Ecotoxicology for Digital Herbicides

Concepts and strategies for the recording of environmental effects of innovative weed control methods require evolved risk assessments

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1. Contents

1 Executive summary

The Zasso Electroherb process is a physical process for weed control.

Unlike many other physical processes, it is characterized by its systemic effect on plants – from the leaves down into the roots. Ploughing, grooming, scorching, hot water and other methods heat or move the entire surface or soil largely unselectively. The Electroherb process, on the other hand, only works on plants that are in direct contact with the applicators, which can also be designed spatially selectively without additional sensors by means of an appropriate applicator design.

As a physical process, Electroherb is not subject to approval according to chemical registration laws.

However, Zasso, out of responsibility for the environmental impact of its products, voluntarily conducts predictive ecotoxicological studies to investigate the effect of the method on the environment, both in its main effect (weed control - mode of action) and in possible side effects on non-target organisms.

Since this is a physical procedure and no generally used standards and test protocols are available for such procedures, Zasso has developed criteria and indications for the execution of tests and method evaluation in the first year of an experimental project lasting several years, and also carried out a series of field experiments in cooperation with experienced institutes for ecotoxicological field tests.

These results are presented here as a progress report. The report is a working document and will be continuously developed further in 2018 and 2019.

The first tests were carried out at a qualified screening level. Springtails, horn mites, earthworms and microbial biomass were investigated.

The results showed that, when applied under realistic field conditions, no or only minor effects were observed in the energy dosages found to be effective for weed control.

Naturally larger acute effects were observed at very high doses and in model ecosystems such as wet extensive meadows in full application, which did not represent a target area for normal device use but were only carried out as Mode of Action experiments.

The trials planned for 2018 with improved and more selective applicators and even more targeted dosing will show more precisely how great the distance between effective concentration for weed control and effectiveness against non-target organisms is.

In assessing these results and safety margins, it will also have to be taken into account that, due to the expected purely acute effect of the physical process, the safety factors for the treatment of uncertainty and local double dosages can be significantly lower than for chemical substances which are absorbed in soil and plants, can be transferred to other environmental areas and can also be absorbed and effective by animals and plants in the long term.

Another aspect of environmental protection is that the physical Electroherb process can be used on a height-selective basis, enabling small plants and closed soil cover if this makes sense from an agrotechnical point of view. Also, the applicators insulated at the lower edge and the general effect characteristics (energy introduction according to Ohm's law always occurs where the least resistance prevails/most plants are) contribute to the treated surfaces only being treated as required and not unselectively the entire surface is treated evenly, even if the weed density is very different.

In order to systematically compare environmental impacts with other weed control methods, especially non-chemical methods, it is also important that the Electroherb method does not move soil and that the treated plant material dries up on site. This keeps the habitat of many organisms that live in the soil, build tunnels, lay eggs, have nests or hide from predators undisturbed. Since not only the soil stratification is not changed and also the organic material layer is not mixed in, the food basis can be maintained, especially for organisms living in this litter layer or using the biomass there.

More detailed summaries of individual organisms can be found in the respective chapters.

2 Introduction

As a non-chemical method of weed control, the Electroherb method is not subject to the regulations for pesticide approval. Nevertheless, out of a sense of responsibility for the environmental relevance of their work, Zasso have decided to carry out their own tests of various application techniques through experienced test facilities, and immediately upon adapting the method to application areas in Europe. This program was started in 2017 and is running until 2020, in parallel to the application development.

2.1 Aim of this paper

The main aim of this paper is,

- to record the differences in assessment approaches for chemical, mechanical, thermal and electrophysical methods,
- to give a short description of the mode of action regarding ecotoxicology,
- to map out the systematical assessment approach for the Electroherb method,
- to lay a content-related foundation for a comprehensive assessment of the Electroherb system,
- to present and categorize the results of the tests carried out by Zasso in 2017.

2.2 Preliminary information

Application of the Electroherb method in FCS-certified teak plantations and no-till agriculture in Brazil over many years has not provided any indications that could lead to expect significant and unexpected environmental impacts.

However, this does not replace any tests based on European standards which have to be adapted as well, corresponding to the special characteristics of non-chemical methods.

2.3 Trials in 2017–2018

In 2017, Zasso tasked several experienced test facilities at several locations in the European heartland to carry out ecotoxicological trials under various impact and application scenarios. In the course of these, tests were carried out to investigate the general impact of electrical high-voltage on soil-dwelling organisms, but also trials in real-life application conditions with unselective and height-selective applicators.

Primarily, the following trial aspects were under investigation:

- acute effects on earthworms, nematodes, spring tails, oribatid mites, microorganisms
- monitoring after several weeks of nematodes, spring tails, oribatid mites
- the impact of various field conditions (type, pretreatment, real-life field, model)
- comparison with thermal and mechanical methods

- dose-response relationship of the energy input,
- comparison of various applicator types

Due to the wide range of test parameters and device options, these trials were carried out in the first trial year 2017 as large-scale screening series. This was necessary as because of the innovative nature of the method and the mode of action for the ecotoxicological tests, there are no formulated test and assessment schemes available.

The trials will definitely be continued and specified in a broader context in 2018. And because of the continued development of the Electroherb technology they will be carried out even more dose-specific and show significantly more dose-response correlations, provided that impacts on non-target organisms can actually be found.

3 Technical and biological principals

4 Assessment basis of Electroherb in comparison

4.1 Comparison of abstract assessment targets

The assessment of different weed control methods requires an outline of the general differences between their modes of action.

From this comparison of chemical, electrophysical and mechanical/thermal control methods it is then possible and much easier to derive useful test systems and basic principles of assessment for the Electroherb system. The mechanical/thermal methods have been grouped together here despite of their great diversity, because for this area hardly any formulated assessments exist. The reason being that there was and is just no necessity for the plant protection act to assess these non-chemical methods as strictly as as it does chemical substances.

Electroherb itself does not require authorization by the chemicals act. However, out of a general feeling of responsibility for the technology, Zasso follows the methodology of the comprehensive ecotoxicological assessments as they are used for chemical substances.

4.2 General impact pathways and contributing factors

4.2.1 Overview

A series of graded test have been developed for chemical substances, from the acute single species test to a test in mesocosms to the large-scale field trial. This corresponds to the possible impact pathways of PPPs on organisms, which are here again summarized.

General impact pathways and contributing factors on non-target organisms

	Type	Chemical	Electrical	Mechanical/Thermal
	Acute transfer of the impact on beneficial organisms	Sprayed onto the organism Ingested with food	Organism is in direct, local contact with plant or root to which voltage is applied	Organism is mechanically damaged, overheated
	Sensitivity criteria for beneficial organisms	Substance disrupts metabolism, nervous conduction	Impact by a lot of plant/soil contact; more impact if organism is larger and its skin is wet	Impact through mechanical fragility; if larger through heat sensitivity
	Longer exposure time Adult organisms	Ingested with contaminated food	None	Habitat destruction (plowing) Oversupply in food, part sterilization (thermal?)
	Subsequent generations of the beneficial organisms (non-existent at time of treatment)	Ingested by emerging young with higher sensitivity	None, if no effect on ground nests can be established	Destroyed ground nests and disturbed habitat (soil)
	Chronical effects on beneficial organisms	Chemical contamination	None	Adjustment to disturbed ecosystems (plowing)
	Genetical in-species effects	Resistance selection through genetic variability	None	None
	Species-dependent effects, selectivity	Large metabolism differences possible	Very unselective	Very unselective
	Phenotype-dependent effects	Small, as strong absorption by any surface or food	Large, as age, size are important for acute effect	Large, as size, robustness important
	Location-dependent effects during treatment	Limited, as many effects only occur over time	Large, when situated close to the applicator or on the root/leaf/litter layer	Large, when situated in the heated or mechanically moved area
	Location-dependent effects after treatment	Large, as area often comprehensively treated with the aim of a longer effect	None, as no long-term effect	Substantial, as soil ecosystem massively disturbed and recreation necessary
	Influence on food sources	Comprehensive extinction Poisoning	Extinction only where directly touched, meaning a higher survival rate in general at the margins (between the crop)	Extinction only where directly tilled, meaning a higher survival rate in general at the margins (between the crop)

	Regenerative stability of populations of impacted non-target organisms	Limited, with general effect as application area often very large and evenly treated as well as long-term effects	As the application dosing depends on inhomogeneous ground resistance and plant density, there are always areas created with widely varied dosages, which actually improves regeneration	Depending on the method, the affected soil capacity can be very homogenous (plowing) or greatly varied (row harrowing systems, thermal treatment of large plants)
	Danger of non-classical impact on the nervous systems of non-target organisms	Can be significant, depending on the substance	None	None

4.2.2 The aspect of regenerative capacity and maintenance of non-target organism populations (insects/plants)

An important assessment factor when it comes to the effects of weed control is the recovery and regeneration capacity after treatment. Depending on the effect, the regeneration does not originate from the directly treated area, but from its immediate surroundings or from more remote areas.

