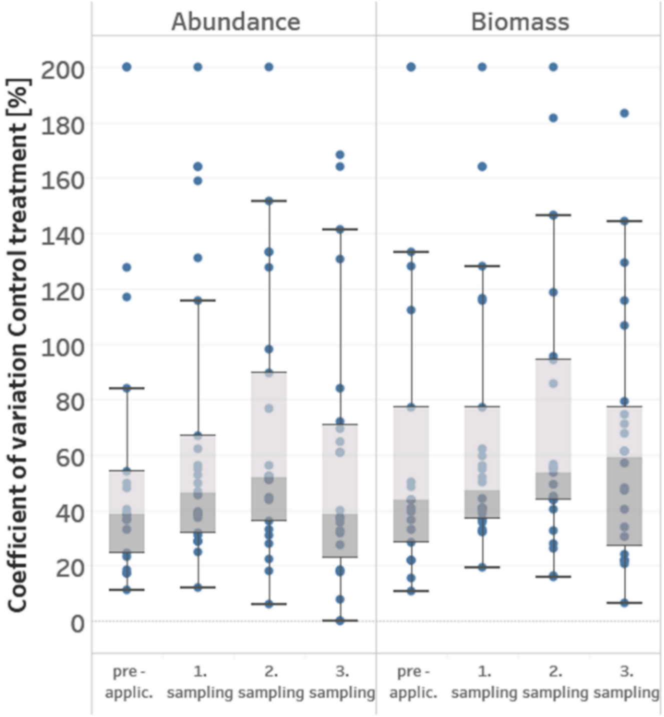
Source: RWTH Aachen University

total adults



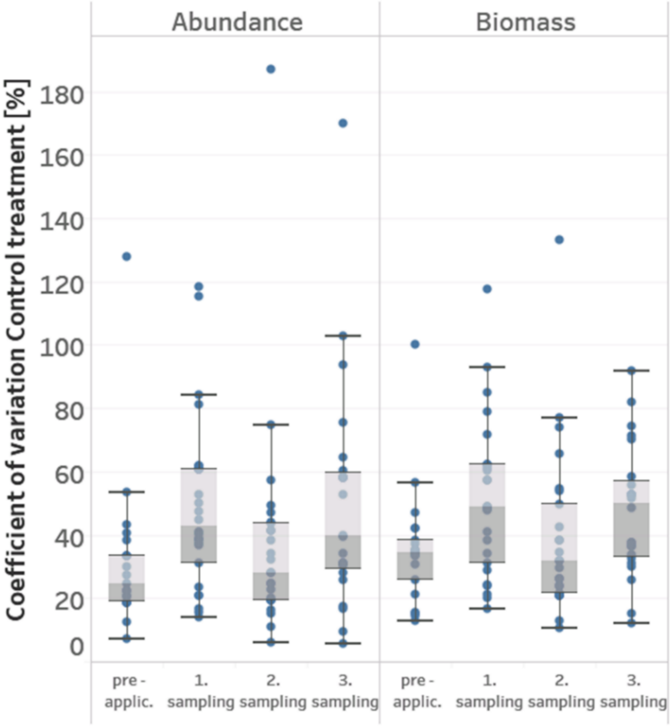
Source: RWTH Aachen University

total anecic adults



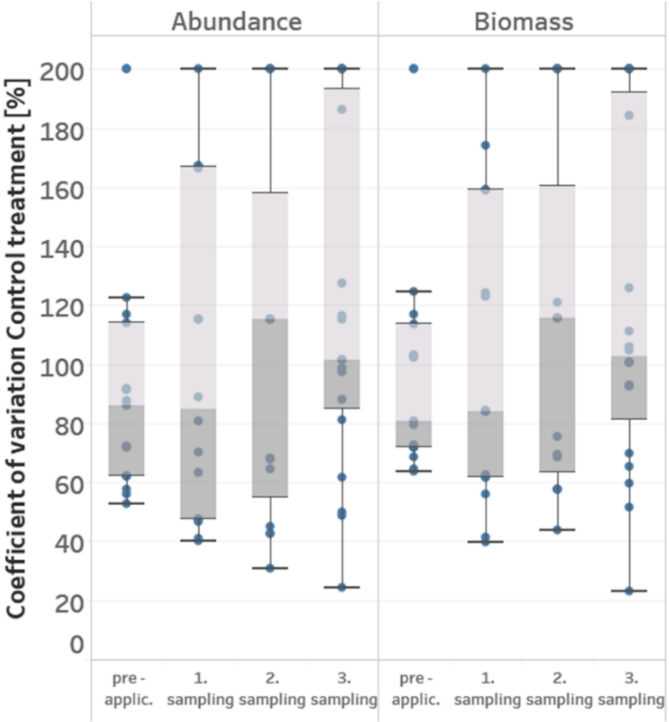
Source: RWTH Aachen University

total endogeic



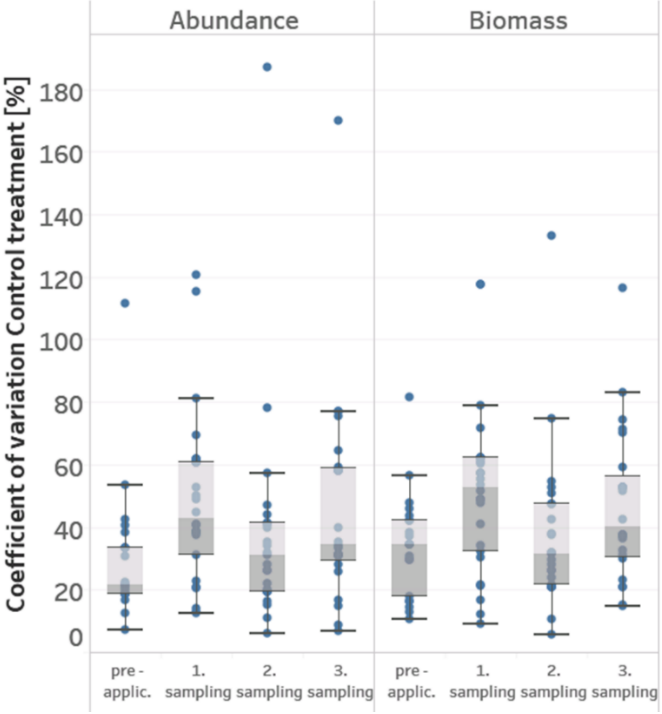
Source: RWTH Aachen University

total epigeic



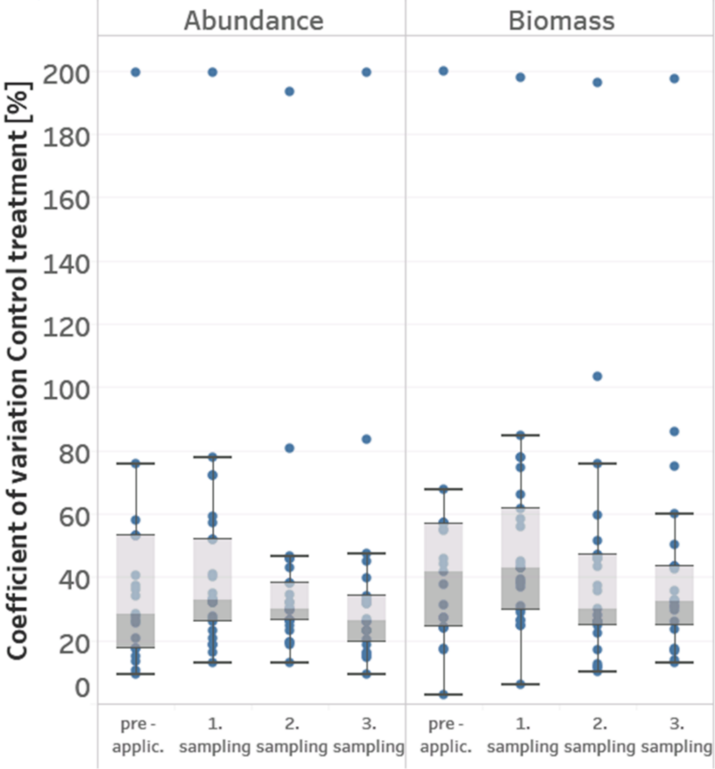
Source: RWTH Aachen University

total epilobous adults



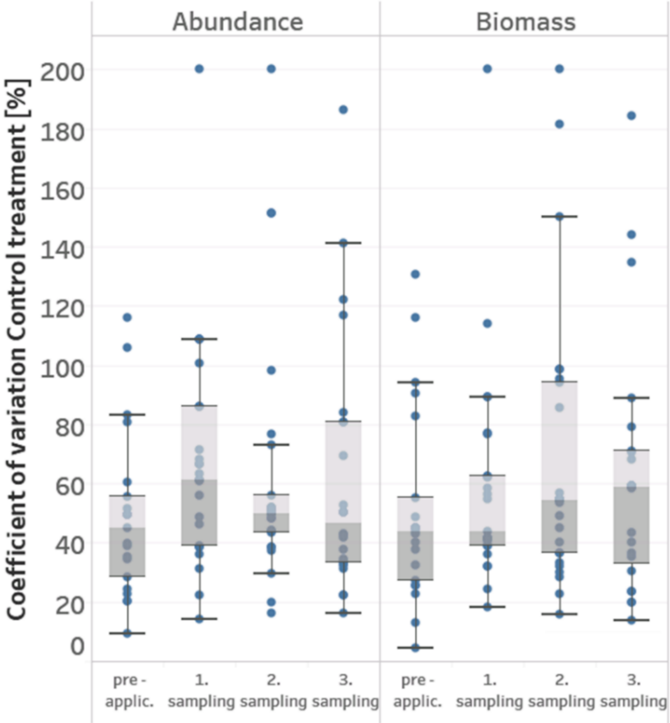
Source: RWTH Aachen University

total juveniles



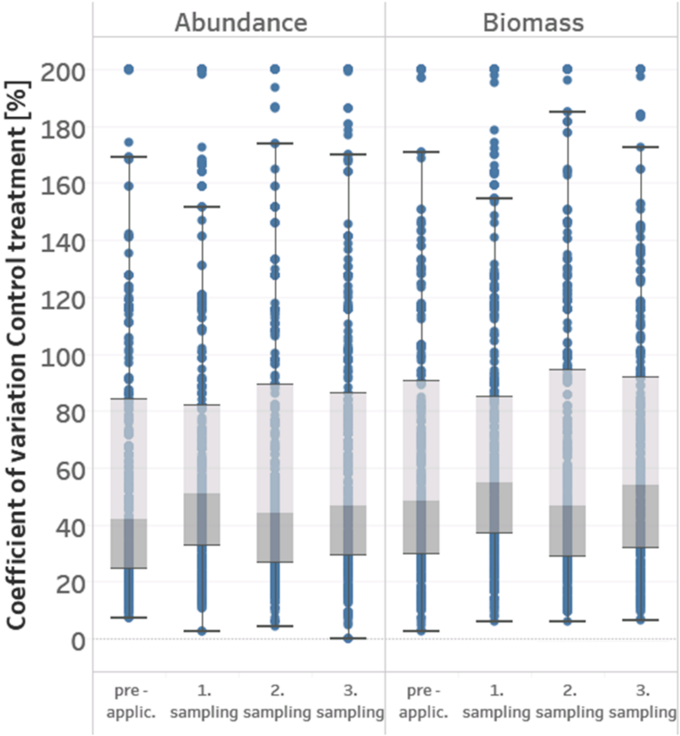
Source: RWTH Aachen University

total tanylobous adults



Source: RWTH Aachen University

All groups



Source: RWTH Aachen University

Table A3-9: Pilot field study: Table of mean coefficients of variation in control treatments (%) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application).

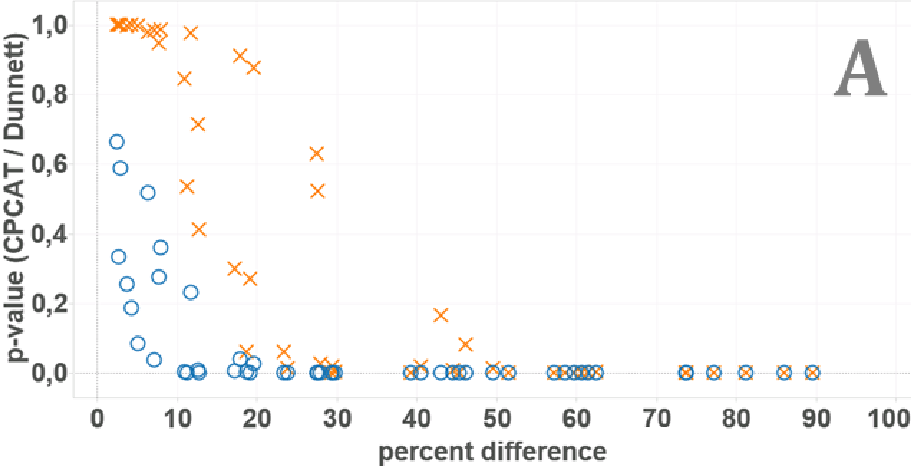
coefficients of variation for control treatments [%] PILOT STUDY								
endpoint measure	Abundance data – pilot study				biomass data – pilot study			
sampling point	pre-application	~ 1 month after application	~ 6 month after application	~ 12 month after application	pre-application	~ 1 month after application	~ 6 month after application	~ 12 month after application
Allolobophora chlorotica adults	33.8	37.8	39.6	34.3	33.8	39.1	45.6	35.5
Aporrectodea caliginosa adults	36.5	52.8	26.9	131.3	31.7	46.6	24.7	133.0
Aporrectodea rosea adults	35.0	54.4	63.5	53.7	37.3	59.2	66.0	56.1
Lumbricus castaneus adults	NaN	244.9	244.9	NaN	NaN	244.9	244.9	NaN
Lumbricus terrestris adults	66.4	51.0	46.4	21.2	41.4	52.1	37.2	14.7
total adults	29.1	31.2	36.2	33.9	22.6	23.9	33.9	15.5
total anecic adults	61.0	45.4	55.2	11.2	39.5	49.5	42.5	11.9
total earthworms	22.4	24.7	11.5	36.9	14.9	14.3	17.5	27.9
total endogeic	31.9	35.6	36.8	36.8	28.9	34.0	39.6	45.3
total epigeic	NaN	244.9	244.9	NaN	NaN	244.9	244.9	NaN
total epilobous adults	30.9	34.1	37.8	37.4	27.4	24.8	46.8	49.8
total epilobous juveniles	26.2	28.6	21.0	40.6	23.8	20.5	19.4	45.5
total juveniles	24.0	26.6	17.5	38.5	22.3	17.5	12.4	36.5
total tanylobous adults	66.4	54.7	45.2	21.2	41.4	52.2	37.2	14.7
total tanylobous juveniles	31.3	42.3	19.6	36.8	55.1	43.5	26.7	47.6

Table A3-10: Differences between database studies and pilot study in mean coefficients of variation in control treatments (%) for the most frequent species and earthworm groups at different sampling time points in the course of the tested year (1. Sampling: ~1-3 month after application, 2. Sampling: ~6 month after application, 3. Sampling: ~12month after application). Positive values indicate the percentage reduction of coefficients of variation in the pilot study compared to the database studies, negative values indicate the percentage increase.

coefficients of variation for control treatments [%] database vs. pilot study								
endpoint measure	**Abundance data – comparison (database – pilot study)**				**biomass data – comparison (database – pilot study)**			
sampling point	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application	pre- application	~ 1 month after application	~ 6 month after application	~ 12 month after application
Allolobophora chlorotica adults	83.9	101.5	78.0	80.6	80.0	99.1	74.3	76.5
Aporrectodea caliginosa adults	4.0	7.1	26.1	-70.6	17.0	18.1	26.0	-73.5
Aporrectodea rosea adults	28.1	15.3	11.5	26.6	24.7	3.2	10.7	22.5
Lumbricus castaneus adults	NA	-122.2	-130.0	NA	NA	-117.3	-127.1	NA
Lumbricus terrestris adults	3.3	16.3	19.5	40.5	28.6	15.5	32.7	50.1
total adults	-0.5	8.9	-6.4	4.2	11.5	16.1	-1.2	24.3
total anecic adults	-1.3	21.7	12.4	46.4	24.7	18.6	28.4	51.7
total e-arthworms	11.6	13.3	21.2	-5.0	20.0	20.4	13.8	5.6
total endogeic	-0.2	13.5	1.1	12.8	6.3	17.6	-0.2	2.4
total epigeic	NA	-143.4	-136.7	NA	NA	-140.3	-131.8	NA
total epilobous adults	-0.6	14.3	-0.2	9.4	6.7	26.6	-9.6	-3.2
total epilobous juveniles	19.4	17.8	25.0	-1.4	23.2	31.1	35.7	-6.4
total juveniles	16.6	17.4	21.4	-2.3	23.9	33.4	29.9	6.7
total tanylobous adults	-17.8	12.9	17.7	40.3	9.5	6.8	30.9	47.1
total tanylobous juveniles	22.4	12.7	20.1	12.2	7.2	23.0	20.7	13.1

A.4 Comparison of CPCAT procedure and Dunnett test

Figure A4-1: Pilot field study - Difference of a treatment compared to the control [%] plotted against the respective calculated p-values for the CPCAT (blue dots) and Dunnett method (orange crosses). Data: Pilot field study, all sampling time points.
A: total earthworms, B: total adults, C: total epilobous adults, D: total endogeic, E:total anecic adults, F: total tanylobous adults, G: *Allolobophora chlorotica* adults, H: *Aporrectodea rosea* adults, I: *Aporrectodea caliginosa* adults, J: *Lumbricus terrestris* adults, K: *Aporrectodea longa* adults, L: *Octolasion cyaneum* adults, M: total juveniles, N: total epilobous juveniles, O: Aporrectodea Allolobophora spp.juveniles, P: total tanylobous juveniles, Q: Lumbricus spp. juveniles



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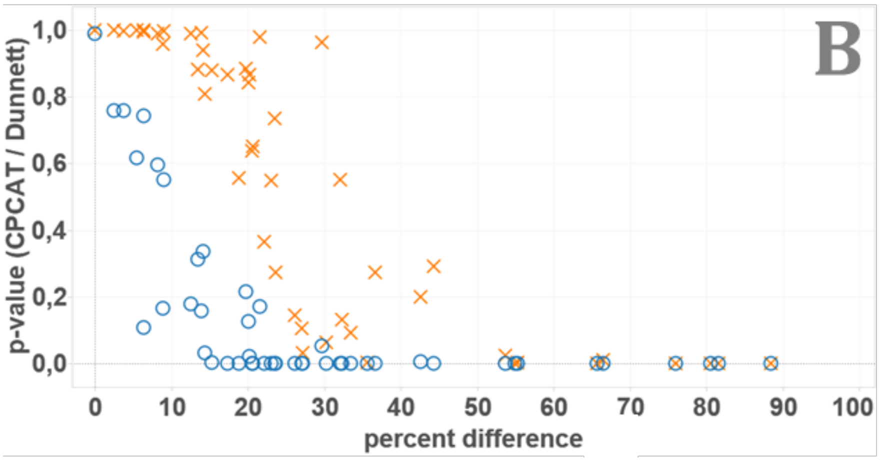