For this very reason, the geometry and compactness of a treated area can play an important role, similar to a biotope network. In addition, depending on the mobility of the organisms or seeds, the absolute size of the continuously treated area is also of importance.

These regeneration patterns cannot be mapped in a meaningful way by using ordinary impact descriptions for the treated area.

For some chemical herbicides, it is particularly the treatment of an entire area across fields of several hectares or square kilometers that poses an ecological problem for the feed supply of wild bees and bumblebees. In these cases, the flower strips around the margins are only of limited use.

But the Electroherb method also offers the possibility, particularly when using the damage threshold principle, to treat 80–90% of the entire area and still distribute the untreated areas extensively across the field, to those locations where there will be none or only slight losses among non-target organisms. For example, depending on the row crop, remaining weeds could be allowed to grow within the row, or in the middle between the rows, or as spontaneous vegetation below a specific maximum height. All of this is possible without decreasing the yield, while at the same time still offering a sufficient food source for insects with a small movement range.

The same applies for the regeneration and protection of rare species within the spontaneous vegetation of fields whose seed bank needs to be controlled, but not completely destroyed.

For any assessment of the Electroherb method it is therefore essential to evaluate the entire system and not only the directly treated area. Additionally, the Electroherb system offers the automatic adaptation of energy intensity and exposure time to various weed species and densities, by employing special applicator types (for example, height-selective or with an insulating protrusion for a minimized introduction of electricity into the soil). By contrast, the total area control methods (spraying, plowing, harrowing, burning, etc.) only work to specific requirements by employing a significant amount of sensor technology.

4.3 Impact on specific populations

The populations listed in the following table are only those that are frequently mentioned in specific discussions about the mode of action. Insects and other soil-dwelling organisms are not mentioned here, because they are routinely assessed in test series.

Zasso does not aim to carry out systematic tests with the stated organisms. But all aspects concerning them should be considered within the framework of literature research, method comparisons and monitoring approaches wherever it makes sense.

Impact on specific populations

	Type	Chemical	Electrical	Mechanic/Thermal
	Damage to overground nests (birds)	Marginal	Optionally substantial if they are touched (but damage unclear)	Reliably high, as mechanical/thermal destruction
	Damage to underground nests (bumblebees, bees lizards, ants)	Marginal	Unclear, potentially very small	Very high if in the mechanically moved area
	Danger for moles	Little to marginal	Unclear if impact reaches deep enough, probably not	Unknown
	Danger for lizards	Little to marginal	Unclear if impact reaches deep enough; strongly dependent on time of day and season	In the affected area large impact; unknown, if high absolute
	Danger for spontaneous vegetation, requiring protection in the target area	If mode of action works, this vegetation is completely eliminated, particularly with residual herbicides	Only eliminated where it has sprouted at the time of application and grown to a sufficient height (can be systematically spared with height-selective application); elimination mostly within the rows or if too low	Limited impact on smaller plants; seedbank mostly not destroyed by plowing/harrowing

4.4 Impact on habitats

Mechanical effects on agricultural habitats are very common and usually have a strong influence on these. But it only becomes obvious in a comparison of conventional fields with those farmed with soil conservation in mind and those that totally forgo any soil movement. A comparison with soil-moving methods is extremely important for the assessment of Electroherb, because its role in farming for soil conservation is considered to be particularly important. If farmers after the abolishing of glyphosate had to work with significant soil movement again, this would immediately cause massive losses of soil-dwelling organisms.

Unfortunately there is very little information in general about the impact of soil movement as an alternative herbicide treatment method, which makes parallel tests in this area particularly important to Zasso.

Impact on habitat is also important when it comes to applying Electroherb for the treatment of invasive plants, and not only those growing in fields, but also on fallow land, in traffic areas and nature reserves.

In the assessment of thermal methods it is important to bear in mind that thermal transfers across different phase boundaries can often take quite a long time. When heating up the target plants over 60 °C, significant environmental impacts are likely to occur. However, there are no detailed studies available about this subject.

Impacts on habitats

	Type	Chemical	Electrical	Mechanical/Thermal
	Danger for spontaneous vegetation requiring protection on field margins	Substantial, because of drift, erosion, evaporation	None, as there is no remote effect	None, as there is no remote effect
	Danger for spontaneous vegetation requiring protection, aquatic	Substantial through leaching/erosion	None	Erosion and adhering substances of other treatments
	Physical habitat destruction of the soil for beneficial organisms	None	None	Often large effects with plowing, and if the thermal method has a real effect in the soil
	Changes to the ecosystem of beneficial organisms	Very variable	Very variable	Very variable, often many
	Changes to the seedbank	Often very significant	Only through less seed entry	Superficial elimination, but also encouragement possible; deep burial can have varied effects, encouraging as well as inhibiting
	Danger for adjacent nature reserves	Significant through drift	None, as there is no remote effect	None, as there is no remote effect
	Danger for water conservation areas, bodies of water	Significant through leaching, drift	None, as there is no remote effect	Through erosion, if any

The impact profile of Electroherb on various habitats also has a significant influence on the meaningful selection of ecosystem sections for any planned field trials.

In general, species-rich, extensively farmed meadows are preferred areas for ecotoxicological impact tests of chemical substances, because they have a high abundance and therefore deliver great statistics. Their large number of species quickly provides a relatively large base of representatives for entire ecosystems.

This choice of test area also makes sense because species-rich field margins can get into contact with the tested substances through drift, evaporation, erosion and solubility. In addition, animals living in these margins can get into contact with the applied substances and be significantly exposed to them when they wander into the treated area and feed there.

However, these impact paths do not exist at all for the treatments with Electroherb, as it is applied locally, does not contain any molecular substances or leave any residues.

Add to that the fact that exposition of non-target organisms to Electroherb is strongly influenced by the environmental geometry (soil, plants, organism) at the exact time of treatment, any extrapolating assessment based on tests in extensive grass habitats will make even less sense.

Such areas should only be used with regards to purely mechanistic matters. Trials with any validity should always be based near-practice testing areas. Application on extensively managed (wetland) meadows, strictly with height-selective applicators only, is conceivable when it comes to controlling large, stubborn weeds (dock, thistles or for example rushes) in this habitat. Only then will tests in various distances from the plants that need to be controlled have an inherent relevance for further assessments.

4.5 Characteristics of the effective component

The most important characteristic of Electroherb as a digital herbicide is that it does not contain any molecular active ingredients. Many potential consequences that are sometimes too complex to model can therefore not occur at all.

Characteristics of the effective component

	Type	Chemical	Electrical	Mechanical/Thermal
	Degradability	Varied and important	Disappeared immediately after treatment	No substance, but potentially thermally significantly changed biomass
	Toxicity of metabolites	Varied and important	None	Decomposition products of substances that have been plowed under (anaerobic?)
	Absorption into the soil	Varied and important	None	Does not apply
	Bioavailability	Varied and important	Only applies with mechanical contact	Only applies with mechanical contact
	Accumulation	Varied and important	None	Possible changes in soil structure and layering
	Later release	Varied and important	None	None

4.6 Distribution channels

As there is no molecule, a distribution of the mode of action beyond the very clearly definable target area is practically impossible with Electroherb .

Overview of the distribution channels

	Type	Chemical	Electrical	Mechanical/Thermal
	Soil: Transfer through solubility, erosion	Varied and important	None	Erosion itself as problem
	Water: aquatic toxicity during application	Varied and important through drift	None	None
	Aquatic toxicity after application	Varied and important through leaching, erosion	None	Erosion itself as problem, sometimes in connection with harmful substances
	Air: Transfer through evaporation	Varied and important	None	Wind erosion itself as problem

4.7 Secondary environmental impacts

A series of environmental impacts can present indirectly or develop over several causal stages. Some of these effects are listed below. They are generally not processed in the framework of normal ecotoxicological studies, or only marginally. But they are still part of an overall assessment of environmental aspects.

Some sub aspects are systematically mapped in life cycle assessments. Therefore the assessments can also be included in such studies at a later time.

Secondary environmental impacts

	Type	Chemical	Electrical	Mechanical/Thermal
	Soil compression	Important for very wide sprayer booms Significant for driving lanes in row cultures and row spraying with narrow sprayer booms	Medium, as working width limited, also frequency of application	Varied, depending on method and frequency; can be very high for narrow width and frequent action; but mechanical treatment can also loosen the soil
	Desorption of substances	Possible through phosphate fertilization (particularly glyphosate) and humus depletion/changes	Not possible	Not possible
	Nitrate release	Minimal as no soil movement	Minimal as no soil movement	Can be very high, for example with plowing up of grassland
	CO_2 release from the soil	Minimal as no soil movement	Minimal as no soil movement	Because of heavy soil movement, often very significant with mechanical and also thermal if it has a real impact on the soil
	Using soils as additional carbon sinks	Very possible	Very possible	Hardly possible, as significant soil movement
	Main energy input with regards to climate	Energy consumption during manufacturing of herbicides Pesticide elimination from water (?)	As environmentally friendly as the currently used electricity producer	Plowing requires significant energy input; thermal energy input probably always the highest of all the methods
	External costs through environmental damage	Complex: reduction in species numbers, accumulation of harmful substances, relocation	Simple: if at all always very strongly related to the type of electrical power production used	Very complex because of erosion, nitrate release, CO_2 release, reduction in species numbers
	Other material transfers			Washing out of fines into deeper layers (bed formation)

4.8 Application parameters

The depiction of differences in the application parameters offers a base for a detailed presentation of the individual methods, to compare and evaluate them despite of their very different impact characteristics.

Depiction of the differences in the application parameters

	Type	Chemical	Electrical	Mechanical/Thermal
	"Formulation"	Chemical additives crucial for effect	Applicator geometry crucial	
	Frequency	Due to permanent effect relatively low, apart from cases of small plants that have to be treated repeatedly	Because of effectiveness right down into the roots often low, also for larger plants	If there is no deep impact (plowing), often medium to high, as only roots of smaller plants damaged (harrowing)
	Global dosage per area	Kg substance/ha	kWh/ha	Mechanically difficult to quantify (disturbed volume ...?); thermally in energy/ha or heating
	Homogeneity of dosage	Generally very high, regardless of the vegetation	Limited by varying conductivity and plant density	
	Beaufschlagter Bereich	Crop plants and all beneficial organisms on the crops are mostly also affected as less sensitive ((Mode of action)	Only weeds affected, sometimes also the soil beneath; organisms on/at crops generally not affected	Only weeds affected and often also the entire soil around the crop; organisms on/at crops generally not affected
	Individual dosing capacity	Only possible with sensor-supported methods	Systemically intrinsic, particularly with height-selective or edgeisolated applicators	With sensor-supported systems for harrowing and thermal methods possible
	Organism-specific dosage for beneficial organisms	Measurable by chemical analysis	Difficult to measure, as short effect caused by electricity, meaning indirect effect measurement	Difficult to measure, as short initial effect, indirect effect measurement; changes in habitat difficult to measure directly
	Application interval in general	Medium to long, if root or residual effect	Medium to long, as root effect and no soil movement	Short/medium, as often limited root effect plus soil movement
	Damage-threshold related application frequency	Often impossible to implement, as preventative for small plants or before germination	Very easy to implement	Often impossible to implement, as area-wide and preventative/early treatment necessary

4.9 Assessment models

While there are very sophisticated assessment models based on simulations available for chemical herbicides, this is not yet the case for the other methods.

It should be evaluated how well the already existing assessment models can be adapted with relatively little effort to Electroherb, by assuming a rapidly degradable active ingredient (e. g. "hydrolysis" in 1 min.).

It is also going to be important to integrate the special practices of soil-conserving agriculture into the models.

The modeling of mechanical and thermal processes could proof more difficult. In the year 2018, Zasso will be able to compare much better the dosage used in ecotoxicological trials with the effective dosage of efficacy tests. This will also result in ways to assess how much larger the ecotoxicologically relevant dosage is in comparison to the actually required dosage. It will also imply the use of safety factors. When specifying safety factors, it should be clearly stated that many risks of chemical herbicides, which occur as a result of residues, metabolites and their persistence in different environmental compartments, do not even occur when Electroherb is applied. Accordingly, such factors can most likely be kept very low.

Assessment models

	Type	Chemical	Electrical	Mechanical/Thermal
	Existence of meaningful model ecosystems	Given	Open	Only limited availability as no legal requirement
	Modeling options	Given, a lot of pre-existing experience	Possible, no experience	Hardly available, unclear

5 Initial assessment of the effects of Electroherb on non-target organisms

5.1 Introduction

As the impact of the electro-physical Electroherb method

- leaves no material residues and decomposition products in the soil (no sorption, accumulation),
- works acutely in a very short time,
- can be applied very accurately,
- does not have any impact paths transferred later (no solubility, desorption),
- is strongly tied to the spatial geometry and structure of the application area,

a whole set of test types from the PPP assessment cannot be applied in a meaningful way.

However, some routine PPP test types can potentially be transferred without many problems or only slight modifications from the laboratory systems or particularly the samplings from mesocosms to the field trial applications to be favored by Electroherb .

By contrast, a series of monitoring field trials for the determination of acute effects and time series for the further development of farming ecosystems would be advisable. These tests are described separately.

For those tests that are considered appropriate, trial concepts and schedules will be mapped for the years 2018 and 2019, so they can then be properly implemented.

5.2 Non-meaningful standard tests

The following table refers to tests with non-target organisms as they are covered in common PPP studies for herbicides, but which Zasso, according to the above-mentioned criteria and impact paths, does not regard as meaningful.

Tests carried out for PPP assessments that are not considered meaningful for Electroherb by Zasso

Organism	Test design/recording path	Assessment of application with Electroherb	Experience/comment by Zasso as of 1/2018
Birds	Acute/oral	Not meaningful*	Not planned
Birds	5 days, food supplement	Not meaningful*	Not planned
Birds	Reproduction, feed	Not meaningful*	Not planned
Rat	Acute/oral	Not meaningful*	Not planned
Rat	2 generations, food supplement	Not meaningful*	Not planned
Accumulation in prey animal	chemical analysis of residues	Not meaningful*	Not planned
Reptiles (only tested if laboratory animals show toxicologically relevant effects)	Acute/reproduction orfeeding test	Not meaningful*	Not planned
Amphibians	Acute/reproduction	Not meaningful*	Not planned
Endocrine disruptions of wild mammals/birds	cellular tests	Not meaningful*	Not planned
Algae	Growth inhibition test	Not meaningful*	Not planned
Duckweed	Growth inhibition	Not meaningful*	Not planned
Fish	Acute, chronical, life cycle	Not meaningful*	Not planned
Daphnia, shrimp, chironomid	Acute/chronical	Not meaningful*	Not planned
Sediment organisms (if accumulation in sediment possible)	Acute/chronical	Not meaningful*	Not planned
Aquatic plants	Mesocosms	Not meaningful*	Not planned
Oysters	Development	Not meaningful*	Not planned
Honey bees	Acute test, contact test/feeding; hatchery trial; blooming Phazelia; semi-outdoor trials	Not meaningful*	Not planned
Bumblebees	Acute contact/food	Not meaningful*	Not planned
Aphid parasites (Aphidius rhopalosiphi, Hymenoptera, Braconidae)	Acute	Standard test not meaningful*; check if possible to cover with oribatid mites	Check prevalence and habitation