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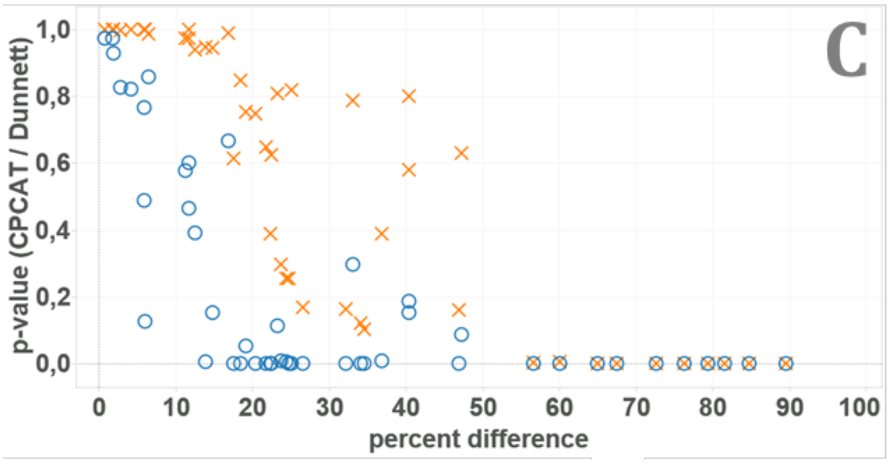

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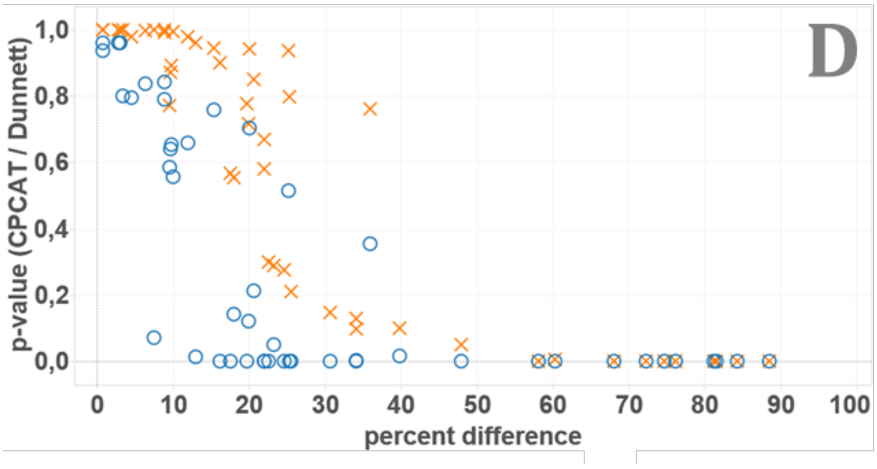

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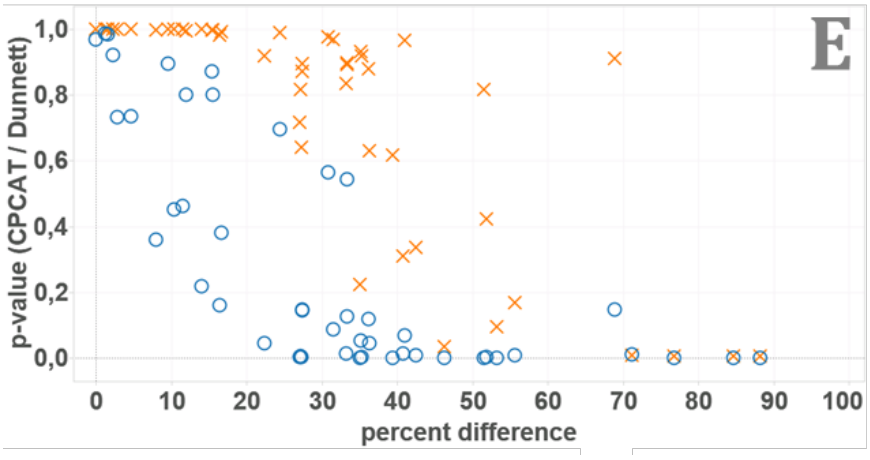

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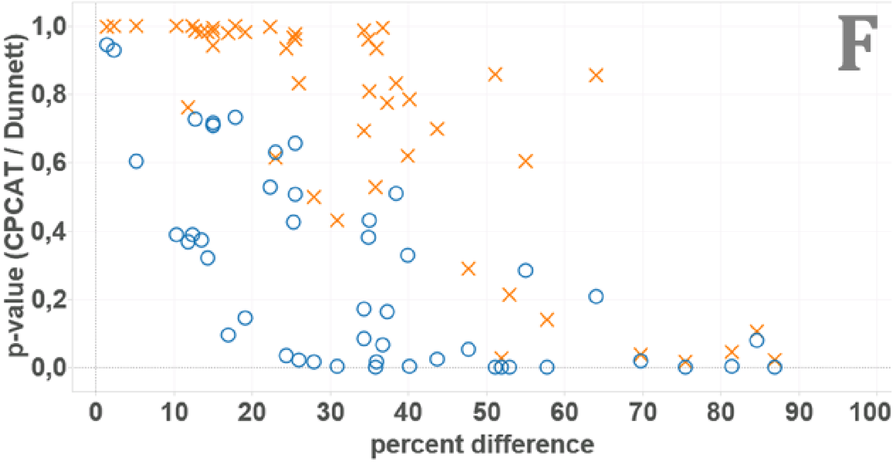

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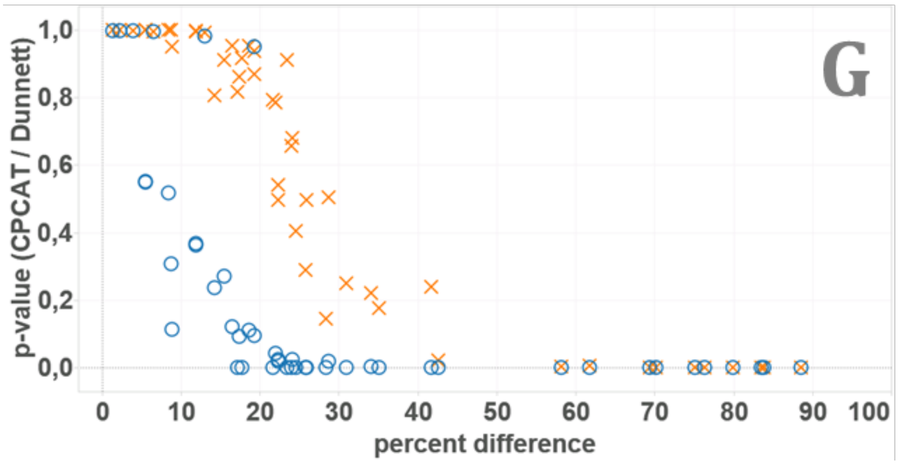

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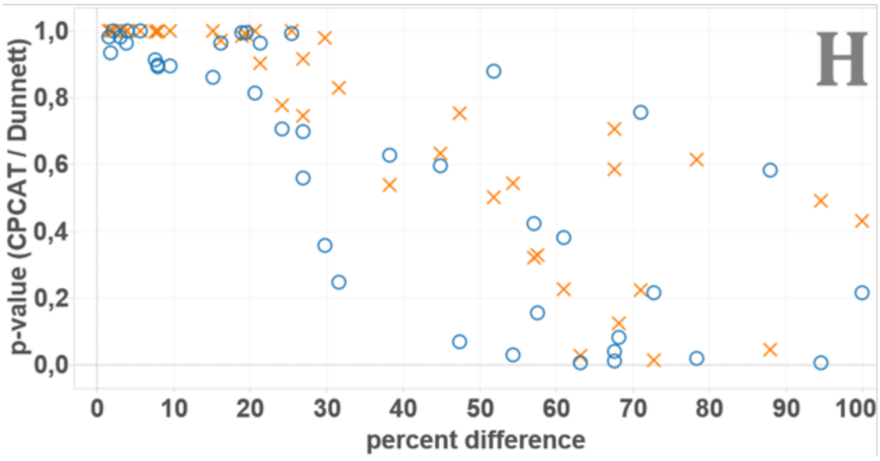

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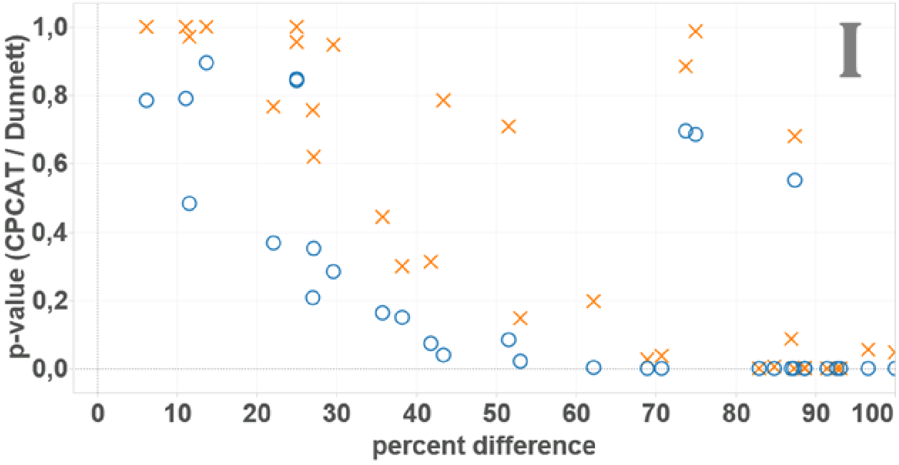

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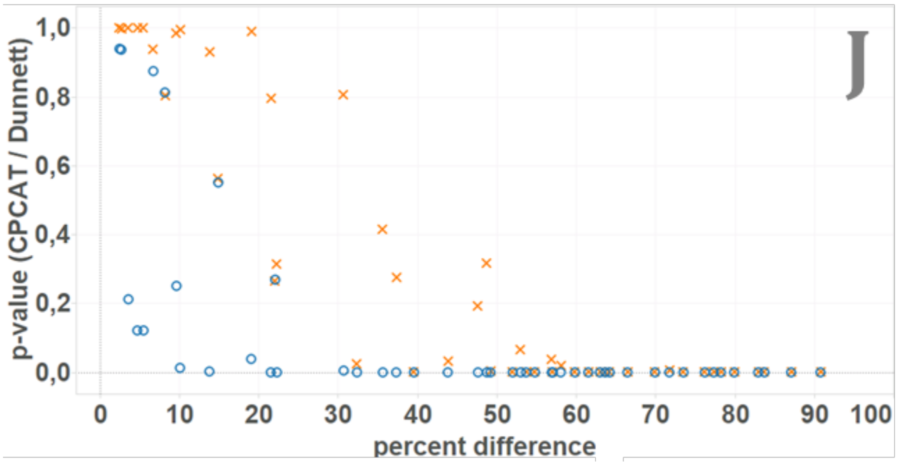

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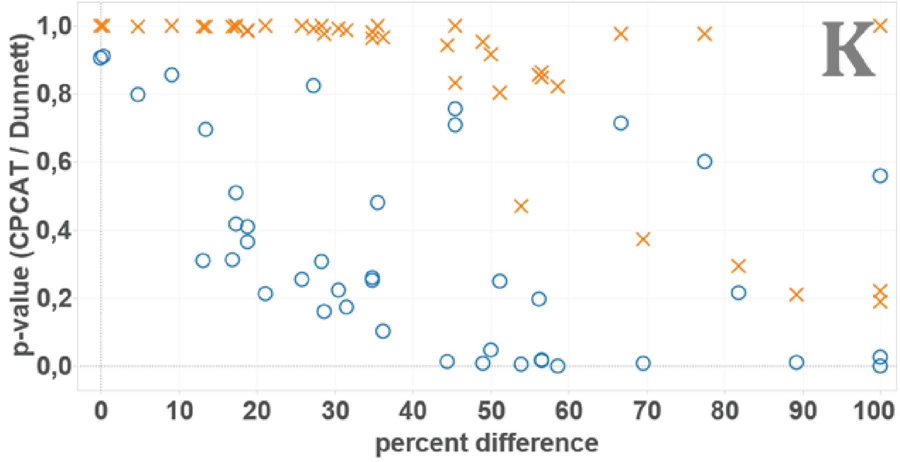

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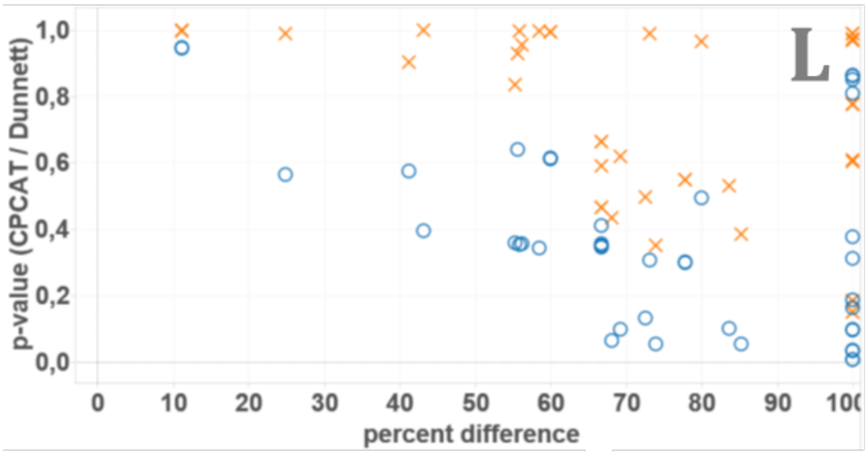

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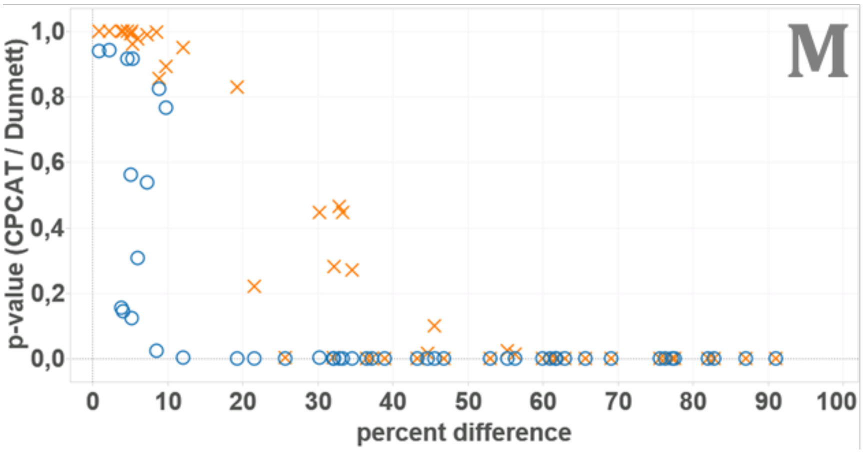

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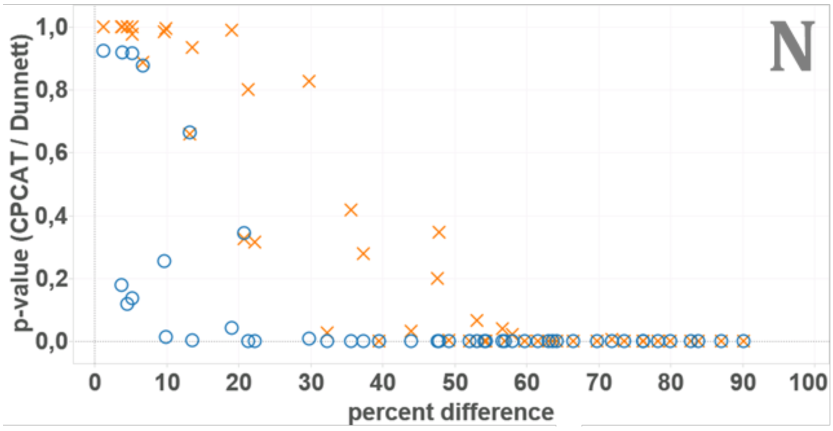

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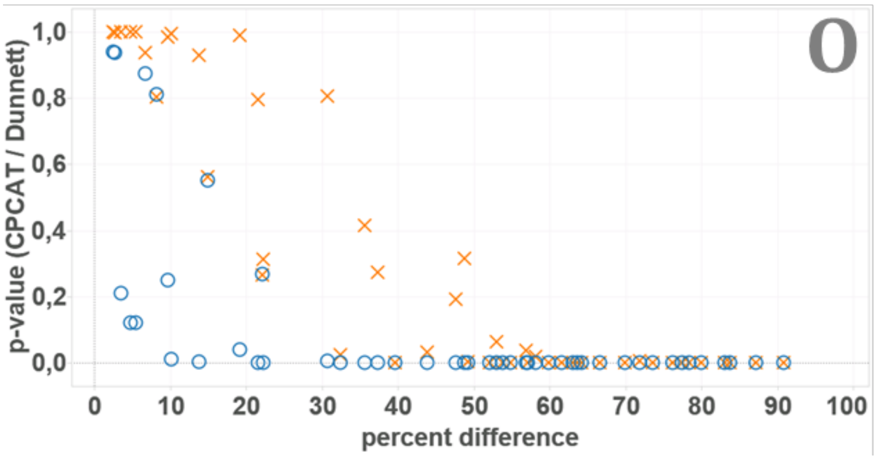

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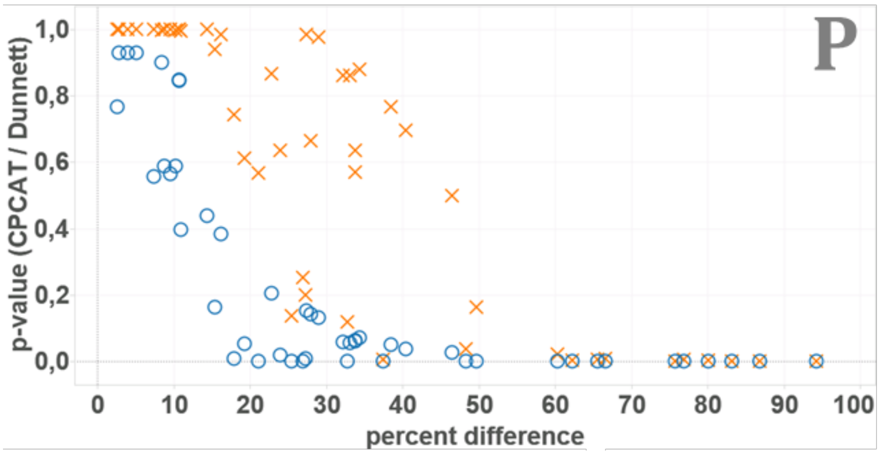