Parasitic mites (Typhlodromus pyri Scheuten, Acari, Phytoseiidae)	Acute/reproduction	Standard test not meaningful*; check if possible to cover with oribatid mites	Check prevalence and habitation
Earthworm (Eisensia fetida)	Acute, reproduction, body weight (laboratory)	Not meaningful*	Not planned
Spring tails	Substance/metabolite test (laboratory)	Not meaningful*	Not planned
Ground beetles	Standard tests??		Not planned
Hypoaspis aculeifer	Substance/metabolite test	Standard test not meaningful*	Not planned
Non-target plants (monocot/dicot)	28 days of sprouting, growth	Not meaningful as control can be confined precisely and happens through contact	Not planned
Resistance studies with plants	Growth test, sprouting	Not meaningful as phenotype and not genotype control	Not planned
Sewage sludge inhibition	3 h, 16 h	Not meaningful*	Not planned

* Not meaningful as no substance

** Not meaningful as no substance and water no contact biotope

Summary: It becomes obvious that a large part of the (close to 30) standard tests cannot be applied to Electroherb in any meaningful way. However, this result does not describe a flaw, instead it shows clearly that the Electroherb method poses no problem in the most important risk areas for chemical herbicides. This has to be included accordingly in the overall assessment of Electroherb impact on the environment.

5.3 Meaningful PPP standard tests

The standard tests from the PPP assessment listed below which are also meaningful for Electroherb are consistently related to soil microorganisms. It remains to be tested if makes sense to carry out these tests with soil material extracted during outdoor application tests. Also to be clarified is if a one-off field trial and a prolonged laboratory monitoring could be more meaningful than multiple field trials as a time series.

It has to be cross-checked with experts if enzyme-specific tests are meaningful or even relevant considering the unspecific effects of Electroherb compared to chemical herbicides.

Tests made for PPP assessments that are also considered meaningful and worth investigating for Electroherb by Zasso

Organism	Test design/recording path	Assessment of application with Electroherb	Experience/comment by Zasso as of 1/2018
Soil, nitrogen conversion	28 days (Lucerne flour decomposition)	Meaningful if field trial	Expert survey in 2018, test design to be checked
Soil, soil respiration	Short-term, 15 days, CO_2 in closed bottle	Meaningful if field trial	Short-term carried out; expert survey to check if longer would be meaningful
Urease activity	28 days	Potentially meaningful if field trial; check if it makes sense	Not done so far; expert survey to check if short-term or longer
Katalase activity	35 days	Potentially meaningful if field trial	Not done so far; expert survey to check if short-term or longer
Soil, microbial biomass	35 days	Meaningful if field trial	Short-term carried out; expert survey to check if longer would be meaningful

5.4 Further tests that make sense for Electroherb

The following table lists especially those test that may have a general relevance for the Electroherb method and can be carried out objectively meaningful. Some of the tests are very complex and undoubtedly only make sense for very specific queries. Trials in particular with vertebrates are not planned. However, it may be meaningful in some cases to schedule enhanced monitoring in the course of impact tests.

Tests normally not carried out for PPP assessments or only on a higher level, but which are also considered meaningful or worth investigating for Electroherb by Zasso:

Organism	Test design/recording path	Assessment of application with Electroherb	Experience/comment by Zasso as of 1/2018
Spring tails	Field trial acute, potentially chronical	Important	Continue acute; check if chronical meaningful (monitoring)
Oribatid mites (not in standard package (field trial))	Field trial acute, potentially chronical	Important	Continue acute; check if chronical meaningful (monitoring)
Earthworms	Field trial acute, potentially chronical	Important	Continue acute, acute* prolonged worm observation/check if chronical meaningful (monitoring)
Earthworms	Feeding tests chemical/mechanical treated leaf/root material	Check if relevant	Expert survey in 2018, potentially tests
Nematodes	Acute abundance tests and monitoring	Potentially important	Continue acute; check if chronical meaningful (monitoring)
Bumblebees	Escape options	Check if relevant	Expert survey in 2018, potentially tests
Bumblebees	Driving over ground nests	Check if relevant	Expert survey in 2018, if necessary tests
Wild bees	Escape options	Check if relevant	Expert survey in 2018, if necessary tests
Wild bees	Driving over ground nests	Check if relevant	Expert survey in 2018, if necessary tests
Honey bees	Escape options	Check if relevant	Expert survey in 2018, if necessary tests
Resistance studies with plants	Field trial chemical resistant plants	Meaningful regarding the reduction of chemical resistance	Planned in 2018 as part of efficiency studies
Resistance studies with plants	Field trial with plants hard to control with Electroherb	Meaningful regarding understanding the mode of action and to optimize devices;	Planned in 2018 as part of efficiency studies

		development of genetical resistance not expected	
Hypoaspis aculeifer	Field monitoring	check if possible to cover with oribatid mites	Expert survey in 2018, check prevalence and habitation
Ground beetle	Escape options	Check if relevant	Expert survey in 2018, if necessary tests
Ground beetle	Field trial acute, if necessary chronical	Check if relevant	Expert survey in 2018, if necessary tests
Lizards	Escape tests for lizards	Check if relevant	Expert survey in 2018
Lizards	Effects of driving over lizard clutch	Check if relevant	Expert survey in 2018
Birds	Effects of Electroherb on nests/eggs of lapwings when driving over	Check if relevant	Expert survey in 2018
Mice	Effects on populations of wild mice in fields and on fruit plantations	Check if relevant	Side survey planned during effectiveness tests
Mycorrhiza	Measurement of root colonization in field	Meaningful, test design to be determined	Expert survey in 2018, if necessary tests
Microorganisms	Litter degradation tests in treated soil or with treated leaf material	Potentially meaningful in comparison with herbicide treatment	Expert survey in 2018, tests planned (test sticks or bags/teabags)

Apart from continuing acute tests based on expulsion methods, their continuation as monitoring is also planned. Escape test with beetles and bees could be helpful to determine how much these species could actually be impacted by the method. Tests with ground nests are complex and need to be planned carefully. It is possible that a first insight can already be gained here by monitoring the treated areas really well.

Litter degradation and feeding tests compared with herbicide-polluted or mechanically treated plant material, for example with earthworms and microorganisms, could provide further indications if the treatment with Electroherb could have particular advantages for soil ecosystems or if for example poisonous plants could be eradicated quicker/more efficiently. A series of observations already indicates that herbicide-treated biomass biodegrades less easy than untreated biomass.

For tests with nematodes it could potentially be very interesting if for example the electricity flow through the roots and the fast mode of action would enable the control of harmful root nematodes in sprouting rape seeds far better than that of other nematodes in the soil. However, this is a trial at the threshold to efficacy testing.

Tests with soil mycorrhiza are of general interest as they are constantly demanded by prospective customers, but unfortunately they also do not seem to be part of the standard repertoire of PPP test series.

6 Information on individual test organisms

The following description of individual test organisms or groups of test organisms is intended to show the current status of test options with regard to the electroherb method. Not all groups of organisms listed here have been tested so far.

6.1 Earthworms

6.1.1 Relevance to the general ecosystem

Earthworms are very important components of soil ecosystems. They make a decisive contribution to the formation of clay/humus complexes, the degradation of plant residues, the permeability of gases and water into the subsoil and the possibility for plants to root in earthworm ducts.

In Germany about 20 species are found more frequently.

The frequency of earthworms in permanent grassland is often more than twice as high as in arable land.

The cultivation of the fields contributes decisively to the earthworm frequency there. Soil movement generally leads to a reduction in frequency. Even the cultivation of beets and potatoes can reduce the earthworm density over years with conservation tillage. Accordingly, the earthworm densities are significantly reduced in ploughed areas. Soil tillage is particularly harmful to anectic (deep-seated) earthworms in the long term.

Soil loosening and material mixing by earthworms is far more important for organic and especially soil conservation/non-ploughing farmers than in conventional agriculture.