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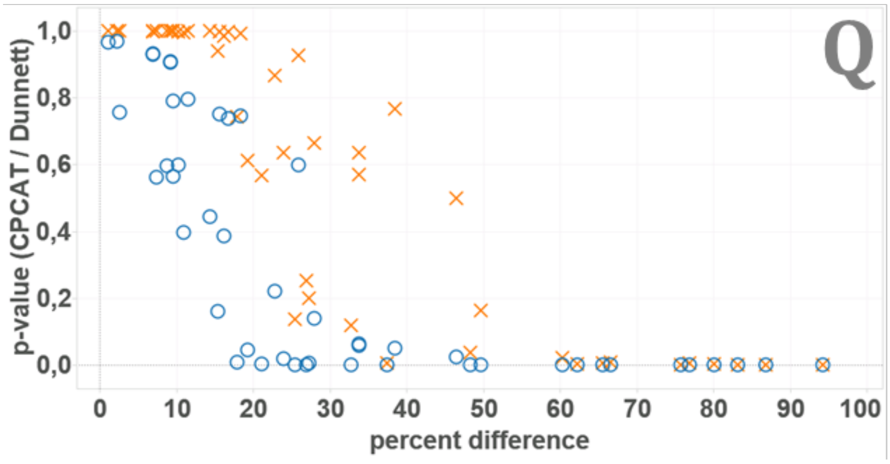
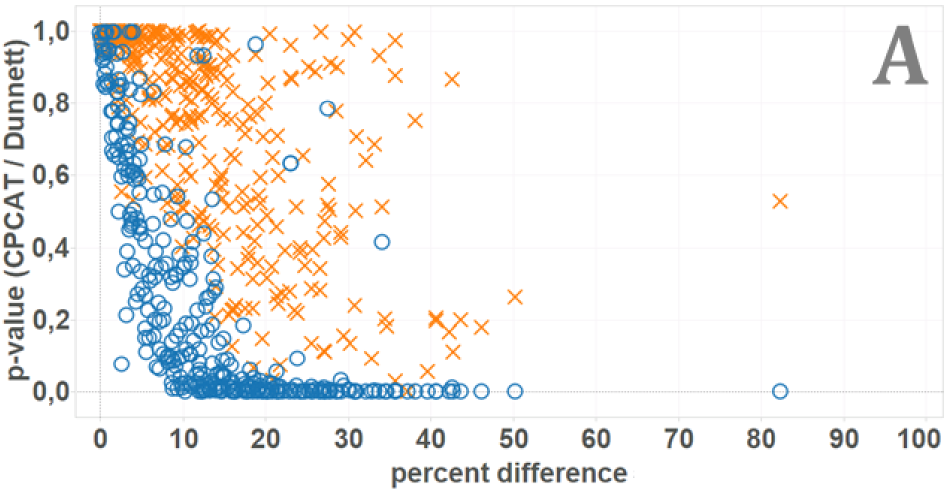


Source: RWTH Aachen University

Figure A4-2: **Database field studies – Difference of a treatment compared to the control [%] plotted against the respective calculated p-values for the CPCAT (blue dots) and Dunnett method (orange crosses). Data: database field studies (4 x 4 plot design) All sampling time points.**
A: total earthworms, B: total adults, C: total epilobous adults, D: total endogeic, E: total anecic adults, F: total tanylobous adults, G: Allolobophora chlorotica adults, H: Aporrectodea rosea adults, I: Aporrectodea caliginosa adults, J: Lumbricus terrestris adults, K: Aporrectodea longa adults, L: Octolasion cyaneum adults, M: total juveniles, N: total epilobous juveniles, O: Aporrectodea Allolobophora spp.juveniles, P: total tanylobous juveniles, Q: Lumbricus spp. juveniles



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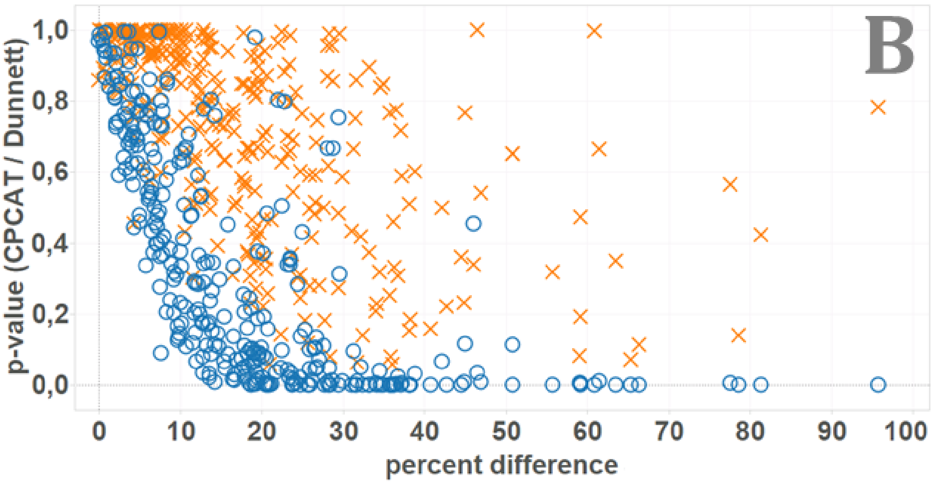

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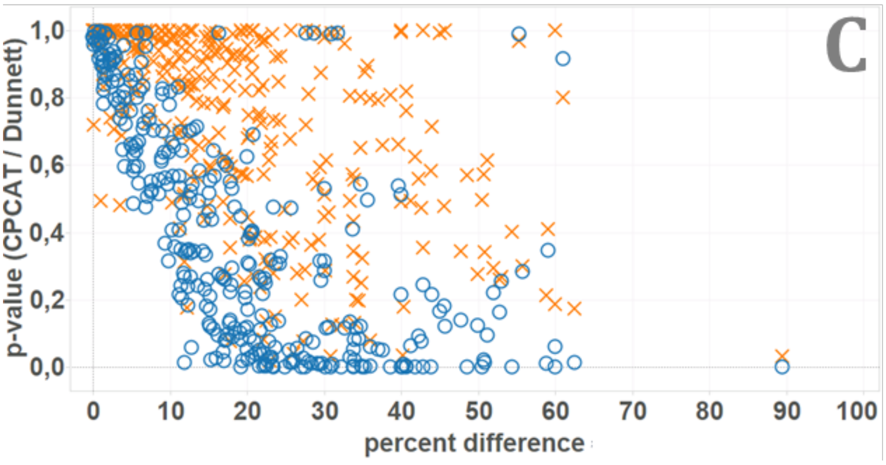

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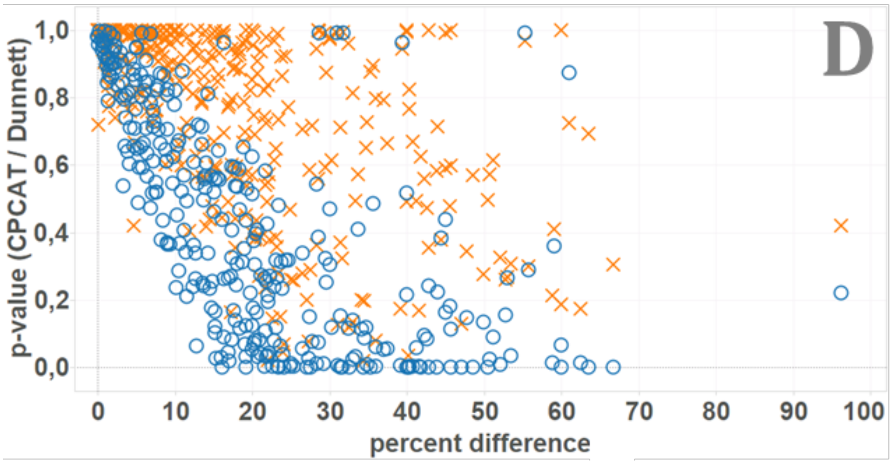

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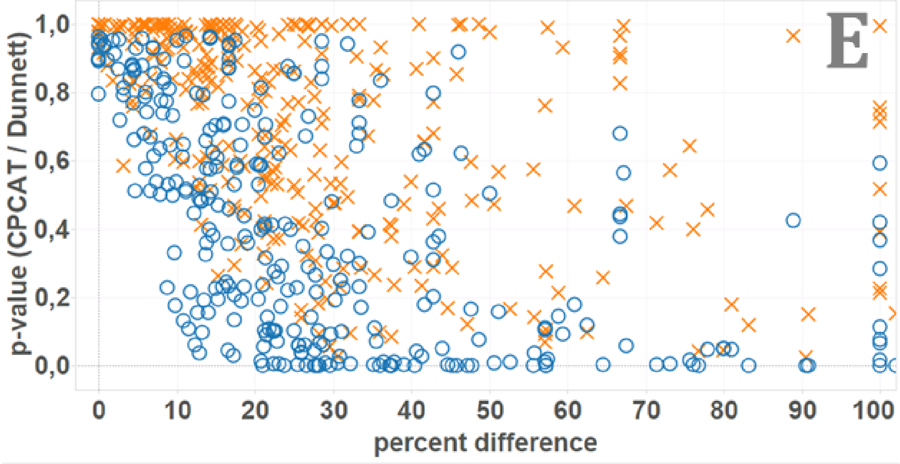

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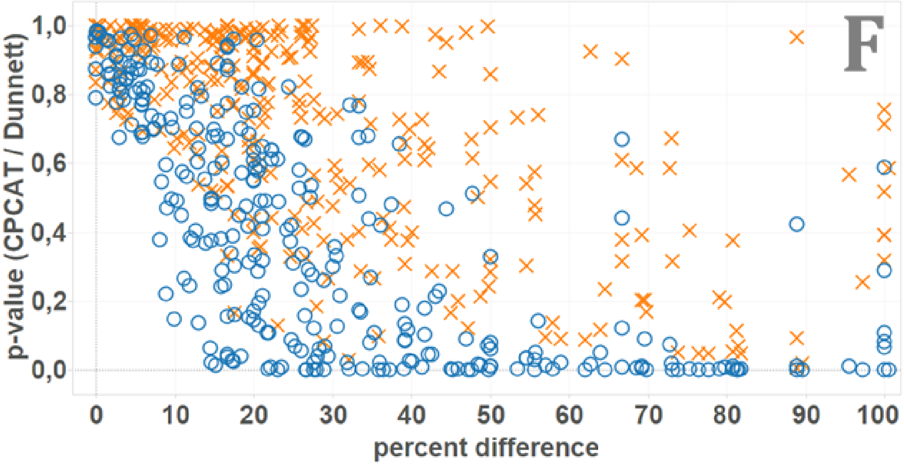

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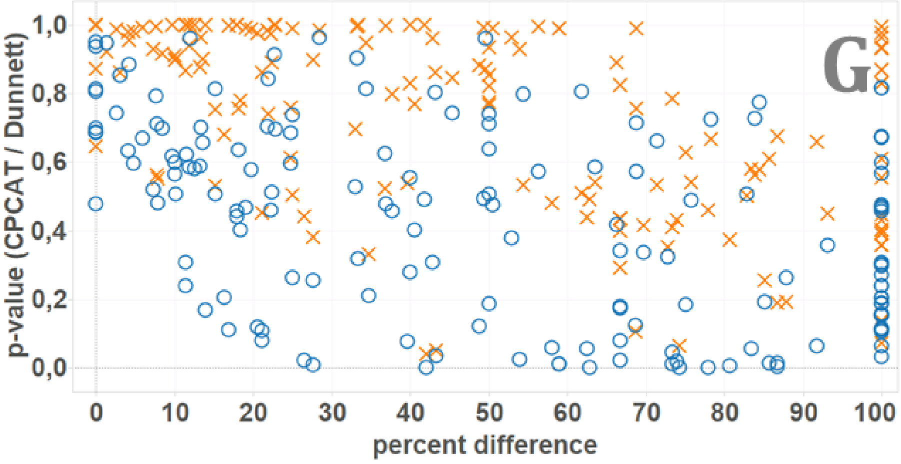

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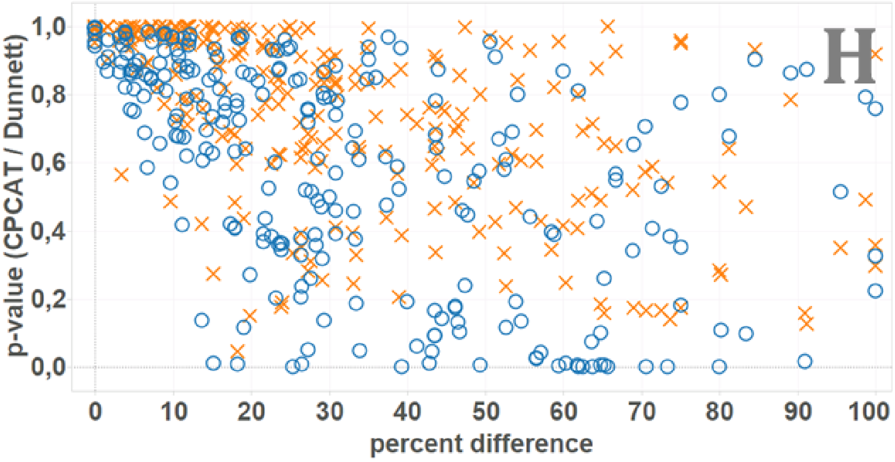

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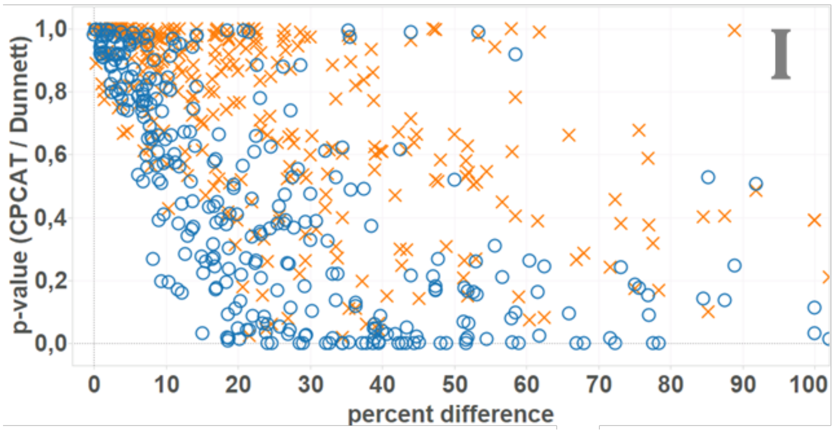

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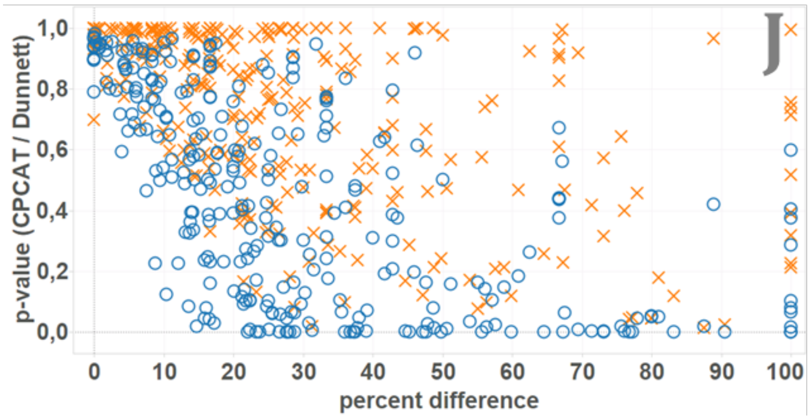

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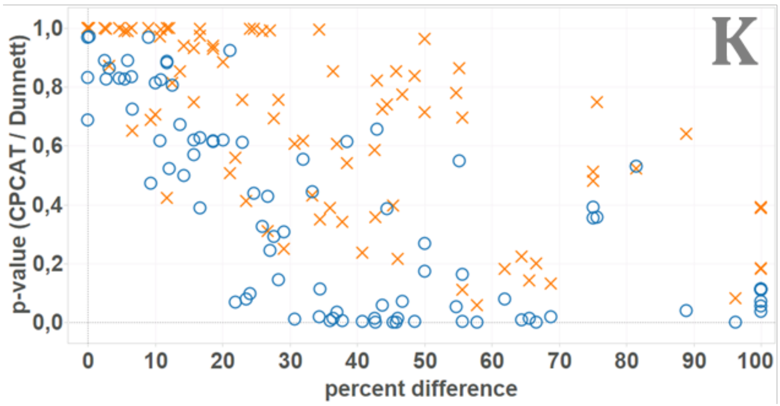

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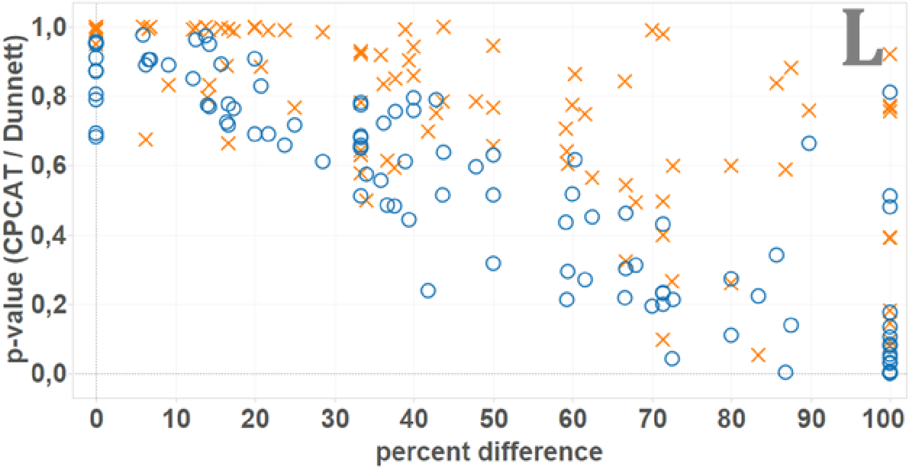

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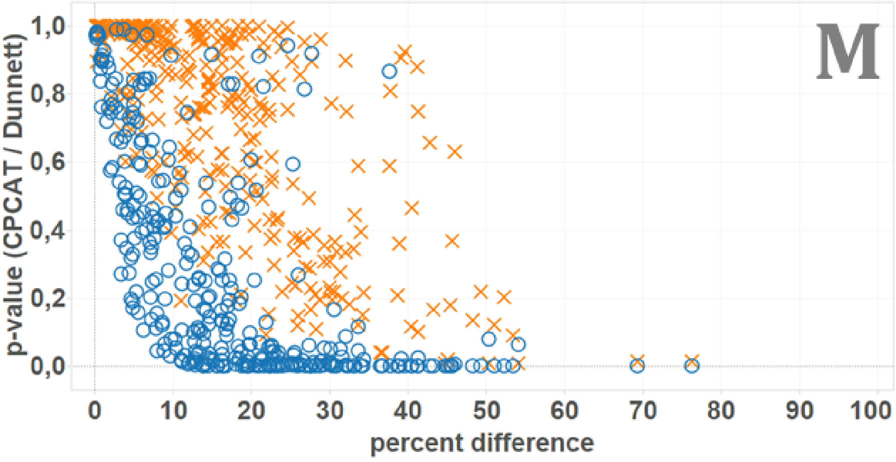

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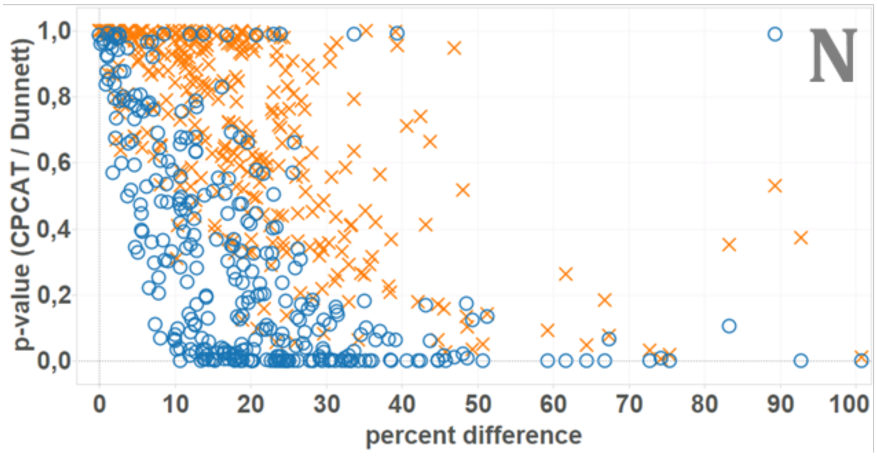

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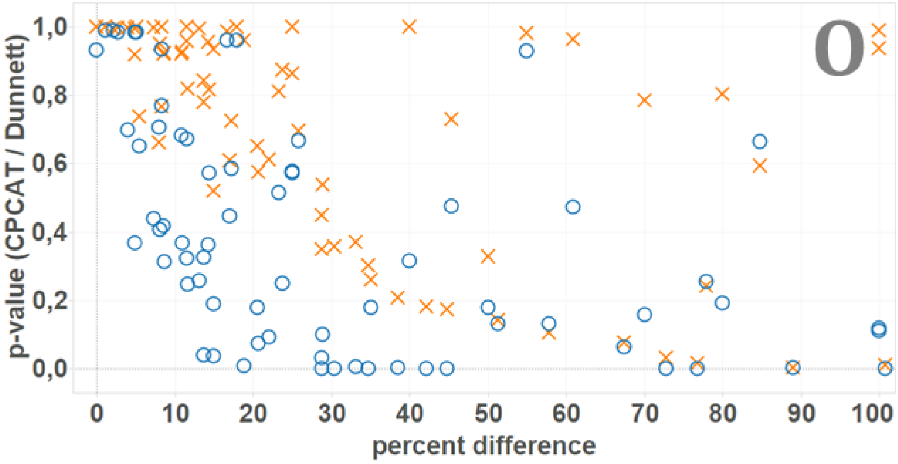

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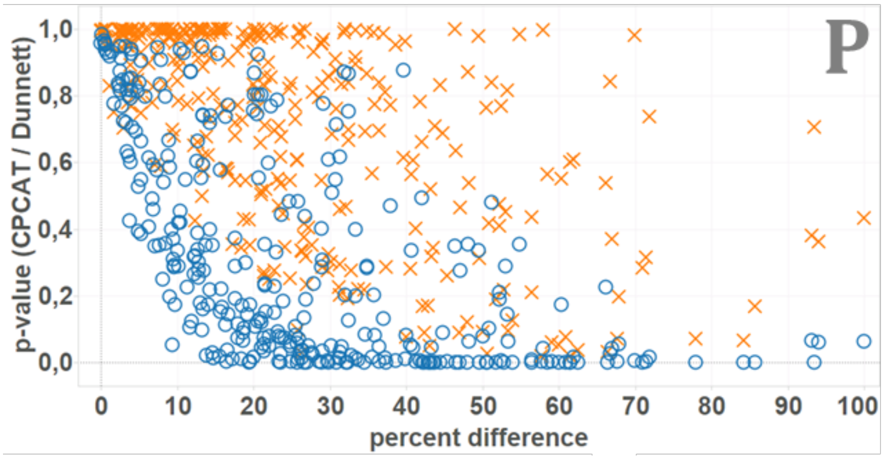

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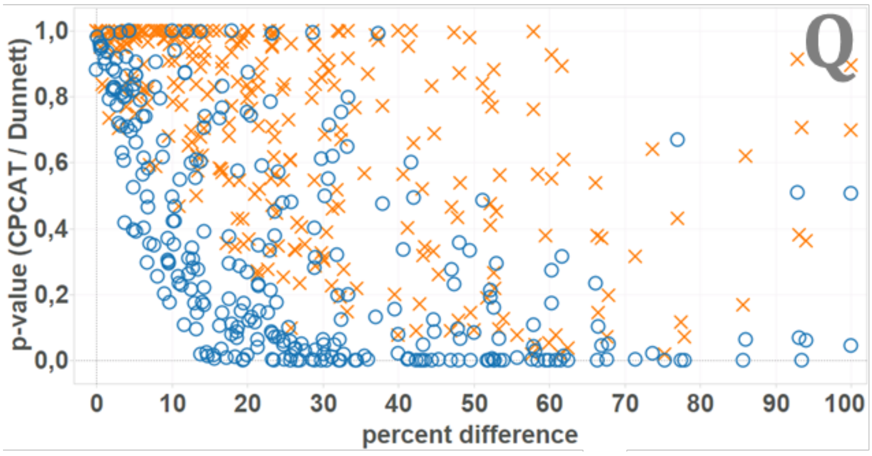

Source: RWTH Aachen University

A.5 Minutes of the two ad hoc SETAC GSIG sub-group project meetings in Flörsheim (2017) and Dessau (2019)

A.5.1 Flörsheim (2017)

Minutes of the meeting of the "OECD-GSIG-Earthworm-Field-Group" (Flörsheim, February 20th and 21st 2017)

Minutes:	Jörg Römbke
Schedule:	See attached (Annex 1)
Attendees/Invitees:	See attached (Annex 2)

1. Preface

These minutes do not contain the discussion in detail but just summarize our contributions, focusing on those issues which are directly addressing the main topic of the meeting, i.e. the design of the earthworm pilot field study to be started in the first week of April, 2017.

Further details regarding the background of this project, the history of the "OECD-GSIG-Earthworm Field Group" were given in slides presented by Jörg Römbke (see Annex 3).

2. Aims of this meeting

The aims of this meeting can be summarized as follows:

- Dissemination of information:
  - Background considerations of the project (ECT)
  - Presentation of data sets from earthworm field studies (UBA)
  - Statistical analysis of these data (e.g. in terms of MDD) (RWTH)
  - Statistical analysis of six BASF field studies (P. Kabouw)
- Proposal for the study design of a pilot field study
- Discussion of the pros and cons of the pilot field study design (e.g. Limit, NOEC, ECx, number of plots and samples/plot)
- Conclusion on the performance of the pilot field study
- Information on the next steps of the project (e.g. the standardization process and the distribution of information)

3. Specific problems

3.1 Use of AITC as extraction fluid

Recently, some ISO member states have questioned the usage of AITC as an extraction fluid for earthworm field work, partly because it is very toxic to aquatic organisms. In addition, it is preliminarily classified by producing companies as being toxic for humans as well (e.g. when being swallowed). However, these are warning signs used for many chemicals. Very briefly the group discussed this problem. According to the ECHA homepage this chemical is still under review by ECHA. So, there is no change regarding its use as an extraction fluid in earthworm sampling programs (e.g. recently ISO reviewed its guideline for earthworm monitoring but without withdrawing the use of AITC).

In addition, several issues regarding the new OECD standard were mentioned, which need clarification (e.g. how much and which residue analysis will be required, how to handle the assessment of individual species versus ecological groups etc.). However, the participants decided to focus the discussions of this workshop on the design and performance of an earthworm pilot field study.

3.2 Availability of the test substance carbendazim

The group discussed briefly the availability of the reference substance carbendazim, which is planned to be used as model chemical in the earthworm pilot field study. Basically, the situation got worse in the last year, since it is more and more difficult to get carbendazim for scientific purposes from outside Europe. Right now, import from Brazil or China seems to be the only possibility, but both options are not easy and are time-consuming. It was proposed to organize a group of “carbendazim users” which has the aim to get enough carbendazim to run several earthworm field studies. However, details are not yet clear.

4. Discussion on the design of the earthworm pilot field study

4.1 Background notes

In mid-2016, the German UBA launched a call for a project entitled “Necessary adaptations of the standard Earthworm Field Test”. Silvia Pieper briefly explained that Germany - in its role as lead country for the preparation of the new OECD standard on earthworm field testing - saw the need for scientific input in order to improve specific aspects of the new OECD standard. This project is going to be performed by a consortium consisting of ECT Oekotoxikologie GmbH and RWTH Aachen University (i.e. the “Project Team”). During the second half of 2016, this team met several times in order to discuss the outcome of the compilation of earthworm field data. This data set was statistically evaluated by the RWTH colleagues. It served as a basis for developing a proposal for a new study design for the earthworm field test.

4.2 Further information

Before starting the discussion on the design of the earthworm pilot study itself, further recent contributions addressing different aspects of the planning, performance or evaluation of earthworm field studies were presented to the group. Tobias Vollmer and colleagues (Vollmer et al. 2016) performed an assessment of the results of 26 standard earthworm field studies performed in Germany, France and Spain according to the ISO Guideline 11268-3. In the context of this project, two results of their work are important:

- The statistical power of the current earthworm field test is suitable to detect medium effects (35 – 65%) for total abundance or dominant species, but is not sufficient for small effects (10 – 35%), especially for individual species.
- Increasing the number of plot replicates beyond 6 is not a practical option to overcome the natural variation of earthworm populations.

The outcome of six standard earthworm field studies was summarized by Patrick Kabouw (Andrade et al. 2017) as follows: The current design of earthworm field studies provides a suitable degree of statistical power when earthworm density is sufficiently high, considering the magnitude of effects that are relevant at the earthworm community level.

4.3 First proposal (starting point at the beginning of the workshop)

The design of the earthworm pilot field study was distributed before the workshop among the members of the whole group. Especially those colleagues who could not attend in person provided valuable input, which was included in the discussions as much as possible. While basic considerations leading to this proposal were summarized in the invitation document, no statistical details had been provided. Thus, Björn Scholz-Starke presented the results of the statistical analyses of existing field studies - mainly done at the RWTH Aachen (for details see the slides presented in Annex 4). The results of the evaluation lead to the first proposal of the earthworm pilot field study design to be performed in 2017 (Fig. 1). This design is characterized by combining a so-called NOEC- with an ECx-design. As in the ISO guideline, four sampling dates are proposed, covering in total a study duration of one year. One control (C) and six treatments (T) are used. The number of plots per treatment differs between six (C, T2, T5) and three (T1, T3, T4, T6). The number of samples per plot is higher than in the currently used guideline (five instead of four). Running such a study means that in total 30 plots with 150 samples have to be covered. This original proposal was considered by the project team as large but still practical in terms of handling (e.g. number of days needed for sampling), field size etc.

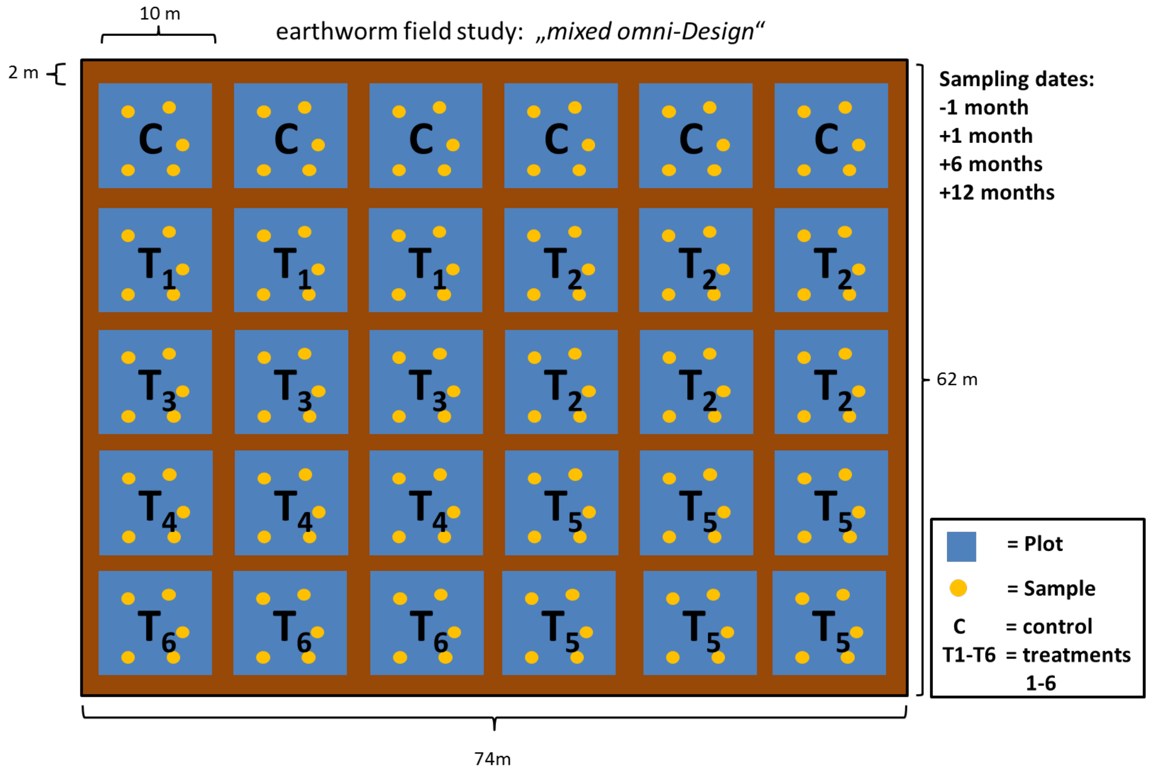

Figure 1: Original proposal for the design of the pilot earthworm field study provided prior the meeting in Flörsheim.

In the discussions during the meeting (and partly even after the meeting, especially with Olaf Klein and Tobias Vollmer) various changes were proposed, all of them with the intention to improve the quality of the output but without increasing the efforts at the same time – without doubt a real difficult task.

In detail, the number of plots dedicated to the NOEC-part of the study was kept constant, i.e. the control and two treatments, each of them with 6 plots per treatment. The number of samples (in the ISO version: four) differed strongly: in the original proposal it was five, but this idea was not really supported by the group due to theoretical considerations (i.e. the difference between four and five seems to be quite small) and to the experiences with eight samples per plot presented by Patrick Kabouw and published quite recently (Andrade et al. 2017). However, such a high number of samples per plot in the planned field study would mean that the total number of samples to be taken would be about or even higher than 200.

In the light of the analysed and expected variability in local site conditions regarding soil properties, history and, thus, earthworm distribution, a compromise was found – a so-called “Balanced design”. It is planned to take the same number of samples per plot in the NOEC- as well as in the EC-plots (six), whereby the number of NOEC plots will be 6 and the ECx plots will be 3 per treatment, respectively.

4.4 Identification of the test substance concentrations

From the very first beginning of this project the chemical to be tested was carbendazim, because it is by far the best-studied pesticide in soil ecotoxicology:

- It has been used as reference substance in ISO earthworm field studies for almost 20 years (partly in parallel with the parent active substance benomyl). Information from (some of) these studies have been collected by the UBA;
- Carbendazim was used in an EU project focusing on the development of a standard semi-field method where Terrestrial Model Ecosystems have been employed (e.g. Knacker et al. 2004; Römbke et al. 2004).

It had been expected that the availability of carbendazim would be problematic. However, with the help of the colleagues from Eurofins (Olaf Klein, Tobias Vollmer) enough test substance could be gathered in the weeks after the workshop. However, there are no reserves left.

Using the available information from these different sources, various carbendazim concentration ranges were discussed. In detail, information from regulatory field studies (in total 16 studies) were compiled by UBA and were assessed by the RWTH colleagues, together with data from the literature (especially the EU TME ring test). For details, see the slides of the presentation by Scholz-Starke et al. during the workshop (see Annex 4). For this exercise, it was assumed that the assessment date about 4 – 6 months after application would be most suitable / robust one in order to decide which treatment rates to select. This was for reasons of comparability of different sources of effect data, because in the literature (Römbke et al. 2004) most information was found on TME that were sampled 16 weeks after application of carbendazim.

The following six application rates (plus a negative, i.e. water-only, control) were finally selected in order to cover a range spanning from concentrations where no effects are expected to concentrations where strong effects are likely (Table 1).

It should be noted that the spacing factor is not fixed between the different treatments. This approach is already used in laboratory tests following the ECx design (e.g. the earthworm reproduction test) where it is stated that “The spacing factor may vary, i.e. less than or equal to 1.8 in the expected effect range and above 1.8 at the higher and lower concentrations” (OECD 222, 2004). While this factor is as high as 3 at the lowest and highest rates, it is about 1.8 at the centre of the treatment range.

Table 1: Application rates of the earthworm pilot field study. Concentrations are given in kg active substance (a.s. carbendazim)/hectare (ha).

Treatments	T1	T2	T3	T4	T5	T6
	0.6	1.8	3.2	5.8	10.5	31.5

In the currently used ISO guideline 11268-3 (1999), the reference substance carbendazim should yield a statistically significant difference of at least 50 % on overall abundance and/or biomass compared to the control at least at one sampling date, when applied at rates of 6 to10 kg a.s. carbendazim/ha. Thus, such effects should be detectable at the three highest application rates. Accordingly, and referring to the experiences made in the EU project mentioned above, no detectable effects should appear at the two lower rates. A priori analyses have shown that an EC_{50} could be expected at rates around 2.5 kg carbendazim/ha.

5. Next steps after the workshop

Note: The activities described in the section of the minutes refer to work performed after the workshop. They were included in order to provide group members with the newest information available.

5.1 Practical work

The preparation of the practical work got highest priority due to the very small "window of opportunity" for running such a study. In detail, the following activities were performed:

- Identification of a suitable study site. Due to long-term relationships with local farmers an appropriate test site (size: 107 m by 52 m was found in early March in a distance of less than 10 km from the laboratory of the ECT GmbH. From the 32 plots which were installed 30 plots (No. 2 to No. 31) are used in the study (see Fig. 2). The plots were surveyed immediately afterwards.
- A full characterization of soil properties is still under way, but the soil type is probably a silty loam.
- The site is almost free of vegetation, since – as usually done in such studies – glyphosate was applied once on March 16, 2017 (1.8 kg a.s./ha).
- In parallel, the amount of carbendazim necessary for the test design described above was delivered from Eurofins GmbH.
- In the first week of April, the first earthworm sampling was performed (Fig. 3). Despite the fact that no detailed evaluation has been performed yet it became clear that the site is well inhabited by earthworms.
- In the second week of April (11.04.2017), the test chemical was applied. Immediately afterwards, the whole site was irrigated. No puddles appeared.
- The first post-application earthworm sampling was performed in the third week of May.

In summary, despite the very tight project schedule, the study is on track. All formal requirements of the current ISO guidelines have been fulfilled.

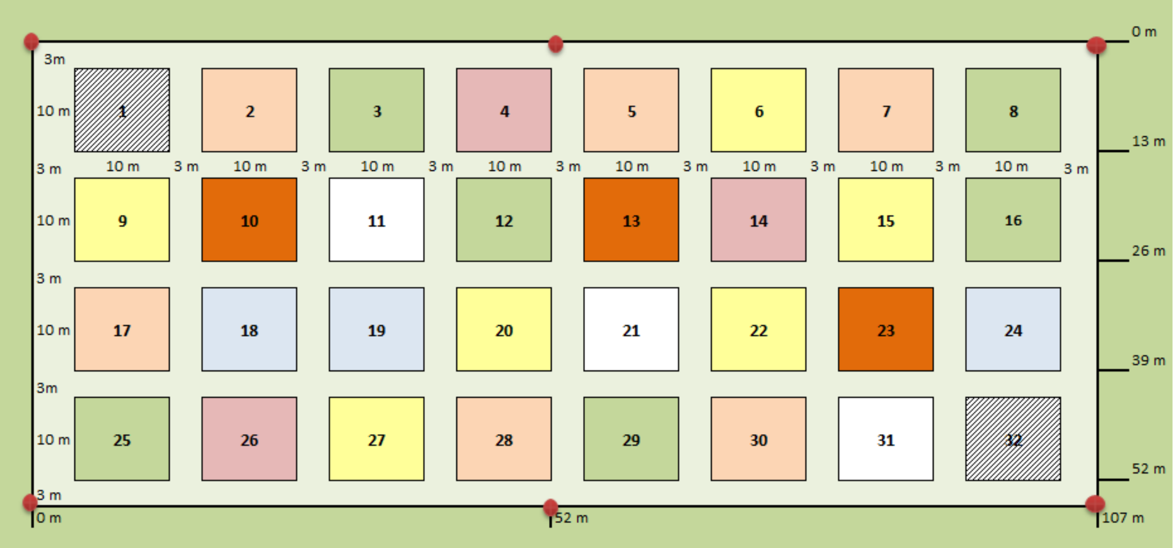

Figure 2: **Scheme of the earthworm pilot field study. From the 32 plots which were installed 30 plots (No. 2 to No. 31) are used in the study (colours indicate different treatments).**

Figure 3: **Impression from the first earthworm sampling (i.e. before application) at the study site near Wicker**

5.2 Statistical evaluation

The statistical analysis of all data collected aims at supporting the guideline developers with reliable information on the probability to achieve sufficient power of a study design to detect effects of substances toxic to earthworms. It will be primarily investigated if applying dose-response designs in earthworm field studies would be a feasible option, as demonstrated by the balanced design of the pilot field study. Furthermore, the project team develops algorithms to simulate different detection levels of alternative experimental designs (varying either the number of plots per treatment or samples per plot, or both) that are covered by or can be extrapolated from the pilot field study. Recommendations for an appropriate approach in the statistical analysis of field study data (dose-response regression and effect threshold detection) will be derived from the statistical analysis of the pilot field study data and the resulting alternative designs.