6.1.2 Special relevance of testing for the general application of Electroherb systems - hypothesis of effect

Earthworms can generally be strongly influenced in their frequency by 3 types of impairments:

1. the attractiveness of the habitat is generally reduced (chronic effect) by strong soil movements combined with redeployment of soil and plant mass.
2. the movement of the soil can destroy channel systems in such a way that earthworms and channel systems are buried and earthworms become less viable because they are cut off from food for longer, have to dig again or die during rainfall (indirect acute effect).
3. earthworms are acutely killed or irreversibly injured during treatment by any method.

In the case of mechanical tillage, all three impairments are likely to occur, although the extent for comparative evaluations has yet to be clarified. According to the current state of knowledge, only acute death can play a role in the Electroherb process without ground movement.

It is known that earthworms start to move under the influence of electric fields/currents (angler method for earthworm recovery with batteries and octet method for the determination of earthworm frequencies).

How deep these effects are and which voltages, currents and durations are necessary is quantitatively unknown. Initial screening tests with outputs of up to 80 kW at 3 - 5 km/h and a working width of 2.6 m on a meadow areas have shown that a larger number of living, but hardly dead earthworms were found in the top 10 cm.

From the above realistic application rates, application times (at approx. 1 m applicator spacing) of 0.8 - 1.4 s can be derived as rough values. It is important to note that the cross section through which the current flows is difficult to quantify in the soil.

Electrical damage requires a considerable current flow through the worm. With the direct current or high-frequency alternating current used, thermal interference is to be expected primarily, but less with special effects on nerves, as they occur with low-frequency alternating current (cardiac influence, muscle contraction).

Its orientation relative to the direction of current is also important for the flow through the organism. Since many earthworms live in rather vertical aisles, but the current flows rather horizontally over the larger distance (entry into the soil through vertical roots better than through soil), the effective dose per earthworm is theoretically difficult to determine.

The contact resistances between soil and earthworm, the real field strength and conductivity of the corridor walls (possibly charge repulsion, because hole in a conductor) are difficult to derive and to measure.

The preferred location of the worms according to time of day and soil moisture could also have a considerable influence on the effect. Very damp soil also has a higher conductivity, which does not necessarily raise the transition from electricity to earthworms.

6.1.3 Special relevance of testing for specific application areas of Electroherb systems

Conventional arable land

Replacing ploughs with Electroherb improves the habitat for earthworms in general. To what extent earthworm populations respond positively to the replacement of glyphosate treatments (e.g. post-harvest) in combination with shallower or no tillage would have to be clarified in long-term experiments, as gradual effects can be expected here.

Organic farming

The reduction of soil tillage with plough, hoe and harrow significantly reduces mechanical intervention in the soil. This can have an impact on the overall suitability of the habitat for earthworms as well as on acute damage and animal loss due to frequent treatment.

Ploughless management of arable land

When using the Electroherb method over large areas to remove intermediate cukltures, and e.g. grazing grass cultures before sowing, either work is carried out on mainly living plants and the applicators touch little soil or mulch directly. When weed growth and fallow seed are removed over the entire area or between rows, mulch and the soil top layer, in which earthworms may be present, are more directly touched.

Meadows/continuous grassland (local application)

The Electroherb method is used to selectively control thistles, knotgrass and other undesirable plants in their local stands on grassland without affecting the main grass area.

Hard substrates (gravel, industrial areas, traffic areas)

Earthworms are generally not to be expected here.

Protected natural areas, fallow land, roadsides (local, area-wide application)

Electroherb will be used particularly where the use of chemical methods is generally prohibited or not appropriate for certain reasons (NSG, proximity to water bodies, etc.). Areas and plant species that cannot be controlled by pruning are treated in particular. General soil cultivation is usually not feasible or desirable in such areas. The Electroherb process therefore represents a minimised intervention here, which may prevent very far-reaching soil work or other very radical measures and therefore generally has less impact on earthworms than comparative processes such as hot water, soil damping, high-pressure water, scorching.

6.1.4 Special challenges for standard tests by Electroherb

As shown above, there is no research model for the influence of electricity on earthworms. This also has an impact on possible test designs. Due to their complexity, it is clear that only biotests as impact tests can achieve relevant results at all. Physical simulations and estimates are likely to have little informative value at present.

An important factor in all tests is likely to be that earthworms are strongly stimulated to move by the current. Whether and for how long the stimulation of movement extends beyond the treatment time and whether the movement always goes in the direction of the earth's surface (or simply in the direction of decreasing field strengths/current flows) is unknown.

In any case, frequency analyses with expulsion tests (mustard powder, formalin, etc.) should ensure that the earthworms:

- may accumulate in certain layers under the influence of current
- can move out of the examined layer
- in case of multiple exposure (several crossings) of an area to increase the dosage (not relevant for practice) come to the surface already after the first pass and then are contacted directly during the second pass, which can lead to unrealistic results
- with slower travel and thus longer application time, they reach the surface so much faster that they are still directly contacted by the second applicator.

To what extent the plants treated by the Electroherb process are being eaten preferably by earthworms or degrade differently compared to the vegetation after mechanical or chemical control is open. However, a change cannot be ruled out, since the Electroherb process physically destroys cells.

6.1.5 Evaluation approaches through comparison procedures

The use of the electric hearth process avoids frequent and deep soil movements. This primarily reduces the acute damage to earthworms caused by soil movement and the reduction of the attractiveness of the habitat in general (indirect and chronic effects).

When removing weeds from permanent grassland, the massive movement of the soil and the use of chemical processes can be minimised. The earthworms would also be considerably disturbed by the often necessary ground movement.

Non-mechanical comparison methods from the urban sector, some of which are also applied to soil, such as hot water, superheated steam and high-pressure water, can disrupt the soil structure and lead to extensive partial sterilisation. Since plants are only permanently damaged at temperatures of locally 60 °C, it can be assumed that all earthworms in the effectively treated soil volume will not survive this.

Under certain circumstances, a direct comparison with the effect of flame-removal devices in the test frame is possible.

6.1.6 Literature and standards

DIPLOMARBEIT ANALYSE UND BEWERTUNG DER ABUNDANZ UND DES ARTENSPEKTRUMS VON REGENWURMPOPULATIONEN (LUMBRICIDEN) IN ACKERBAULICH GENUTZTEN BÖDEN DES LEHR- UND VERSUCHSBETRIEBES GLADBACHERHOF, JUSTUS–LIEBIG–UNIVERSITÄT GIESSEN, Fachbereich Agrarwissenschaften, Ökotrophologie und Umweltmanagement, Institut für Pflanzenbau und Pflanzenzüchtung II, Professur für Organischen Landbau, gestellt von: Prof. Dr. G. Leithold, eingereicht von: cand. agr. Renate Regina Gnan, Giessen im Juni 2002

Direktsaat und Pflug im Systemvergleich; Wolfgang G. Sturny, Andreas Chervet, Claudia Maurer-Troxler, Lorenz Ramseier, Moritz Müller, Roland Schafflützel, Walter Richner, Bernhard Streit, Peter Weisskopf und Urs Zihlmann, AGRARForschung 14 (8): 350-357, 2007
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Powerpoint presentation, Kanton Bern, Amt für Landwirtschaft und Natur des Kantons Bern Fachstelle Bodenschutz, Claudia Maurer-Troxler, Bodenbiologische Erhebungen auf der Dauerbeobachtungsfläche «Oberacker»

6.2 Microorganisms

6.2.1 Introduction

Microorganisms are important for soil fertility and must be investigated, even if there is no evidence to date of any significant influence of the Electroherb process on them.

6.2.2 Special relevance of testing for the general application of Electroherb systems - hypothesis of effect

Microorganisms are generally small and live in the water phase in soil pores and on material surfaces. They therefore bridge only very small voltage differences and have no sensitive nervous systems. This is probably the reason why soil sterilization methods are generally based on heat, pressure and chemical substances, but electrical current methods are not known.

Nevertheless, it makes sense to investigate the general influence of the Electroherb method on microorganisms, as these are decisive for long-term soil fertility.