5.3 Additional work

Several participants provided ideas on how the performance of an earthworm field study could be modified in order to increase the amount and/or quality of the information gained in such an exercise. For example, the following issues were mentioned:

- Analytical chemistry: right now, this is mainly used to confirm the exposure of the earthworms. It was discussed whether a more detailed chemical sampling would be envisaged, but such an effort was not scheduled in the project plan and budget. Therefore, the application of the test chemical was checked as follows: the volume of the actually applied spray solution per plot was measured and recorded.
- However, in order to address the exposure of earthworms in a more direct way, soil samples (diameter 5 cm; depth: 0 - 10 cm) were taken in parallel to the first sampling and at the end of the study. These samples will be kept deep-frozen at ECT GmbH, since the performance of the analytical work (in particular the costs) is not clear yet.
- The question was raised whether and how quickly earthworms can re-enter plots applied with the test chemical. In this context, the broadness of the strips between plots was discussed: currently, 2 m seem to be common, but 5 m have been used as well. Therefore, we used a width of 3 m between plots for reasons of practicability (see Fig. 2). In addition, it was recommended to study the recolonization (extent, time frame) of earthworms after the application of a test chemical in detail.

5.4 The OECD standardization process

Since early 2017, this project activity is running in parallel to the preparation of an OECD Earthworm Field Guidance Document. At a later stage, OECD will provide draft templates in order to facilitate the process of preparing the guidance document. The last sampling will be in April 2018, followed by the evaluation of the results. By the end of 2018, a meeting of the OECD-GSIG group is envisaged, with a clear focus on the evaluation of the results. In the beginning of 2019, OECD is expecting a first draft of the new document. Afterwards, the draft document will be discussed by national experts. Assuming that no substantial problems will occur, the document could be approved by OECD in the second quarter of 2019. The minutes of this meeting will be provided to OECD by Susanne Walter-Rohde.

5.5 Further meetings

The next meetings of the Project Team or of the “OECD-GSIG-Earthworm-Field-Group” are not fixed yet. However, the start of the pilot study was briefly presented at the GSIG meeting, held during the SETAC Europe Conference in Brussels, in the second week of May. The next meeting of the Project Team will happen when the first results of the earthworm samplings will be available, i.e. during late summer at the earliest. Probably, the next meeting of the “OECD-GSIG-Earthworm-Field-Group” could be organized during winter, assuming that at that point earthworm data from three sampling dates are ready.

6. References

Andrade, T.O., Bergtold, M. & Kabouw, P. (2017): Minimum significant differences (MSD) in earthworm field studies evaluating potential effects of plant protection products. J. Soils Sediments doi:10.1007/s11368-017-1662-z.

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Vollmer, T., Klein, O. Frank, S. & Knaebe, S. (2016): Statistical power and MDDs in Earthworm Field Testing. Poster presented at the SETAC Europe Conference in Nantes.

Annex 1: Schedule

Monday: February 20, 2017	
13:00	Welcome address, technical notes and brief introduction round *Jörg Römbke (ECT)*
13:15	Background and aims of the project *Silvia Pieper (UBA)*
13:30	Work done since Nantes and aims of this workshop *Jörg Römbke (ECT)*
13:45	Assessment of existing data on earthworm field studies *Björn Scholz-Starke (RWTH Aachen)*
14:30	Summary of a new study (Andrade et al. 2017) *Patrick Kabouw (BASF)*
14:45	*Coffee break*
15:00	Discussion of the new proposal *ALL*
17:30	*End of the first day*
19:00	Workshop dinner at the Restaurant BOOTSHAUS (Flörsheim)
Tuesday: February 21, 2017	
09:00	Summary of the discussion on the previous day Silvia Pieper
09:30	Practical performance of the pilot study (and additional work) Jörg Römbke
10:30	*Coffee break*
11:00	Status and follow-up of the OECD-Standardization process *Susanne Walter-Rohde (Umweltbundesamt)*
11:30	How to proceed: who is going to do what and when? When and where will this group meet again? *Silvia Pieper*
12:00	Public relations *Jörg Römbke*
12:15	Final discussion *ALL*
13:00	*End of the workshop*

Annex 2: Attendees/Invitees

Note that the list of invited participants is given in alphabetical order. Names of colleagues who were attending the meeting in person are given in bold.

Name	Employer	Country	Comment
Annette Aldrich	Agroscope	Switzerland	No feedback
Sophie Campiche	Ecotox Centre	Switzerland	Excused
Tamara Coja	AGHFS	Austria	No feedback
Mike Coulson	Syngenta	United Kingdom	Excused
Benjamin Daniels	**RWTH Aachen**	**Germany**	
Frank de Jong	RIVM	The Netherlands	Excused
Axel Dinter	**DuPont**	**Germany**	
Gregor Ernst	**Bayer CropScience**	**Germany**	
Matthias Ganssmann	IBACON	Germany	No feedback
Simon Hoy	PSD	United Kingdom	Excused
Stephan Jänsch	**ECT**	**Germany**	
Patrick Kabouw	**BASF**	**Germany**	
Olaf Klein	**EAS Ecochem**	**Germany**	
Pia Kotschik	**UBA**	**Germany**	
Silvo Knäbe	EAS Ecochem	Germany	Excused
Christine Kula	**BVL**	**Germany**	
Visa Nuutinen	MTT	Finland	Excused
Pascal Pandard	INERIS	France	No feedback
Celine Pelosi	INRA	France	Excused
Silvia Pieper	**UBA**	**Germany**	
Juliska Princz	Environment Canada	Canada	Excused
Jörg Römbke	**ECT**	**Germany**	
Martina Ross-Nickoll	**RWTH Aachen**	**Germany**	
Stefanie Schabio	IBACON	Germany	No feedback
Lisbeth Schnug	Bioforsk	Norway	No feedback
Björn Scholz-Starke	**RWTH Aachen**	**Germany**	
Lennart Schulz	BioChem agrar	Germany	No feedback
Els Smit	RIVM	The Netherlands	Excused
Kees van Gestel	FU Amsterdam	The Netherlands	Excused
Tobias Vollmer	**EAS Ecochem**	**Germany**	
Susanne Walter-Rohde	**UBA**	**Germany**	

A.5.2 Dessau (2019)

Minutes of the Meeting of SETAC GSIG / OECD ad hoc Working Group and final expert discussion of the UBA project „Necessary adaptions for a harmonized field-testing procedure and risk assessment of earthworms (terrestrial)", Dessau, 28./29.03.2019

Participants

Name:	Affiliation:
Benjamin Daniels	RWTH Aachen University
Axel Dinter	FMC Agricultural Solutions
Gregor Ernst	Bayer CropScience AG
Bernhard Förster	ECT Oekotoxikologie GmbH
Stephan Jänsch	ECT Oekotoxikologie GmbH
Florian Kaiser	ECT Oekotoxikologie GmbH
Olaf Klein	Eurofins Agroscience Services Ecotox GmbH
Pia Kotschik	Federal Environmental Agency
Christine Kula	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
Richard Ottermanns	RWTH Aachen University
Silvia Pieper	Federal Environmental Agency
Jörg Römbke	ECT Oekotoxikologie GmbH
Martina Roß-Nickoll	RWTH Aachen University
Björn Scholz-Starke	RWTH Aachen University, currently darwin statistics
Tobias Vollmer	Eurofins Agroscience Services Ecotox GmbH
Susanne Walter-Rohde	Federal Environmental Agency

Note: These minutes do not repeat the information given on the presented slides at the workshop but focus on the discussion within the group. They do not strictly reflect the chronology of the discussion during the workshop. Some issues (e.g. possible use of sub-plot replicates, analytical measurement, application of plateau concentration, "extended ISO design") were discussed controversially and no conclusion could be drawn at the meeting. These discussions will be followed up in the further process of the test guideline development.

1 Introduction

To avoid redundancies, the various discussion contributions and arguments have been summarized and separated into the following topics:

- Evaluation of UBA-studies as well as finding the design of the pilot field study;
- Practical performance of the pilot field study;
- Proposed new test designs;
- Statistical evaluation of future studies;
- Analytical measurement of the test chemical;
- Application of a plateau concentration;
- Experimental field management, testing of herbicides and other special cases;
- OECD standardization process.

2 Evaluation of earthworm studies in the UBA database as well as finding the design of the pilot study

In order to answer the two major research questions, the internal database of the UBA has been checked, focusing on two topics:

i) the description of variability within and quality of earthworm field study data;

ii) the assessment of statistical effect detection thresholds (e.g. MDD) and the analysis of dose-response relationships in the studies,

In a first step, 151 field studies included in the UBA database have been analysed according to the following quality criteria:

i) validity according to the ISO guideline,

ii) extraction method (formalin & hand-sorting),

iii) number of concentrations tested,

iv) reporting of subplots.

Twenty-one studies were identified that fulfilled the above listed criteria, describing biomass and abundance of 17 earthworm species, aggregated taxa and juveniles (on genus level). Based on the results of the analysed data of the 21 remaining field studies (variability, statistical power), a possible design for a pilot field study was investigated. The aim was a) to analyse the feasibility of increased sampling efforts in order to improve the statistical power and b) to explore the performance of a field study following a so called ECx- design. It should be noted that the chosen design for the pilot study is not the proposed one for the OECD Guideline, but it is the one that best suits the scientific questions above. For the pilot study, a so called “mixed omni-design” has been chosen (**Fig. 1**).

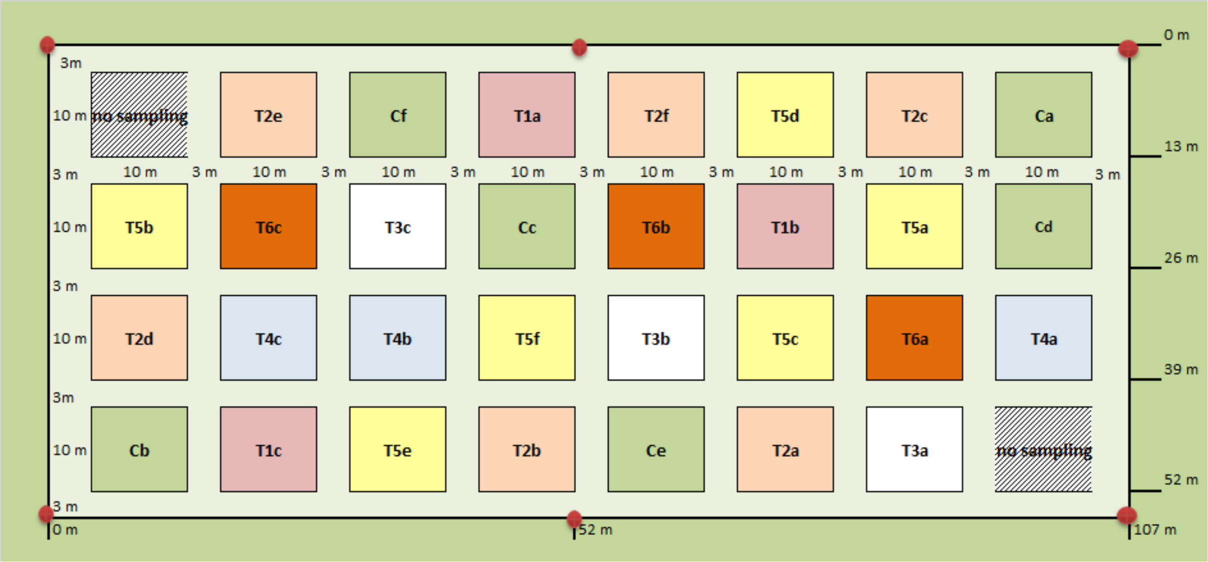

Fig. 1: Scheme of the trial area in a "mixed-omni design" with randomized allocation of the plots (squares). C (replicates a-f; control), T1, T3, T4, T6 (replicates a-c) and T2, T5 (replicates a-f; test chemical treated). The size of each plot was 10 m by 10 m and the distance between plots was 3 m

As carbendazim shows at given concentrations a high acute and chronic toxicity towards earthworms and has been used as reference substance for many years in field studies for earthworms, it has also been used in the pilot field study. The choice of the test rates was based on an evaluation of several test reports on field studies with earthworms, terrestrial model ecosystems as well as laboratory data with carbendazim (**Tab. 1**):

Tab. 1: Tested rates in the pilot test study with carbendazim. T = treatment.

C	T1	T2	T3	T4	T5	T6	Unit
0	0.6	1.8	3.2	5.8	10.5	31.5	kg/ha

Tab. 2: Important characteristics of the performed pilot study

Site information	Arable land near Flörsheim, Wicker (Germany)
Soil properties	pH ($CaCl_2$): 7.2; C_{org} [% dm]: 1.46; N_{tot} [% dm]: 0.17; $CaCO_3$ [% dm]: 0.4; Soil type: silt loam; WHC [% dm]: 55.3; CEC [cmol/kg dm]: 14.6
Site management	Glyphosate application (1.8 kg/ha, 16.03.2017)
Test item	carbendazim applied as Carbomax 500 SC suspension concentrate
Test rates	0.6, 1.8, 3.2, 5.8, 10.5, and 31.5 kg a.s./ha;
Earthworm sampling	8-6 DBA (pre-sampling), 34-36 DAA, 188-190 DAA, 377-379 DAA; Combined hand-sorting and AITC extraction with 5 to 10 l AITC solution (0.1 g/l) according to ISO 23611-1; 6 random samples per plot - distance between two samples at least 2 m; Sample area: 0.25 m^2 (50 x 50 cm) ~20 cm depth. Preservation 70% ethanol

3 Discussion on the practical performance of the earthworm pilot field study

The preferred standard method to sample earthworms in field tests is a combination of hand sorting and subsequent allyl isothiocyanate (AITC) extraction. Thus, this was used in the pilot field study. AITC is the current replacement for the formerly recommended formalin which has been banned for human health reasons. However, AITC is currently under discussion, actually for similar reasons. Therefore, other alternatives might emerge in the future. The octet (electrical) method is not recommended anymore as it is less efficient than the standard method and may not expel some earthworm species, especially under dry conditions often present in agricultural fields.

No crop was sown on the experimental field used for the pilot study for the following reasons:

- Sowing prior to application poses the risk of a vegetation cover and thus spray interception may be present during application (especially if the application would have to be postponed due to unfavourable weather);
- Sowing after application is technically difficult (due to small sized plots agricultural machinery can hardly be used due to the risk of cross-contamination);
- Although sowing a specific culture would enlarge the possibility of homogenous vegetation cover, a homogeneous growth of the culture across the experimental field cannot be guaranteed;
- Instead, natural vegetation was allowed to grow in the pilot field study.

The group discussed that, generally speaking, no-management could lead to an inhomogeneous development of the vegetation across the field – even if this was not the case during the pilot field study. Inhomogeneous vegetation could then influence the earthworm population due to varying soil moisture, soil temperature, and food availability. An alternative to no-management could be sowing of a crop shortly before application. A problem persists if an herbicide is to be tested, as varying vegetation cover between the plots may lead to differences in earthworm population (see also section on experimental field management and herbicide testing below). An alternative to using a broadband herbicide like glyphosate before test start in order to create homogenous conditions could be cutting the present vegetation as short as possible prior to application.

The strong effect of carbendazim in the pilot field study is probably due to the good food availability after application (i.e. the plant residues left on the field after glyphosate application) and the irrigation of the plots directly after application. The NOEC/NOER of carbendazim determined in the pilot field study was even lower than – on average - in the laboratory (< 0.6 mg a.i./kg DW vs. 1 – 5 mg a.i./kg DW). The duration of the effects is not equivalent to the persistence of the chemical in the soil (delayed effects) as the DT50 of carbendazim in soils is about 40 days. Regarding the practical efforts, the pilot field study worked well - but the actual area required for such an experimental field should not be increased further, because homogenous conditions at such a large scale are difficult to be found in reality.

4 Discussion of the proposed new test designs for earthworm field studies

Based on the evaluation of the dataset as well as the results of the pilot field study, two new test designs for the performance of earthworm field studies have been proposed for suggestion to OECD: the ECx/ERx test design as well as the mixed test design (**Tab. 2**).

Table 2: Study designs proposed by the consortium

Test design	Number of plots per treatment (4 samples per plot)									No. of plots (total)	No. of samples (total)
	C	T1	T2	T3	T4	T5	T6	(T7)	R		
ECx/ERx Design	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**(3)**	**3**	**24 (27)**	**96 (108)**
Mixed Design	**6**	**2**	**6**	**2**	**2**	**6**			**3**	**27**	**108**

C: Control; T1-T7: Test Chemical; R: Reference

In general, the aim of the proposed new ECx/ERx and mixed test design is to better characterize the risk of the test chemical under field conditions at different time points, regarding low risk/no observed effects levels (NOEC/NOER; EC10/ER10/20), small effects (e.g., EC/ER30) or higher effects (e.g. ≥ EC/ER50). However, the low number of replicates for the toxic reference might hamper the statistical power to detect differences from the control. This issue needs to the further discussed. In addition to the ECx/ERx and the mixed design proposed here as the result of this project, participants of the meeting proposed a third variant, i.e. a revisited NOEC/NOER design, which should be added to the OECD guideline to have more options under certain conditions. Ideally, several doses (e.g. three) with 6 plot replicates each and the same plot design for control replicates (called either "extended ISO design" or "NOEC/NOER revisited") could be applied (Tab. 3). This design was proposed by some participants, as 1) a lack of clear effects for majority of the test substances will impede a calculation of robust ECx for the majority of the substances and 2) that for some test chemicals the determination of an EC/ER50 is deemed difficult as a dose-response design cannot be achieved for technical reasons, e.g. herbicides, seed treatments, and other special use patterns.

Tab. 3: Additional study design suggested at the workshop for specific research/regulatory questions

Test design	Number of plots per treatment (4 samples per plot) each									No. of plots (total)	No. of samples (total)
	C	T1	T2	T3	T4	T5	T6	(T7)	R		
Revisited NOEC/NOER	**6**	**6**	**6**	**6**					**3**	**27**	**108**

Even if the design should be adaptable to some extent because of particular properties of the test chemical or the purpose of the study, the OECD guideline should fix the number and nature of possible test designs to be able to give guidance but to still include some options.

It was discussed that in some cases test chemicals might show less effect in the field than in the laboratory. It was argued that the ECx/ERx design might pose a problem for deriving the necessary application rates - as no range-finding test can be easily performed in the field. Also, it was argued that at higher application rates there might be no visible concentration-response. However, the performance of a so called "revisited NOEC/NOER design" is not the preferred option.

The suggested additional third design should be formulated within strict limits. In addition, it should be kept in mind that by testing one single rate only for deriving a NOEC/NOER in specific cases, no information is available about the expected effect threshold in the field. It was suggested to test e.g. the 2-fold, 5-fold, or 10-fold recommended application rate. This would also allow to extrapolate to different chemical application rates. The question was raised if a specific margin of safety would be needed. It was agreed that this can only be answered in a regulatory context, e.g. by evaluating the finalized study and the uncertainties in the data (e.g. design, statistical power).

It was discussed that some chemicals cannot be tested at very high concentrations due to technical limitations of preparing such high application rates. Also, higher concentrations would be difficult to apply with seed or tuber treatments. One option would be to sow them more densely -but this would require an additional control treatment. Alternatively, the active substance could also be tested by spraying on the soil surface. However, this would not mirror a realistic case, especially for large seeds (e.g., tubers), since there are large differences between row testing of treated seeds / tubers and a spraying application. Very high application rates may also pose a problem regarding field availability and authorization of the trials which has not been a problem so far.

It should also be kept in mind that the new OECD guideline is not exclusively designed for pesticides. Thus, some flexibility should be in principle also possible because of other regulations that might change in the future (even if earthworm field studies are not known from other regulations except for fertilizers). Annexes to the Guideline in preparation could cover other chemicals than pesticides. In any case, there should be a clear separation between practical testing (e.g. validation criteria, test procedures, statistical evaluation) as described in the Test Guideline and specific regulatory requirements (e.g. assessment factor, in which context data from the TG is acceptable, etc.). Summarizing, the discussion on the test design is part of the test guideline, the discussion on a possible assessment factor accounting for uncertainties in the test outcome is part of a regulatory decision framework.

As shown in the pilot field study, the workload of the proposed test design seems to be manageable, since the pilot study had a more complex design than the ones suggested for the Guideline. However, due to increased costs and practical efforts needed, problems might arise for smaller test facilities. This could be an argument to limit testing efforts. However, both from a scientific and regulatory point of view, testing one rate in a so called limit test may not be the state of the art anymore. Actually, such a design might be still common for metabolites tested in the laboratory, but this is not comparable with the situation in the field. One solution to overcome workload problems might be the set-up of task forces, e.g. when testing active substances used in several products.

5 Discussion of the statistical evaluation of future studies

5.1 Use of plots vs. sub-plots as replicates

It was discussed whether sub-plots (i.e. individual samples within one plot) can be used as replicates to increase the power of the statistical analysis. Using sub-plots as replicates requires homogeneity of the experimental field. The use of sub-plots as replicates should be ecologically and systematically justified and the interdependencies between samples and plots should be explored statistically. The main question is whether there is a closer relationship between samples of the same plot than between plots. It was argued that this may be a specificity of every tested field. If sub-plots are used as replicates, information on the earthworm distribution within plots might be gained. For example, the presence of outliers within one plot can strongly influence the mean and can result in higher variability between plots of the same treatment. On the other hand, there were some reservation in using replicates, arising from the fact that they can be considered pseudo-replicates. Ecologically speaking, the question of replication in a given field is always difficult. The additional information that can be extracted from addressing sub-plot replicates should however not be completely disregarded and the approach should not be rejected for dogmatic reasons. The use of sub-plot replicates instead of plot means should also be seen as an exercise to understand how much additional information can be gained from the data evaluation. It was argued that if a subplot would not be treated homogeneously with the pesticide or is different in terms of earthworm densities, then the influence of this higher variability would be more pronounced if based on a sub-plot level than on mean plot level.

It was discussed that the length of autocorrelation of earthworm abundance data might be larger than the plot size - but autocorrelation is possibly (or even should be) interrupted by chemical application. Earthworm migration between plots might also be a problem, because of the possible arising interdependency between treatments and controls. In the pilot field study, the distance between samples on different plots was at least 7 m. Earthworm dispersal has been estimated in the literature to be around 10 m/a, but differs between age stages, species, soils and weather conditions. This factor should be assessed from an ecological point of view in a greater framework, since it cannot be included into the evaluation of single studies, as geostatistical analyses would be necessary. Enlarging the experimental field might increase the variance and pose a problem when selecting possible test sites.

There was a feeling of a general trade-off between practicability of the test and statistical power, which might question in general the test system if no reasonable compromise can be found.

5.2 NOEC/NOER and ECx/ERx test designs

It was discussed by some participants that calculating an ECx/ERx from field studies may be difficult as there are often no strong effects visible by applying the current test design according to ISO 11268-3 (2014), also because of the few rates tested. In order to overcome this problem, higher rates than the recommended field rate might be tested. It was argued that for chemicals other than carbendazim, the necessary range of rates is not known a priori as there are usually no other field studies available for comparison. This needs careful evaluation of the available information on the test item before performing the field test.

The choice of the model for calculating an ECx/ERx is described in OECD guidance document No. 54 (e.g. model with 3 or 4 parameters). Determination of a sound EC_{10}/ER_{10} in the field might not be always possible. It was discussed that it needs to be agreed on how the goodness-of-fit of such methods will be assessed. It was suggested that with the help of an F-test, the quality of the dose-response relationship can be assessed. Also, a comparison of a NOEC/NOER with an EC_{10}/ER_{10} or EC_{20}/ER_{20} would be possible. The slope of the dose-response curve and the width of the confidence interval resulting from the analysis of the data from the field need to be considered. An ER_{50}/EC_{50} is statistically easier to determine than a sound NOEC/NOER or EC_{10}/ER_{10}. The choice whether to concentrate on a test design to determine a sound EC_{10}/ER_{10} (or EC_{20}/ER_{20}) or alternatively on an EC_{50}/ER_{50} does not depend on the setting of a specific protection goal, since these endpoints represent so-called measurement endpoints and not the assessment endpoints (i.e. the specific protection goal). It would however be preferable to derive both in the same field study. Regulators could possibly use the ER_{50}/EC_{50} and apply an assessment factor to extrapolate to a more conservative protection goal. It was discussed, in order to finally conclude on these specific points, more ECx/ERx data would be needed and that these decisions are outside the remit of the OECD guideline.

5.3 The use of the CPCAT method to analyse differences between treatments

The consortium elucidated why the CPCAT-method is the appropriate statistical tool for Poisson-distributed data. However, experience available regarding its application and/or limits is not exhaustive. An R-script is available for free download and a standard application of the method it is currently being implemented into the data evaluation program ToxRat. The question was raised regarding the scientific and regulatory acceptance of CPCAT as a new evaluation technique that is currently not being used as standard assessments for laboratory tests. It was pointed out that already an OECD guideline for testing of chemical effects on molluscs refers to a statistical approach for analysing Poisson-distributed data with CPCAT. It was discussed that generally the recommendation of a new method e.g. in a guideline is a prerequisite for its wider acceptance. CPCAT seems to be of interest and, although there are few publications so far, it is a promising approach. While an ECx/ERx is the statistically stronger endpoint, CPCAT would be an improvement regarding NOEC/NOER calculations. It was agreed that the CPCAT method should be included in a future update of the OECD statistical guidance documents but statistical evaluation should be kept flexible as CPCAT is not necessarily the best choice in each case.

5.4 Statistical power of the field tests

Currently, there are often problems to detect statistically significant differences between treatments in earthworm field studies even if deviations from the control can be noted possibly due to the low statistical power of the tests. This is a problem when biologically relevant effects are not statistically significant. Minimal Detectable Differences (MDD) as one measure of statistical power depend on the variance of the data, effect size and earthworm abundance. When discussing the two options of i) increasing the number of plot replicates or ii) increasing the number of (sub-)samples per plot, the research consortium stated that in this respect it is important to decide whether it is possible to use sub-samples as replicates. The use of (sub-)samples introduces more variability in the data, but this is counterbalanced by the higher number of replicates, especially in the current test set-up.

The improvement of statistical power through the increase of plots per treatment is limited by the spatial and methodological capabilities in the field. It was discussed whether an option would also be to increase the number of control replicates, as it is often done in the laboratory. This would help if data fulfil the prerequisite for parametric testing. It was mentioned that the increase in statistical power could be calculated for conventional methods but not for CPCAT as there is no standard power analysis for this method available so far. It was agreed that statistical power is not a validity criterion in the OECD guideline proposal so far. However, the magnitude of the so-called beta error (i.e. false negative, the probability of not detecting effects that are effectively there) should be minimized. It was discussed that it is a regulatory decision about which power needs to be achieved in a study and that for specific questions, even studies with a low statistical power can be useful to draw conclusions (e.g. if great abundance shifts are to be assessed).

It was agreed that the best methods to achieve regulatory requirements should be described in the guideline (test design, statistical evaluation). However, the reality of the test performance may differ from optimal requirements. In order to meet the realized variability in the specific trials, the current focus is finding a possibly homogeneous field with high earthworm abundance. Such homogeneity of earthworm distribution previous to chemical application can be statistically investigated. In the previous ISO test design, the randomized complete block design was a possibility to account in the data assessment for the presence of a gradient in the field. This is not possible for asymmetric designs. Maybe an Analysis of Covariance (ANCOVA) could be applied to account for continuous variables that cannot be controlled in the experimental field. It would also be possible to once randomly re-arrange the allocation of treatments to the plots after pre-sampling based on an analysis of total earthworm abundance, even if time to reallocate plots in case of inhomogeneous distribution is short. Also, outliers could be excluded if additional plots are available.

However, it was discussed that there is no guarantee for an overall high statistical power at all sampling dates due to the development of the experimental field throughout the year. This may result in a study not being fully useful in all points for risk regulation. It was discussed, how the statistical power of higher tier studies should be reflected in the size of an assessment factor, but regulatory guidance for identifying such factors is missing. It was pointed out that - according to EFSA - an uncertainty analysis should be performed for risk assessment in the future. A reference how to describe the statistical power of a field test should be included in the test guideline and the power has to be stated in the study report (a priori power analysis based on the pre-sampling; alternatively, a retrospective (post-hoc) power analysis).

5.5 PRC – principal response curve

In order to detect effects at the earthworm community level, a principal response curve (PRC) should be included in the study report. The PRC for the conducted pilot field study - in contrast to the NOEC/NOER and ECx/ERx approaches - did not show any effects after one year. Hence, as also indicated in other assessment areas apart from earthworm field studies, PRC should only be used in combination with other evaluation techniques.

6 Discussion of analytical measurements of the test chemical

The analytical measurement of the test chemical is needed in order to confirm the applied application rate and/or to assess earthworm exposure. Possibly, measurement should be performed separately for soil and grass/root layer.

One key question is: Which is the ecotoxicologically relevant type of concentration that best explains observed effects? Lately, it has been proposed to include aspects of environmental exposure assessment in earthworm field studies to evaluate the exposure profile behind the observed effects and to decide whether the study results could be extrapolated to other sites (comparison of exposure profiles). For example, in the EFSA Opinion on soil organisms (2017) strong recommendations are given to measure exposure and not regulate on nominal rates/concentrations. In aquatic semi-field-tests, the effect thresholds are always based on measured values. Obviously, there are huge differences in the determination of representative concentrations of the test chemical in the two compartments.

Four levels of analytical measurements can be differentiated:

1. Petri dishes to confirm the applied rate are very useful, e.g. if several applications are envisaged. They have a lower variability than soil core samples. They can be waived if one of the more in-depth analytical measurements are performed;
2. Soil cores at test start (10 cm depth): to confirm the application rate and assess initial earthworm exposure which often drives the effects due to uptake by earthworms;
3. Soil cores (10 cm depth) at each of the earthworm sampling time point. Could be possibly waived at later time points for fast-degrading chemicals but metabolites should be tracked anyhow;

4. Spatial exposure profile (e.g. separated for 0-1; 1-2.5; 2.5-5; 5-10 cm) over time (if required by regulatory questions in particular studies): to better link exposure and effect, to separate persistence of chemicals from delayed effects and to best extrapolate from one site to another.

Analytical measurements would give very useful information to link exposure and effects, they are however an additional cost in studies. It has to be checked, how high sample costs actually are and what the benefits/regulatory use of the chosen level of analytics would be. For example, the measured vertical distribution could have implications for the calculation of the Predicted Environmental Concentrations (PEC). It was discussed which determination level should be chosen, and that the answer is partly a regulatory one. The detailed requirements should be addressed elsewhere than in the guideline. However, a guide to sampling earthworm field plots for analytical measurements could be included in the guideline. It was argued that the aim of field tests should be to pursue the worst case (and not perform a parallel e-fate study). However, this is currently not demonstrated. The degradation/dissipation of substance (e.g. DT50) is often different in the field compared to modelling results, where often a worst case is assumed. The aim is not to determine DT50 values of tested substances in earthworm field tests – as these are usually experimentally determined in the laboratory, or investigated in dedicated field studies. It was argued that linking exposure and effects in the field is difficult and that the test system needs to be suitable. It was suggested that determining the effective exposure would be useful to transfer effects observed in e.g. a grassland site towards a crop site. Differences regarding the biological activity and the degradation pattern of the test chemical are expected when comparing arable crop with grassland sites. So far, there are few study pairs where field trials have been performed with the same chemical in different land uses. In the future, measurement of pore water concentrations may also be of regulatory interest - but these are even more difficult to determine over time.

7 Discussion of the application of a plateau concentration

In the past and in terms of testing persistent substances, the expected amount of chemicals to reach a calculated plateau concentration has been sprayed on top of the surface in several cases. Often, this procedure does not reflect the calculated distribution of chemicals, as in many cases an incorporation elicited by land management to a soil depth of 20 cm is assumed. Therefore, mechanical incorporation of the plateau concentration into the soil to a depth of 20 cm (e.g. in autumn) before a respective study start (e.g. in spring) has been suggested. It was discussed whether this procedure would be too deleterious for the earthworm fauna. High abundances of earthworms in arable land can often be expected when no deep tillage has been performed for several years before study start. It was discussed, if incorporation could be achieved through watering, which would however be poorly effective for adsorptive chemicals. It was discussed whether an alternative would be to apply the chemical in spring and incorporate it along with sowing. This would occur at a depth of 5-7 cm (not 20 cm). The question in this case is which concentration to apply - as the calculated background concentration is based on a soil depth of 20 cm. In addition, the plateau concentration in reality would undergo ageing over several years in the soil. Up to now, there is little experience of tests performed with a plateau concentration. Hence, it would be advisable to coordinate this issue beforehand with the regulatory authority. The increase in the overall test concentration should be kept in mind when evaluating the results.

8 Discussion of experimental field management, testing of herbicides and other special cases

As already discussed in the chapter on the performed pilot field study, the initial management of the experimental field depends on the chosen field/system. In general, grassland should be mowed to facilitate the exposure of earthworms in the soil, whereby the clippings should be removed from the test site. In terms of testing a 'bare soil scenario', mechanical surface treatment (e.g., harrowing) should be performed. The application of an herbicide before test start is not preferred.

When testing an herbicide, indirect effects due to differences in plot vegetation development are problematic. Initial treatment and management of the experimental field depends on the mode of action of the chemical, i.e. whether the applied herbicide is a leaf-active or a soil-active chemical. Bare soil is the best option for all herbicides at test start. It was reasoned that, however, it is not feasible to keep bare soil all through the field trial duration. Alternatively, clover/grass or cereals can be sowed, with subsequent regular mowing in the course of the trial. Additional parameters to be measured are soil water content and temperature (especially when testing herbicides) and soil coverage/interception by vegetation at the sampling dates. The crop addressed in the intended use can be sowed when testing selective herbicides. It was clarified that higher application rates than the intended one must not have an effect on the crop and, in a best case, a homogeneous plant cover should develop over time. There might be herbs growing in the control plots if these are not suppressed by the intended use crop. The intended use crop usually does not remain on the field until the end of the study, since often harvest is in late summer. It was discussed how to proceed afterwards, but no agreement was found. One solution would be to leave the vegetation develop and cut again later in the year if needed.

In general, it was agreed that for some cases e.g. herbicides, the envisaged management and the conditions of the experimental field over the trial should be discussed with the interested authorities beforehand. In any case, the field conditions need to stay as homogeneous as possible through the test. If different scenarios are applicable and one is to be tested, the worst case should be chosen. However, it was discussed that it is not entirely clear what the worst case is. Currently, grassland sites are preferred because of the higher number of earthworm species, but chemicals might adsorb stronger to grass roots resulting in decreased bioavailability and mobility. In turn, this may cause a higher exposure of juveniles in the root mat. However, grasslands are increasingly difficult to find for trials due to their high legal protection status. It was discussed that a rich and abundant earthworm community can also be present at crop sites. High individual densities of earthworm species can increase the statistical power of the test but do not necessarily represent the worst case state of earthworm communities.

9 OECD standardization process

The current aim is to have the new OECD guideline published as soon as possible (at the very latest in 2021). The ongoing guideline process started in 2013 as OECD project no. 2.47. It was clarified that –next to the OECD working group members, further stakeholders will be involved through "Business at OECD" (BIAC), which is the industry representation at OECD.

Along with the draft guideline, a validation report (VR) needs to be prepared. This will not be a "classic" VR including ring-testing results, since this is not fully feasible for the earthworm field test. However, this method has been established for 20 years. The experience gained in the performance of the earthworm field studies up to now will be analysed and included in the VR. In addition, the criteria laid down in OECD Guidance Document 34 on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment related to the VR structure and content are in part adaptable to the specific guideline in preparation. The VR should be available at due time in order to better comment the draft test guideline, since OECD focusses also on the VR during test guideline development. Therefore, the evaluation of the available data basis (dedicated UBA project) should form a part of the VR, together with the results of the pilot study. Overall, this approach equals a retrospective validation, which has already been performed for VRs without ring-testing. The group was well aware that, generally, OECD doesn't fund ring-testing but provides a framework for interest groups.

The next steps are planned as follows (amended October 2019):

- Until 28.05.2019: comments of the GSIG / OECD Expert Group (= extended project group) on the draft guideline sent to J. Römbke. DONE
- The consortium will use a commenting table with responses to each comment to be sent around; ONGOING, completion planned for end 2019
- If necessary, September 2019: subsequent nominations to the OECD Expert Group of 2013. DONE
- Revision of the draft guideline by the project consortium and preparation of the Validation report. ONGOING, completion planned for end 2019
- Face to face meeting of the SETAC GSIG / OECD ad hoc Working Group planned (Aachen, Frankfurt, Berlin) for discussing the OECD guideline and VR before it is sent to OECD. Meeting postponed to January/February
- Spring/Summer 2020: OECD expert group commenting of draft TG + VR;
- Summer/Fall 2020: WNT commenting rounds.
- Spring 2021: WNT approval

Annex 1: Schedule

Thursday, 28th March 2019, Room 0.163, UBA Dessau	
13:00	Welcome, background and aims of the project *UBA*
13:10	Overview of the activities *Jörg Römbke (ECT)*
13:20	Statistical background, evaluation of field studies with earthworms and planning of a pilot study *Richard Ottermanns (RWTH Aachen), Björn Scholz Starke (Darwin Statistics / RWTH Aachen)*
14:00	Performance and results of the pilot earthworm field study *Bernhard Förster (ECT)*
14:30	*Coffee break*
14.45	Evaluation of the results of the pilot field study and comparison with the available data base *Benjamin Daniels (RWTH Aachen)*
15:30	Discussion of the data evaluation *All*
15:50	Break
16:00	Proposals for the future earthworm field study design and evaluation *Martina Roß-Nickoll (RWTH), Jörg Römbke (ECT)*
16:30	Discussion of the proposal *All*
17:30	*End of the first day*
19:00	Workshop dinner at a Restaurant in Dessau (to be decided)

Friday, 29th March 2019, Room 0.163, UBA Dessau

09:00	Summary of the discussion on the previous day *Silvia Pieper, Pia Kotschik (UBA)*
09:15	OECD status and expectations regarding the OECD-standardization process *Susanne Walter-Rohde (UBA)*
09:30	Discussion of draft OECD test guideline proposal *Jörg Römbke (ECT)*
10:30	*Coffee break*
10:45	Discussion of draft OECD test guideline proposal (on-going) *Jörg Römbke (ECT)*
11:45	How to proceed *Silvia Pieper (UBA)*
12:00	Public relations *Jörg Römbke (ECT)*
12:15	Final discussion *All*
13:00	*End of the workshop*

Annex 2: Attendees/Invitees

Note that the list of invited participants is given in alphabetical order. Names of colleagues who are going to attend the meeting in person are given in bold.