A relevant effect by heating the soil and the bacteria contained in it is to be excluded, since the amounts of energy used (max. 45 kJ/m²) would be sufficient to heat approx. 1 l of water per m² by 11 °C). Even if this energy were to heat the soil completely and not also dissipate in the plants standing on it, it is clear that no relevant bacteria can be destroyed by thermal effects. In practice, even superheated steam often takes hours to sterilize the soil.

Toxicity, if at all, could only occur through direct electrical effects, which, however, would have to have an effect on the small bacteria, which are often coated with water and biofilms.

It is certain that the Electroherb process destroys plant cells, bursts them open and dries up the plant. This can lead to a change in the proportion of readily bioavailable material in the soil and the bacteria may react with increased metabolism.

If the input of electrical energy into the soil has any relevant effect directly on the soil, it could consist of changes in the bioavailability or solubility of organic substances already present in the soil. This could also have an impact on the availability of food for the bacteria. For this reason, metabolic activity could increase at least in the short term as a result of this effect.

6.2.3 Special relevance of testing for specific application areas of Electroherb systems

Since bacteria are generally present and important for the ecosystem, there are no specific areas where these organisms need special testing or are particularly relevant.

6.2.4 Special challenges for standard tests by Electroherb

The number and activity of organisms depends on a number of factors and can only be measured cumulatively in soil or soil samples using specific metabolic products (e.g. CO_2 or e.g. dimethyl sulphide/DMS).

The basic claim of an influence study must therefore be to exclude as many trivial influencing variables as possible and then to measure whether the metabolic activity changes significantly through an experiment with Electroherb.

6.2.5 Planning and options for test design

In order to exclude the influence of growing but also dying plants on soil respiration, the soil respiration tests should be carried out on an unvegetated soil area or a vegetation-free fallow land. This is the only way to ensure that soil respiration can be safely traced back to a direct effect on the bacteria.

The two techniques refer to microorganisms and do not systematically exclude soil fungi.

For the test, the sampled area is treated once with an agricultural machine with a high but typical area output. The measurement of DOC as another quantitative measure of the presence of microorganisms is a complementary measurement. If there are differences between the two methods, these could be explained by changes in the availability of soil-borne DOC in the soil.

6.3 Bees

6.3.1 Introduction

While a number of methods for the ecotoxicological evaluation of chemicals for bees are known, the test design situation is significantly more difficult with physical methods.

The following text therefore does not describe test procedures, but only initial approaches for possible tests and general considerations.

6.3.2 Ecosystem and Electroherb relevance

Bees and bumblebees are very important components of agroecosystems and ecosystems in general as pollinators. The pollination performance seems to be optimized by a good mixture of honey bees, wild bees and bumble bees and not only affordable by a group. The different species live and reproduce in very different ways.

The Electroherb process does not lead to chemical residues on plants. For this reason, a chemical-based chronic effect can be ruled out.

The wide ranging definition and control of weeds can reduce the feed sources of bees and bumblebees. However, since the death of plants through the use of herbicides is the desired effect, this cannot be the aim of an ecotoxicological assessment. It is therefore assumed that weeds are only removed where they actually exceed damage thresholds by means of strict definition (integrated management).

The following ways of influencing bees and bumblebees are therefore under discussion:

1. bee or bumblebee sits on a plant that is caught by a high-voltage applicator and can escape
2. bee or bumblebee sits in a blossom covered with applicators
3. nest of bumblebees or solitary bees in the ground is covered by applicator.

To what extent bees and bumblebees flee from electric charges/potentials is unknown and must be tested. In general, organisms rarely bridge an area of more than 1 cm and seem to stand primarily on thin, poorly conductive legs. Whether a generally relatively high insensitivity can be deduced from this has yet to be clarified in a meaningful way.

6.3.3 Special relevance of testing for specific application areas of Electroherb systems

<u>Conventional arable land (full area)</u>

Weeds on conventional fields are generally controlled long before flowering, so that there is no danger of bees or bumblebees being caught on flowers.

In general, siccation does not take place in blooming stands.

Whether relevant quantities of flowering plants can be found in the post-harvest treatment, e.g. on the mowed cereal fields, is unlikely, but still needs to be finally clarified.

Whether earth bees and bumblebees nest on such fields in relevant numbers in the seasons in which weeds are still controlled remains to be seen.

<u>Greening areas</u>

Greening areas have a high number of flowers but are generally not herbicidally controlled. Should it make sense in individual cases, e.g. to kill thistles from greening strips by height-selective control with Electroherb, this is certainly done at the very beginning or before the flowering phase of the thistles. As composite flowers with very open flowers, the organisms' general chance of evading treatment by flying away appears high.

<u>Ploughless management of arable land (No-till)</u>

In general, non-frozen or grassy intermediate seeds or definitive weeds are treated before sowing, which should normally also be before the plants bloom. Whether there are exceptions remains to be clarified.

The extent to which nests can be damaged must be clarified. It is important to determine whether such nests actually also occur in the usually relatively dense plant stands.

<u>Meadows, lawns</u>

Selective treatment of meadow weeds generally takes place in a non-flowering state. Whether there are exceptions remains to be clarified. Meadows with recognizable larger colonies should be omitted from the treatment and are normally not areas with weeds to be treated anyway due to their meagreness.

Whether the treatment of large weeds can affect nests at the foot of the plants is unknown and not very likely. It would have to be tested.

<u>Hard substrates (gravel, industrial areas, traffic areas)</u>

Depending on the use and accessibility of such areas, plants are treated at all stages of development in order to kill and control them in the long term. Depending on the speed of the treatment systems, an escape start could become difficult. Here it would have to be checked whether a preceding active deterrent (pre-beam, air jet etc.) can be effective. At the same time, it should be checked how the organisms start flying when the plant is generally applied relatively far down from the side with current and then pushed over before the applicator sweeps it over.

<u>Nature reserves (local application)</u>

In the case of nature conservation applications of Electroherb, it is always checked in advance whether bees or bumblebees could be affected and treatment is certainly placed in periods that are more compatible with the organisms.

Areas with spontaneous vegetation in gardens

It is also strongly recommended to check weeds before flowering in the garden. A particular danger for wild bees and bumblebees can arise for their nests if weed areas on the ground are covered with the applicators. Here tests are meaningful.

6.3.4 Special challenges by Electroherb for standard tests by electricians

There are probably no standard tests that can be modified appropriately.

6.3.5 Planning and options for test design

The following test designs are conceivable and are hereby put up for discussion:

- For general sensitivity determination, static tests can be carried out in which the bees/bumble bees are placed on an area with grass, dry soil, moist soil, flowering plants (e.g. clover) which can be supplied with electricity above ground by the applicators standing on the both side of the soil area.
- It is known in this scenario that non-contacted plants continue to grow undisturbed, although potential differences in the range of up to 1-10 V/cm between applicators with high voltage (5000-15000 V) can be measured. The behaviour of organisms could allow initial statements to be made here.
- In a separate experiment - preferably outdoors - it is possible to test whether or not the bees flee before or shortly after the plants have touched the trunk with a phase applicator using highly attractive plants (bee pasture, etc.). From a physical point of view, a direct damaging effect through current flow is not to be expected, as the current flows downwards through the plant and the flower area should have a spatially constant potential (similar to birds on high-voltage lines).
- With flowering plants, from which the bees come out only slowly, parallel experiments should be made to the reaction on current supply and escape times with mechanical and electrical impact of the plant.
- In order to determine effects on below ground nests, they would have to be run over with a device. Small devices would be available for this purpose. The behaviour of the bees/bumblebees must be observed and a statement on juvenile development must be made later.