Name	Employer	Country	Comment
Mike Coulson	Exponent	United Kingdom	Excused
Benjamin Daniels	**RWTH Aachen**	**Germany**	
Frank de Jong	RIVM	The Netherlands	Excused
Axel Dinter	**DuPont**	**Germany**	
Gregor Ernst	**Bayer CropScience**	**Germany**	
Kathleen Götz	**BioChem agrar**	**Germany**	
Simon Hoy	PSD	United Kingdom	Excused
Stephan Jänsch	**ECT**	**Germany**	
Patrick Kabouw	BASF	Germany	Excused
Olaf Klein	**EAS Ecochem**	**Germany**	
Pia Kotschik	**UBA**	**Germany**	
Silvo Knäbe	EAS Ecochem	Germany	Excused
Christine Kula	**BVL**	**Germany**	
Visa Nuutinen	MTT	Finland	Excused
Celine Pelosi	INRA	France	Excused
Silvia Pieper	**UBA**	**Germany**	
Juliska Princz	Environment Canada	Canada	Excused
Jörg Römbke	**ECT**	**Germany**	
Martina Ross-Nickoll	**RWTH Aachen**	**Germany**	
Björn Scholz-Starke	**Darwin Statistics**	**Germany**	
Lennart Schulz	BioChem agrar	Germany	Excused; colleague coming
Els Smit	RIVM	The Netherlands	Excused
Kees van Gestel	FU Amsterdam	The Netherlands	Excused
Tobias Vollmer	**EAS Ecochem**	**Germany**	
Susanne Walter-Rohde	**UBA**	**Germany**	

A.6 Draft OECD Guideline (as of March 2019)

Earthworm (Oligochaeta, Annelida) test in the field

INTRODUCTION

1. This Test Guideline is designed to be used for assessing short- and long-term effects of chemicals on earthworms in soils under field conditions. The method was developed mainly based on experience from arable and grassland sites in temperate regions of the world. However, the test can as well be performed in other regions - but the experience is very limited so far.

2. The earthworm field test is based on a method developed by the German Federal Biological Research Centre for Agriculture and Forestry for the testing of pesticides (BBA 1994). Later, it was internationally standardized by the International Organization for Standardization (ISO), taking into account results and recommendations of an international workshop on the "Ecotoxicology of Earthworms" in 1991 in Sheffield, United Kingdom (Greig-Smith et al. 1992; ISO 2014). In two meetings organized by the German BBA (Braunschweig 2002) and by the German Federal Agency for Consumer Protection and Food Safety (Lille 2005), an ad-hoc working group of experts from various countries and institutions proposed further improvements (Kula et al. 2006), which were incorporated in ISO 11268-3 (2014). At the same time, it was decided to transfer the ISO Standard to OECD.

3. About 6,000 earthworm species are known worldwide, roughly 670 of which belong to the family Lumbricidae (Blakemore 2003). They are the dominant family in the temperate regions of the world (North America, Europe, and Northern Asia), while other families are dominant in Africa (Almidae), South America (Glossocolecidae), Asia and Australia (Megascolecidae). Mainly lumbricids, but also individual species of other families have spread globally (hence called peregrines), often due to anthropogenic activities.

4. Earthworms belong to the saprophagous soil macrofauna and are often considered to be the most important soil animals. This appraisal is based on their high biomass as well as their strong contribution to ecologically and agronomically important functions and ecosystem services (e.g. Van Groenigen et al. 2014). These include the bioturbation of soils, the acceleration of soil organic matter decomposition, the enhancement of nutrient supply for plants as well as the improvement of the water holding capacity of soils by generating clay-humus-complexes (Darwin 1881, Edwards and Bohlen 1997; Brussaard 2012). Some key lumbricid species are called ecosystem engineers, e.g. *Lumbricus terrestris* in temperate regions (Lavelle et al. 1997). Earthworms are generally divided into three ecological groups (Bouché 1977): mineral dwellers (= endogeics), litter dwellers (= epigeics) and vertical burrowers (= anecics).

DEFINITIONS

5. Definitions of the most relevant terms used in the document are provided in Annex I.

INITIAL CONSIDERATIONS AND LIMITATIONS

6. This Test Guideline is intended to be used for the registration of chemicals designed to be applied in the environment, probably most prominently for the testing of pesticides (e.g. in the European Union, EC 2009; EU 2013). Before the use of the Test Guideline for the testing of a

chemical mixture intended for the purpose of providing information for approval prior to placing chemicals on the market, it should be considered whether, and if so why, it may provide adequate results for that purpose. Such considerations are not needed when there is a regulatory requirement for testing of the mixture.

PRINCIPLE OF THE TEST

7. Species and numbers of earthworms collected from sampling plots in the field treated with a test chemical are compared with those collected from untreated control plots. Sampling is performed by a combination of two methods: hand-sorting and an extraction fluid (ISO 23611-1 (2006); Bartlett et al. 2010). The test duration is usually one year but may be prolonged depending on the properties of the test chemical and/or the effects observed after one year. A reference chemical (= positive control) should always be tested in parallel to the test chemical. Application of the test and reference chemicals as well as sampling dates are chosen to lie within the periods of activity of the earthworms (in temperate regions in spring and autumn). In other regions, application and sampling have to be adapted according to the local climatic conditions. Test endpoints include total abundance and biomass of earthworms, of species, of different life stages, of morphological groups, and of the three ecological groups. Statistical analysis of numbers of each species, life stages or grouped taxa collected at each sampling occasion is used to determine the response of the earthworm community to the tested chemical, e.g. by estimating effective concentrations (ECx) and/or by comparing abundance and biomass between control and treated plots.

INFORMATION ON THE TEST CHEMICAL

8. In accordance with OECD GD 82 (2016), the following information on the test chemical (and transformation products if available) should be available: Description of the chemical, or the formulated product and active substance(s) therein, solubility in water, vapour pressure, Henry’s law constant, n-octanol-water partition coefficient. Additional information on the fate and behaviour of the test chemical in soil, such as degradation half-times, is desirable. Details of the source, batch or lot number and purity of the test and reference chemicals also need to be provided.

9. In principle, this Guideline is applicable to all test chemicals. The method may not be applicable to chemicals, for which the air/soil partition coefficient is greater than one, or to chemicals with a vapour pressure exceeding 0.0133 Pa at 25°C. Other factors - such as water solubility or high adsorption to soil limiting the volatilisation potential - should be taken into account when deciding whether or not the chemical can be tested.

10. Rates of application of test chemicals are expressed as the weight of active substances per hectare (e.g. kg a.s./ha) and volume of formulated product per hectare (e.g. l prod./ha). The concentrations of test chemicals in soil are given as mg a.s./kg soil dry mass (dm). This unit is used to compare the results of the field test with laboratory studies (see also §32).

REFERENCE SUBSTANCE

11. The parallel testing of a reference chemical (positive control or toxic standard) is necessary to demonstrate the sensitivity of the test system under the specific experimental site conditions. For example, the active substance carbendazim, which is toxic to earthworms, is suitable for this purpose (Edwards and Brown 1982; Römbke et al. 2004). One single application of the

reference chemical of 6 to 8 kg a.s./ha in parallel to the first application of the test substance is considered to be sufficient (independently from the application pattern of the test chemical) (Kula et al. 2006).

12. The applied reference substance should lead to a statistically significant reduction of at least 50% of total earthworm abundance or biomass at the first, latest at the second sampling time point. Experience has shown that the effects of carbendazim on earthworm populations (e.g. 50% reduction of abundance) can be demonstrated already four weeks after application (Römbke et al. 2004).

VALIDITY OF THE TEST

13. For the test to be valid, the minimum mean earthworm density required for testing of chemicals in the field as determined by a pre-application sampling (identical in efforts to the post-application samplings) present at the start of the test is:

- Grassland: 100 individuals/m^2;
- Arable land: 60 individuals/m^2.

It should be noted that these values refer to Central/Northern, i.e. temperate, Europe. Respective ranges for other regions of the world need to be defined. For further details see Annex II. The pre-sampling should occur after all planned treatments (e.g. grass cutting) and close to test start and chemical application.

14. In addition, the experimental site should harbour a population of earthworm species which are ecologically important for the type of environment selected. For example, in temperate agricultural areas, anecic (e.g. *Lumbricus terrestris*) and endogeic (e.g. *Aporrectodea caliginosa*) species should be present at a sufficiently high density (at least 10 % of the community of adults) at the pre-sampling. Due to natural reasons, certain ecological groups possibly do not occur in some regions. In such cases, expert knowledge is required in order to identify the ecologically most important species of that region (e.g. *Lumbricus friendi* instead of *Lumbricus terrestris* is found in western Europe; Bouché 1972).

DESCRIPTION OF THE METHOD

Equipment and Material

15. Installation of the experimental site:

- Adequate device for georeference, e.g. GPS receiver;
- Measuring devices to position the basic points of the plots;
- Weather-proof markers for the plots.

16. Sampling of the earthworms in the field:

- Adequate equipment to excavate the sub-plots in specific size and depth (e.g. measuring device, spade or shovel);
- Adequate equipment for carrying or storage of excavated soil (e.g. a piece of thick plastic or big containers) during hand sorting;

- Options to wash the earthworms before fixation in ethanol if advisable, e.g. small vessels filled with water to clean earthworms from adhering soil particles;
- Watertight containers (e.g. 500 ml) for first fixation and storage of the earthworms in ethanol (70% volume fraction);
- Extraction fluid for earthworm extraction (e.g. allyl-isothiocyanate (AITC));
- Adequate containers to pour the extraction fluid uniformly (e.g. 10 – 20 L watering cans);
- Adequate devices for measuring air and/or soil temperature, e.g. thermometer;
- Weather-proof markers for sampled sub-plots;
- Equipment to avoid/reduce cross-contamination (for samplings after application), e.g. overshoes, cleaning supplies;
- Various utilities like forceps, protection gloves etc.

17. Application of the test chemical in the field:

- The reference chemical, e.g. carbendazim (preferably applied as water soluble formulation);
- Appropriate equipment to set up the application solution(s) in the field (e.g. volumetric flasks, dilution water, graduated cylinder);
- Adequate application device (e.g. plot sprayer).and equipment for its calibration in the field;
- Wind velocity measuring device;
- Equipment to confirm
- the total applied amount of application solution (i.e. capacity measuring);
- the application rate per plot (e.g. by placing Petri dishes on the ground);
- Wind velocity measuring device;
- Irrigation measurement device.

18. For the work in the laboratory:

- Use standard laboratory equipment, such as

  preservation medium for earthworms (e.g. ethanol, 96%);

  dissecting microscope;

  balance (precision at least 0.01 g).

Test design

19. The experimental design depends on the objectives of the study and the amount and quality of information available from the study site. In general, it should be taken into consideration that a dose-response design clearly facilitates environmental risk assessment as compared to single-dose studies. In any case, the reasons for the selected test design shall be explained in the study report.

20. The test follows a randomized design with four samples per plot and sampling time point (see Table 1).

The performance of a dose-response design (i.e. ECx-design; Effect concentration for x% effect, e.g. EC_{50} or EC_{20}; see Table 1) with at least seven treatments and three plots per treatment is recommended. It should be noted that the application rates for the dose-response testing would need to be estimated with sufficient confidence before the definitive tests, based on existing information (e.g. laboratory test results, range-finding tests).

Otherwise, a mixed-design with two treatments of six plots (for sufficient statistical power in the determination of No/Lowest Observed Effect Concentration, NOEC/LOEC) and at least three more treatments with two plots (additionally for ECx determination) should be carried out. In this case, six untreated control plots are required (Table 1, 'Mixed Design'). The treatments for NOEC/LOEC calculation should have the second lowest and one of the two highest application rates among all tested treatments.

Depending on the experimental design, the NOEC, LOEC or the ECx can be determined.

Table 1: Number of plots and treatments for the ECx- and the mixed-design in earthworm field tests. More information on the design type in the text above. C control; T 1-x treatments; R reference substance

Test design	**plots per treatment (No.)**									**plots (sum)**	**samples (total No.)**
	C	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**(T7)**	**R**		
ECx Design	3	3	3	3	3	3	3	(3)	3	24 (27)	96 (108)
Mixed Design	6	2	6	2	2	6			3	27	108

Selection and description of the experimental site:

21. In general, the test site should be as homogenous as possible in terms of earthworm distribution to improve the statistical power of the test, as proven by the pre-sampling. Gradients in environmental conditions should be avoided, e.g. adjacent ditches, canopy influences such as woodland borders or compacted tracks on the site. The site should be on level ground and should have similar vegetation and soil characteristics throughout. In cases where effects due to gradients cannot be excluded, the sampling design and the statistical evaluation should be adapted (please see point 26 Plot set-up). Extreme soil types, e. g. with a very high sand content, or a pH <4.5 should be avoided when selecting the test site, since both abundance and species diversity decrease considerably at such sites (Jänsch et al. 2013). The history of the test site should be known (e. g. applications of pesticides, mineral fertilizers, sewage sludge, etc.) and reported for the last three years.

In case of pesticides, no product with an active substance having the same mode of action as the substance to be tested should have been applied in the last three years before the test, unless the specific regulatory question requires for it.

22. Earthworms are not evenly distributed in soil, mainly due to the uneven distribution of physico-chemical soil properties and food sources. In addition, the reproductive potential and dispersive powers of the individual species plus historical events (e.g. disturbance) might also be responsible for this pattern (Edwards & Bohlen 1996; Palm et al. 2013). In order to select an adequate field site, a screening of abundance, diversity and distribution of the earthworm community prior to the study can be performed to decide whether a particular site is suitable.

23. In case the intended use of the test chemical is focusing on one specific land use (e.g. arable land), the test site can be selected accordingly. Otherwise, grassland is the preferred study site for testing effects of chemicals on earthworms, due to higher individual densities and species diversity in these environments compared to arable land. Orchards are not recommended for testing –unless specifically required– because of the heterogeneity of the site due to tree rows and strips without trees. If an orchard is used, it shall be ensured that the higher variability is compensated by taking more samples or restricting sampling to specific areas.

24. A description of the test site should contain the following physico-chemical and biological information (it should be determined in the A-horizon using standard, preferably ISO, methods):

- Particle-size distribution (texture) (%) (ISO 2009);
- Organic carbon content (%) (ISO 1998);
- pH-value ($CaCl_2$) (ISO 2005);
- cation exchange capacity (CEC) (
- Maximum water holding capacity (WHC_{max}) (%) (ISO 1998);
- Description of vegetation, e.g. crop type(s) of the last season;
- Daily air temperature and precipitation, e.g. from the nearest weather station.

The soil density is usually assumed to be 1.5 in agricultural soils (adapted from PEC calculations).

Plot set-up

25. The study design determines the number of plots and therefore the surface area of the field site. Each plot should have the same size of at least 100 m2 (i.e. at least 10 m x 10 m). The individual plots should be geo-referenced. The earthworm samples are taken exclusively from the central area of the plots so that around the sampling area there is an at least 2 m wide edge strip which is also treated (see Figure 1 as an example).

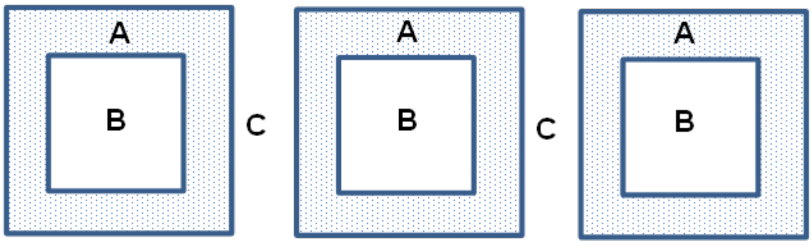

Figure 1: Scheme of three neighbouring plots, each at least 10m by 10m large (A) with inner sampling area, e.g. 6 m by 6 m wide (B), and space between two plots, at least 2 m (C)

Allocation of plots to the treatments

26. The plots should be allocated randomly to the treatments. If at pre-sampling the distribution of the earthworms is manifestly uneven, it should be avoided that a given random allocation of plots will lead to diverging earthworm abundances between treatments already at test starts and prior to chemical application. In this case, a re-run of random assignment is advisable. The distribution of earthworms between plots and treatments at pre-sampling should be analysed before concluding on the test outcome, in order to identify uneven distributions. Uneven distributions prior to the chemical application need to be taken into account, in order to avoid i) attributing differences between treatments after application to chemical effects (i.e. false positive) and/or ii) interpreting even distribution between treatments after application to an absence of chemical effects (i.e. false negative). Please see point 49 for suggested analyses.

Application of the test chemical

27. Test and reference chemicals should be applied at latest within two consecutive days and during periods of earthworm activity (e.g. in temperate regions in spring or autumn). It should be avoided to apply the chemicals after longer periods of drought, due to reduced earthworm activity and minor permeability of the soil surface layers.

28. When chemicals are designed for soil application (e. g. pesticides), application rates, formulations and modes of application are specified by the suppliers. In such cases, these specifications should be used. In case of testing pesticides, time of application should mirror the intended uses (e.g. spring or fall). Ideally, application in the test should be carried out using application equipment similar to that used in practice. For example, when testing pesticides, the application should be carried out using appropriate agricultural equipment such as a boom with low drift spray nozzles designed to deliver equivalent volumes in the same manner. All equipment should be calibrated prior to use to confirm the correct functioning necessary to evenly apply the desired rate.

29. In case of chemicals applied with water as carrier, a water application rate according to Good Agricultural Practice (e.g. 200 l/ha to 800 l/ha) should be used. If several applications are planned, they should be carried out at intervals corresponding to usual application procedures. The application should be performed at local wind speeds less than 3 m/sec at the height of the spraying device.

30. The total amount of the test chemical applied and the application rate per plot should be confirmed after application by appropriate measures, e.g. by analysing soil exposed in Petri dishes placed on the soil surface of all treated plots during test item application.

31. If no or little rainfall (i.e. <10 mm) occurs within two days after each application, irrigation of the site is considered necessary to achieve optimal conditions for exposure. At least 10 mm of precipitation (natural plus irrigation) are required within the first three days after application.

32. Immediately after application, the concentration of the test chemical in soil shall be determined by residue analysis to verify the actual exposure concentration in soil. Collection of soil samples (X individual samples per plot) for residue analyses should be performed according to standard protocols (e.g. OECD 2016). Since the first residue analysis in the earthworm field study is particularly needed to confirm the application rate, a soil sample depth of 20 cm (including the “biologically active” zone) should be sampled, also in grassland studies. In light of the

high variability in field studies, a recovery of 50% to 150% of the nominal concentration in soil should be achieved (OECD 2006). Concentrations are calculated based on the soil density and the required horizon calculation depth. If required by the specific study, soil sampling can be performed for different depths and concentration given based on measured values (e.g. 0-1, 1–2.5, 2.5-5 cm depth).