6.3.6 Intrinsic evaluation approaches of the Electroherb process

The following measurement parameters are conceivable

- Irritation/change of behaviour relative to control
- Escape speed relative to control

- lethality at different doses and times

6.3.7 Valuation approaches through comparative procedures

The following method comparisons would be conceivable, whereby it is primarily to be clarified whether there are references to this:

- Influence compared to mechanical machining processes in the field
- Influence of thermal processes (hot air, water) or high water pressure in the soil
- Effect of scorching tests
- Effect of chemical herbicides

7 Individual test descriptions

7.1 Earthworms – saturated, extensively farmed meadow

Test objective: Initial effect assessment on areas with a high abundance of organisms typical for chemical ecotoxicology

Table overview

Test title	Saturated, extensively farmed meadow
Test species	Earthworms
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso
Test design	Acute screening after expulsion with subsequent husbandry to determine vitality
Ecotox. end point	Abundance of earthworms, detection dead/live
Applied norms	Extraction of earthworms with glucosinolate
Deviation from standard	Subsequent husbandry to determine vitality
Application area	Wet, extensively farmed meadow
Device specification Electroherb	Non-selective applicators for total area treatment of all plants (2 loop rows), approx. 200 kWh/ha
Material and methods	On a marked area of 30 x 30 cm^2 a 0.6% solution was applied and any surfacing worms were collected. Then the soil in the marked area was excavated to a depth of 30 cm and sifted for more earthworms. All earthworms were kept overnight in plastic containers with soil and checked the following day. In the process the biomass of the live and dead earthworms was weighed. The expulsion occurred at three marked spots before and after the treatment on adjacent places between the lanes.
Execution	The surface was treated once. Immediately afterwards the earthworms were expelled (see above) (3 replicates each).

Comments about the test

Wet meadows are no agricultural target areas for the Electroherb method, especially not with non-selective applicators.

In a parallel test at another site using the same procedure, mortalities of more than 20% occurred in a pre-treatment control. For this reason, the results shown here may need reproduction only because of the methodology.

Results

Before the treatment 73 g earthworms per plot (30 x 30 cm) were found. This total value dropped to 59 g after the treatment, which may indicate an escape reaction.

While almost no mortality was found prior to treatment, the survival rate after 24 hours was 25% after treatment.

Conclusions and evaluation

As the Electroherb treatment is not used in agricultural practice comprehensively on extensively farmed meadows, the test is only an indication that earthworms can be damaged at all if high energy dosages are used on such areas. In addition, application was on a wet meadow, which indicates that the earthworms were very close to the surface or were in considerable numbers in the wet litter layer. The presumed escape of earthworms could be an indication that in the case of local treatment, e. g. of thistle nests or dock plants, the escape reaction can contribute considerably to the protection of earthworms.

Outlook

In any case, the manual of the Electroherb devices will, within the framework of good agricultural practice, contain a note that a comprehensive use on extensively farmed meadows is not advisable and driving on wet ground in particular should be avoided.

Trials planned for 2018 with agronomically sensible height-selective applicators for the selective treatment of docks or thistles are going to prove how narrowly the effect is limited to the immediate surroundings of the treated plants or if there will be any effect at all on non-target organisms with direct application only on the target plants.

7.2 Earthworms – monitoring of green manure – comparison with flaming

Test objective: Comparison of Electroherb with flaming on agricultural areas

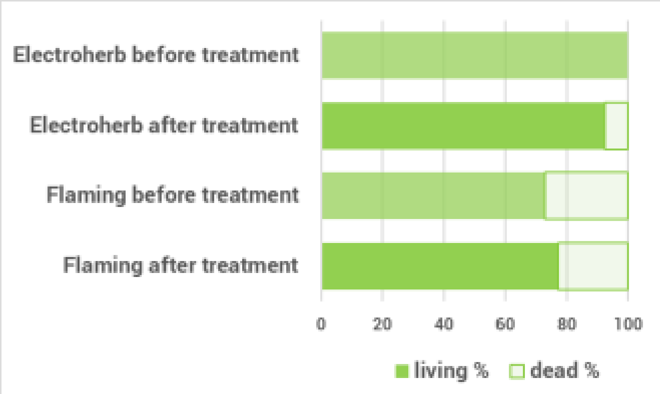
Overview table

Test title	Dry soil for vegetable production with greening – comparison with flaming
Test species	Earthworms
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso
Test design	Acute screening after expulsion with subsequent husbandry to determine vitality
Ecotox. end point	Abundance of earthworms, detection dead/live
Applied norms	Extraction of earthworms with glucosinolate
Deviation from standard	Subsequent husbandry to determine vitality
Application area	Dry soil green manure. Vegetable field
Device specification Electroherb	Non-selective applicators for total area treatment of all plants (2 loop rows), approx. 200 kWh/ha
Material and methods	On a marked area of 30 x 30 cm^2 a 0.6% glucosinolate solution was applied and any surfacing worms were collected. Then the soil in the marked area was excavated to a depth of 30 cm and sifted for more earthworms. All earthworms were kept overnight in plastic containers with soil and checked the following day. In the process the biomass of the live and dead earthworms was weighed. The expulsion occurred at three marked spots before and after the treatment on adjacent places between the lanes.
Execution	The surface was treated once. Immediately afterwards the earthworms were expelled (see above) (3 replicates each).

Comments about the test

In a partial test for flaming using the same procedure, mortalities of more than 20% occurred in a pre-treatment control. For this reason, the results shown here may need reproduction only because of the methodology.

Results



Variability of the three replicates was high. Still a clear trend can be observed. Whereas there was no mortality before the Electroherb treatment, it was 8% after the treatment. The total mass was on average the same for both sample packages. However, it must be taken into account that already before the flaming a mortality of 27% could be found, which had decreased to 23% after the treatment. The relative decrease in mortality results from a higher overall biomass of the worms with a constant mass of dead worms.

Conclusions and evaluation

The results of this test show that Electroherb applied on green manure, even with a method that may well cause significant control mortality, does not lead to a significant reduction of earthworms. It is yet unclear in how far the found mortality is an artifact of the test method. But it is safe to say that with application of the Electroherb method in real-life agricultural conditions (dry soil, green manure) earthworms do not take massive damage.

Outlook

Zasso is going to continue the trials with samples on agricultural areas under real-life application conditions on various soils and cultures in 2018.

7.3 Earthworms – comparison of mulching and no-tilling

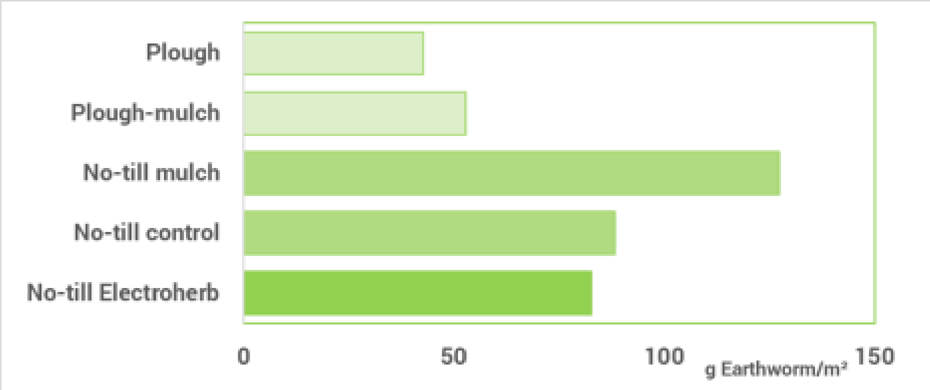
Test objective: Using Electroherb in soil-conserving agriculture

Overview table

Test title	Comparison of mulching and no-tilling
Test species	Earthworms
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso

Test design	Acute screening comparison between various treatments
Ecotox. end point	Overall number of expelled earthworms
Applied norms	
Deviation from standard	None
Application area	Arable land with green manure treated long-term with plow and no-till
Device specification Electroherb	Non-selective applicators for total area treatment of all plants (2 loop rows), approx. 200 kWh/ha
Material and methods	The expulsion of the earthworms was done with a 0.1% formalin solution (maximum 10 liters) over 50 minutes. For hand selection, 0.1 m^2 of soil of the treated area was then supplanted and tested. The collective earthworms were immediately preserved in a 4% formalin solution.
Execution	The total abundances and biomasses (mean value from four parallel samples) were determined. Prior to sampling, the individual areas were pre-treated or generally managed as follows: Plow: green manure on permanently plowed area, the day before sampling cultivated approx. 12–15 cm deep by ONLAND plow Plow-mulch: green manure on permanently plowed area, mulched the day before No-till mulch: green manure on permanent no-till area, mulched the day before No-till control: green manure on permanent no-till area, sampled standing No-till control: green manure on permanent no-till area treated with Electroherb immediately before sampling

Results



As was to be expected, the density of earthworms in no-till areas is always significantly higher than in mainly plowed areas. Mulching the day before leads to an increase in earthworm abundance, possibly because the feed basis is significantly improved.