33. Each biological sampling should be combined with analytical measurements of the test item, in order to monitor the exposure profile over time and to possibly support extrapolation of the test results to other conditions.

34. Test chemicals toxic to earthworms may induce avoidance behaviour, i.e. earthworms migrating to the soil surface. Therefore, for the first two days after each application of the test chemical and/or after irrigation, the soil surface should be systematically searched for alive and/or dead earthworms.

Inclusion of plateau concentration (see Annex III)?

Site management

35. Management procedures during the experiment should be kept to a minimum but ensure that similar conditions are maintained across the experimental site. On experimental grassland sites, the grass cover should be regularly cut and the remains left on the soil surface (in the following: mulching) in order to keep the vegetation cover short and to facilitate earthworm sampling. Mulching should be carried out “on demand” but at least within one week before the application of the test chemical to ensure that the clippings on the surface, which acts as a food source for some earthworms, has been in contact with the test material. The last mulch before application of the test chemical may remain on the site provided that it does not create a coherent mat. In the case of mulching over the course of the year, the mulch should remain on the field as it serves as food for some earthworm species. If a test is carried out on arable land, ploughing and other soil treatment measures should be avoided during the experiment.

36. Apart from the test and reference chemical application, no other chemicals should be used on the experimental site during the experiment (see also §20). If chemical treatment for site management is unavoidable, then the chemical chosen should be applied in rates far below toxicity thresholds (NOEC, EC_{20}) to earthworms. With respect to the interpretation of the test results, it shall be kept in mind that, even if the additionally applied chemicals are used at low doses, interactions between residues of the non-toxic chemical and the test chemical could occur.

37. In case an herbicide is to be tested, all experimental plots should be kept free from vegetation. It is recommended to treat the plots mechanically by hand, in order to keep the disturbance as low as possible. The use of another herbicide, which is not the test chemical, is not recommended. As already outlined above (point 36), interaction with the tested chemical cannot be assessed and might change the response of the earthworm community to the test chemical.

38. In cases of very dry soils, artificial overhead irrigation of the experimental site can be useful as earthworms only become active and rise to the surface when sufficient soil water content is present. Irrigation one to two weeks before sampling can facilitate sampling. An even water distribution over the experimental site shall be assured at any irrigation treatment.

Sampling of earthworms

39. Sampling dates are chosen to lie within the periods of activity of the earthworms, in temperate regions usually in spring and autumn. In other regions, sampling dates have to be adapted according to the local climatic conditions (see Annex II). Samples taken on the same date should be at least 2 m apart, and it must be assured that during the time of the study sampling has not been done twice at the same sampling place (e.g. by using markers or by pre-defining a chess-board sampling structure). Sampling of all plots should be completed within seven consecutive days per sampling date.

40. Related to the date of the first application of the test chemical, at least four samplings should be performed:

Pre-sampling:	within four weeks prior to the first application and after the last site management measure;
First sampling:	three to five weeks after first application;
Second sampling:	five to seven months after first application;
Third sampling:	11 to 13 months after first application.

The test duration is usually one year but may be prolonged depending on the properties of the test chemical and/or the effects observed after one year. If additional samplings are planned, they should be carried out at appropriate intervals during periods of earthworm activity.

41. A combination of two different methods, hand-sorting followed by application of the extraction fluid AITC into the excavated hole, is recommended for the sampling of earthworms in temperate regions. Based on several comparative studies, this combination is clearly recommended in the various reviews on earthworm ecology (e. g. Vetter 1996; Coja et al. 2008; Smith et al. 2008; Bartlett et al. 2010). All earthworms per sub-plot and sampling date are combined and stored as one sample. In temperate regions, most adult earthworms have approximately a length between 1 cm and 20 cm. Hence a square of 50 cm • 50 cm (0.25 m^2) hand-sorted to a soil depth of at least 15 cm is sufficient. For details of the sampling procedure and special cases see Annex II. Care should be taken that the mouth of earthworm burrows is not blocked, and therefore operators should avoid walking on sampling areas.

42. All earthworms collected (i.e. hand-sorting and extraction combined) per one sub-plot and sampling date should be fixed in ethanol in two steps: in order to preserve important morphological features, the earthworms are firstly fixated in 70% ethanol, followed by a final fixation in 96% ethanol within one week after sampling. Fixation and preservation in formalin is not recommend since this chemical destroys the genetic material (DNA) (Römbke et al. 2015).

Taxonomic identification, counting and biomass determination in the laboratory

43. Individual earthworms should be determined as follows: adults to species and juveniles to the genus level. Fragments or unidentifiable remains of worms are counted as “indet”. Identification follows the relevant identification keys (e.g. for Central and Northern Europe: Sims & Gerard 1999), and current taxonomic nomenclature should be used (e.g. Blakemore (2003). Adults and juvenile worms are counted separately. For juvenile worms which are difficult to identify, a distinction between tanylobous and epilobous individuals should at least be made (Edwards & Bohlen 1997). Adults should be additionally allocated to their ecological group, so that numbers and biomass of epigeic, endogeic or anecic worms are given. Currently, morphological identification is recommended. However, assuming that an appropriate taxonomic level

and resolution is achieved, DNA-based molecular methods are also acceptable (e.g. James et al. 2010; Perez-Losada et al. 2009; Richard et al. 2010).

44. The mass of the preserved worms is recorded on the basis of species or genus and life stage per sample, i.e. juveniles and adults separately (for details see ISO 23611-1, 2018). In case the worms are covered with soil or plant particles, they have to be washed in water. Before weighing, worms are dabbed on a piece of soft tissue to remove adhering liquid. Subsequently, the mass as the sum of all worms per group and sample is determined using a suitable balance.

EVALUATION OF RESULTS

45. Depending on the chosen test design, effects of the tested chemicals are assessed using suitable statistical methods (see below, § 49). Statistical testing and inference depend on the underlying distribution and homogeneity of variance for both non-aggregated and aggregated or pooled replicate endpoint measures (abundance or biomass). The reference chemical should not be included in the statistical comparison of treatment and control.

46. Due to the change of mass during preservation and the soil content in the gut, the measurements can be corrected by using factors published in the literature to determine the biomass of the animals. According to e.g., Dunger & Fiedler (1997) or Lee (1985), earthworms seem to lose about 10 % to 20 % of their mass during fixation. This is approximately the same mass as the mass of the gut content. Therefore, compensation between loss via fixation and gut content is not necessary. Afterwards, the measured fresh mass could be converted to dry mass by multiplying by a factor of 0.15 (Petersen & Luxton 1982). However, this factor has been determined on the basis of mineral dwellers from grassland sites and, site-specifically, it can vary considerably depending on the land-use form (e.g. in litter dwellers, it is smaller than in mineral dwellers).

Data treatment

47. The evaluation of the earthworm population at each sampling date shall include:

- Total abundance and biomass of earthworms;
- Abundance and biomass of all determined species and groups. Evaluation shall be performed at the species level or for each of the respective ecological or morphological group;
- Abundance and biomass of juvenile and adult earthworms as described above. Since juvenile earthworms of the same genus can often not be identified to the species level, the evaluation can be restricted to morphological groups (tanylobous and epilobous).

48. Despite the fact that the selection of the test site was based on the occurrence of at least one dominant species from the two most important ecological groups (anecics and endogeics), it cannot be assured that during the course of the study these species are always dominant.

Analysis of data / Evaluation of test results

NOEC estimation and statistical assessment of pre-application conditions

49. The application of powerful tests should be preferred. If it can be excluded that data follow a Poisson or generalized Poisson distribution (e.g. metric response measures of biomass), multiple t-test procedures such as Dunnett's or Williams' test ($\alpha = 0.05$, two-sided for unclear direc-

tion of response) should be performed (Dunnett 1955; 1964) for multiple comparisons in randomized plot design. The prerequisite of normally distributed data and variance homogeneity has to be tested using e.g. Shapiro-Wilks and Levene's test procedure, respectively. If data do not fulfil the criterion of normality, generalised linear models or non-parametric tests e. g. the Bonferroni U-test in accordance with Holm (1979) or the Jonckheere-Terpstra Step-down-test (homogeneity of variance required) can be applied (Figure 2).

The theoretical distribution assumption of earthworm abundance field test data follows a Poisson model. Therefore, the application of the CPCAT approach (Lehmann et al. 2016) is highly recommended for abundance count data due to more powerful test statistics (Lehmann et al 2018). Nevertheless, if abundance data show homogeneity of variances, the null-hypothesis of normal distribution is not rejected and mean abundances per replicate are > 5 (Gupta & Guttman 2014), the application of parametric test procedures (Williams, Dunnett) is also feasible. For multiple t-test procedures and with unequal replication, the table t-values must be corrected as suggested by Dunnett and Williams.

Multiple comparisons should also be performed to detect possible differences between treatments at pre-sampling.

In all cases data transformation is not recommended, the significance of statistical test results using transformed data cannot be interpreted straightforwardly for the non-transformed data. It is noted that from an ecotoxicological point of view relevant increases in abundance and/or biomass are in principle considered as abundance and/or biomass decreases: they are deviations from control situation. Increases, as well as decreases in measured endpoints, need to be considered for their biological relevance and statistical significance.

Test data should be evaluated at two different levels: on a plot level (pooled samples of 1 m^2 in total used as replicates) and on a sub-plot level (single samples as replicates of 0.25 m^2). If the calculated NOEC of the conducted statistical tests vary on both levels, differences should be reported and the NOEC with the lowest concentration should be used for evaluation.

In addition to uni-variate methods multi-variate statistical tools such as PRC (Principal response curves) should be conducted (Van den Brink et al. 2003). This is strongly recommended for tests with multiple treatments (e.g. ECx design).

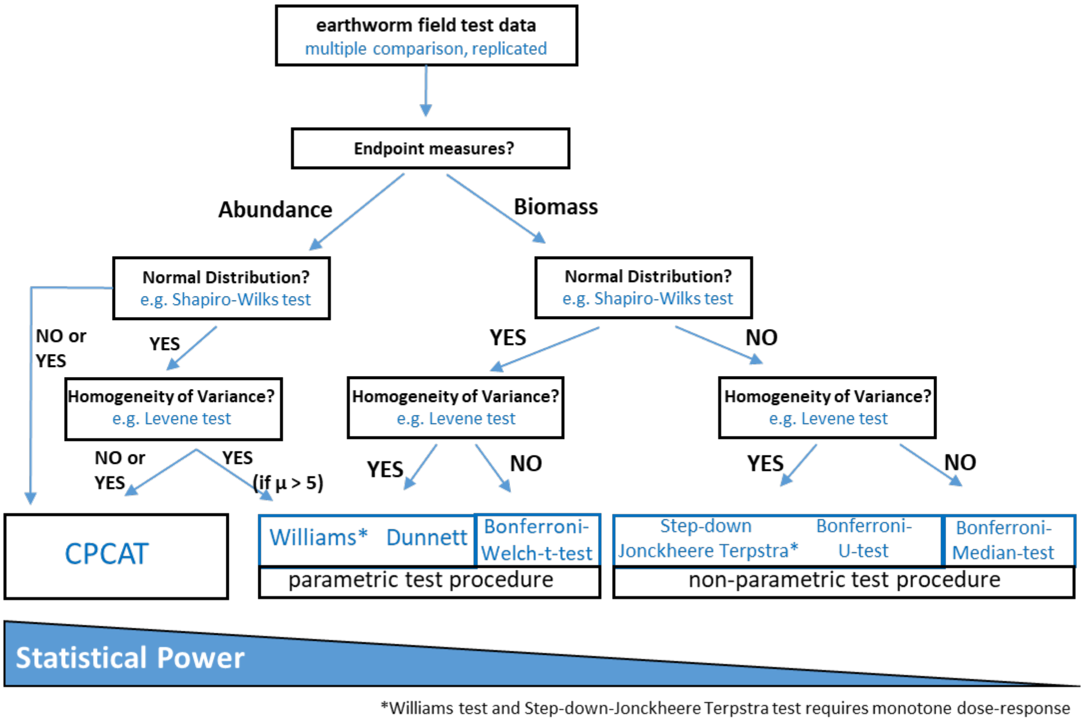

Figure 2: Scheme of the statistical testing procedure for earthworm field study data when assessing differences between treatments and controls (e.g. for No Observed Effect Concentrations, NOEC; calculation in Mixed Design).

Dose-response function and ECx estimation

50. To compute any ECx value, the per-treatment means are used for regression analysis (linear or non-linear), after an appropriate dose-response function has been chosen. For the biomass of earthworms as a continuous response, ECx-values can be estimated by using suitable regression analysis (Bruce and Versteeg, 1992). Among suitable functions for quantal data (number of sampled individuals) are the normal sigmoid, logistic or Weibull functions, containing two to four parameters, some of which can also model so-called hermetic-type of responses. If a dose-response function was fitted by linear regression analysis, the significance of r^2 (coefficient of determination) and/or the slope should be tested before estimating the ECx. ECx values are computed by inserting a value corresponding to x% of the control mean into the equation found by regression analysis. 95%-confidence limits are calculated according to Fieller (cited in Finney 197) or other modern appropriate methods.

Alternatively, the response is modelled as a per cent or proportion of model parameter which is interpreted as the control mean response. In these cases, the normal (logistic, Weibull) sigmoid curve can often be easily fitted to the results using the probit regression procedure (Finney, 1971). In these cases, the weighting function has to be adjusted for metric responses as given by Christensen (Christensen, 1984). However, if a hormesis-type of response has been observed, probit analysis should be replaced by a four-parameter logistic or Weibull function, fitted by a non-linear regression procedure (Van Ewijk and Hoekstra, 1993). If a suitable dose-response function cannot be fitted to the data, one may use alternative methods to estimate the ECx, and its confidence limits, such as Moving Averages after Thompson (Finney, 1978) and the Trimmed Spearman-Karber procedure (Hamilton, 1977).

DATA AND REPORTING

Test report

51. The test report shall include the following information:

Test chemical, reference chemical and control:

- Test chemical (name, common name, chemical name, Batch no., purity etc.)
- Reference chemical (name, common name, Batch no., purity etc.)
- Properties of the test chemical (e.g. log Kow, Koc, water solubility, vapour pressure and information on fate and behaviour), if possible
- Description of the preparation of test and reference chemical dosing solutions
- Application rate based on actually (documented) applied volumes

Test conditions:

- Characteristics of the test site (land use, soil texture, pH, WHCmax, CEC, OM content, etc.)
- Weather conditions during the test period: air temperature and precipitation
- A detailed description of the test design and the management of the test site (size of test plots, number of replicates, number of samples);
- The extraction method used for sampling;

Test results:

- The overall abundance and mass of the earthworms collected per sampling date;
- Tables showing the percentage change per test plot, treatment and date compared to the control;
- The overall abundance and mass of each species for all sampling dates together;
- Tables showing the numbers and mass per sample and date for each species;
- A graphical representation of the abundance and mass change for each species during the test period;
- The results obtained with the reference chemical;
- Any operational details not yet mentioned, and any incidents liable to have affected the results.
- Evaluation of the test results:
- Review/discussion of results obtained
- Conclusion reached.

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ANNEX 1

DEFINITIONS

The following definitions are applicable to this Guideline:

EC_x (Effect concentration for x% effect) is the concentration that causes an x% of an effect on test organisms within a given exposure period when compared with a control. For example, an EC_{50} is a concentration estimated to cause an effect on a test end point in 50% of an exposed population over a defined exposure period. In this test the effect concentrations are expressed as a mass of test chemical per dry mass of the test soil or as a mass of the test chemical per unit area of the soil.

LOEC (Lowest Observed Effect Concentration) is the lowest test chemical concentration that has a statistically significant effect ($p < 0.05$) In this test the LOEC is expressed as a mass of test chemical per dry mass of the test soil or as a mass of test chemical per unit area of soil. All test concentrations above the LOEC should normally show an effect that is statistically different from the control. Any deviations from the above must be justified in the test report.

NOEC (No Observed Effect Concentration) is the highest test chemical concentration immediately below the LOEC at which no effect is observed. In this test, the concentration corresponding to the NOEC has no statistically significant effect ($p < 0.05$) within a given exposure period when compared with the control.

DEFINITIONS FOR THE FOLLOWING TERMS WILL BE ADDED TO THE FINAL VERSION

TEST AREA: Name des Dorfes/Ortes in dessen Nähe die Test Site liegt bzw. wo der Test stattfindet GPS-Koordinaten, Schlagnummer

EXPERIMENTAL SITE – ganzes Feld mit allen experimental verwendeten Flächen

PLOT: 10 * 10 m Fläche auf der die Probenahmen stattfinden, repliziert (t.b.d.)

SAMPLE = Einzelprobe, statistisches Repliakt bei der Auswertung auf Subplotebene

SAMPLING DATE

TEST CHEMICAL

REPLICATE = Einzelne Samples oder Plots, abhängig vom räumlichen Level der statistischen Auswertung

POSITIVE CONTROL

NEGATIVE CONTROL

GOOD AGRICULTURAL PRACTICE

EARTHWORM BIOLOGY

JUVENILE

ADULT

EPILOBOUS

TANYLOBOUS

ENDOGEIC

EPIGEIC

ANECIC

DNA?

ANNEX 2

BIOGEOGRAPHICAL REGIONS OF EARTHWORM FAMILIES

(TO BE AMENDED)

Currently, this document focuses on crop and grassland sites in temperate regions of the world.

ANNEX 3

DESCRIPTION OF AN ADAPTED TEST PROCEDURE FOR SUBSTANCES REQUIRING THE APPLICATION OF A SO-CALLED PLATEAU CONCENTRATION

(TO BE AMENDED)

In terms of testing substances having long degradation times (e.g. DT90 > 365 d), the predicted plateau concentration accumulating in the soil might need to be tested additionally to the freshly applied chemicals, depending on the regulatory question. The calculated plateau concentration should be applied well before test start (e.g. in the autumn if test starts in spring). The test substance should be incorporated into the soil up to a depth of 20 cm. The mechanical maintenance should also be conducted on control and reference plots.

Immediately after application, followed by incorporation, the concentration of the test chemical in soil should be determined by residue analysis, in order to verify the actual exposure concentration in soil. Collection of soil samples for residue analyses should be performed according to standard protocols (e.g. OECD 2016). Since the residue analysis in the earthworm field study is needed to confirm the application rate, a soil sample depth of 20 cm (including the "biological active" zone) is sufficient also in grassland studies. In light of the wide variability in field studies, a recovery of 50% to 150% of the nominal concentration in soil should be achieved.

Pre-sampling of earthworms should be conducted in autumn before the application of the plateau concentration as well as in the following spring before the application of the fresh initial test rate.

A.7 Public presentation of the project at scientific conferences

During the annual SETAC-Europe meetings in Brussels (May 2017) and Rome (May 2018), the ad hoc SETAC GSIG sub-group was informed about the progress of the project.

Additionally, the test design and first results of the pilot study as well as an analysis of the application of the CPCAT approach in earthworm field studies were presented in two posters at the SETAC-Europe annual meeting in Rome:

Daniels, B.; Jänsch, S.; Kotschik, P.; Ottermanns, R.; Pieper, S.; Roß-Nickoll, M.; Scholz-Starke, B. (2018): The application of the CPCAT approach reduces shortcomings of effect detection in earthworm field studies.

Römbke, J.; Daniels, B.; Förster, B.; Jänsch, S.; Kotschik, P.; Ottermanns, R.; Pieper, S.; Roß-Nickoll, M.; Scheffczyk, A.; Scholz-Starke, B. (2018): Adaptation of the earthworm field test method: conceptual overview and first results.