Relative to the control there are 6% less earthworms after the Electroherb treatment. This may be due to an escape reaction or patchiness of the area, which could not be solved even through replicates.

As with no-till Electroherb was sampled directly after the treatment, the dying biomass was here not (yet) able to attract the earthworms.

The density of earthworms after the Electroherb treatment is significantly higher than the values for plowing with and without mulching, even though the layer of mulch was still able to attract earthworms.

Conclusions and evaluation

In this test under real-life agricultural conditions the Electroherb method also only shows a small reduction in the biomass of the earthworms found directly after sampling. This can probably have been caused by escape reactions or scattering of the results. No significant mortality is detectable.

The attraction dynamics for earthworms could generally be different here because of the weather-related slower wilting of the plant material.

Earthworm densities significantly above all plow treatments (which were low) even with the Electroherb treatment show clearly that the Electroherb method is able to provide a significant contribution to habitat conservation for earthworms, if it is used to exclude plowing and replace chemical herbicide treatments.

Outlook

For 2018 and 2019 trials under real-life agricultural conditions are planned, ideally on no-till and plow-cultivated arable land, with earthworm monitoring at selected points of time across the entire season. This will be used to improve characterization of the seemingly already very limited impact of the Electroherb methods and describe the positive impact of systemic Electroherb use compared to soil movement.

It makes sense to run parallel trials with glyphosate to depict the attraction to earthworms as well as the glyphosate-replacing systemic effect of Electroherb by direct comparison.

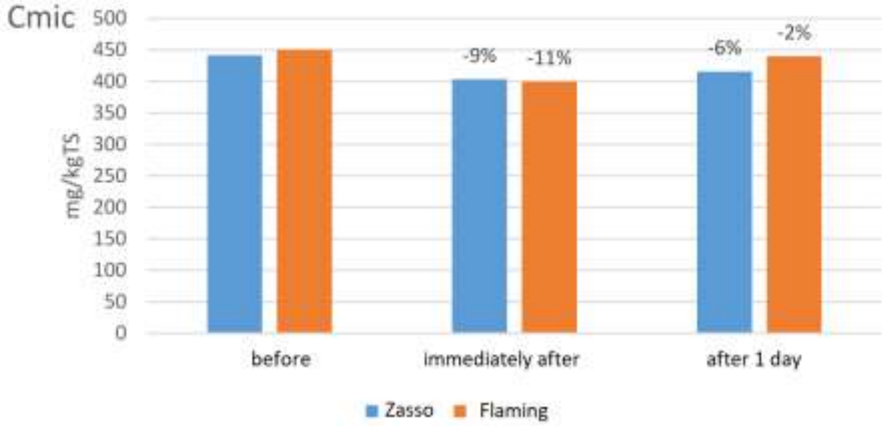
7.4 Microorganisms – effects in field vegetable areas

Test objective: Assessment of acute effects on microorganism populations

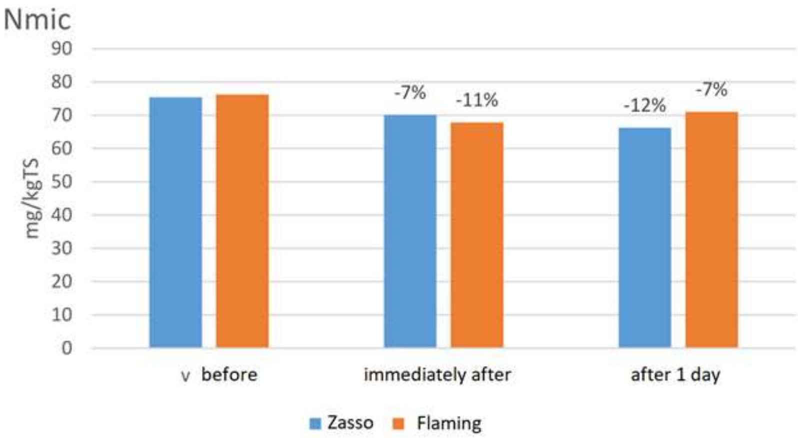
Overview table

Test title	Dry soil for vegetable production with green manuring – comparison with flaming
Test species	Microorganisms
Test year	2017
Testing facility	Not published, test facility with long experience commissioned by Zasso
Test design	Acute screening
Ecotox. end point	Cmic, Nmic, basal respiration $C0_2$
Applied norms	
Deviation from standard	
Application area	Dry soil with green manure. Vegetable field
Device specification Electroherb	Non-selective applicators for total area treatment of all plants (2 loop rows), approx. 200 kWh/ha
Material and methods	• Mixed sample of 10–15 sample rings (triple measurement per sample) • Soil samples are stored at 2 °C until analysis • Measurement of basal respiration respectively C-mineralization performed according to standard method • Determine microbial biomass according to CFE-method (Chloroform-Fumigation-Extraction)
Execution	• Samples are drawn immediately before and after the treatment • Further soil samples are drawn several days after the treatment

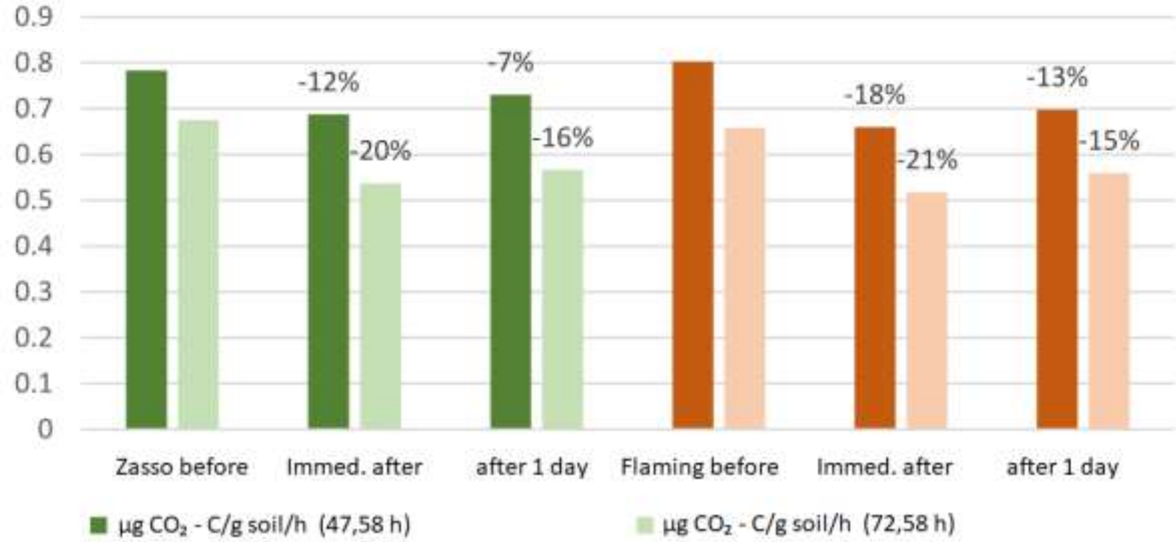
Results



The measured microbial carbon content decreases after treatment with flaming as well as Electroherb to the same amount and recovers already the next day. The overall effects are small.



The measured microbial nitrogen content decreases after treatment with flaming as well as Electroherb to the same, small amount and recovers already the next day with flaming, but not with Electroherb. The overall effects are small.



Basal respiration decreases directly after Electroherb treatment to a lesser degree than with flaming and recovers already on the next day slightly more than with flaming. The measurements for 48 and 73 hours show the same tendencies, with both methods showing the lowest value of 20/21% immediately after treatment in the 73-hour measurement.

Conclusions and evaluation

At this location, flaming and Electroherb seem to have a very similar influence on basal respiration, Cmic and Nmic, which is quite remarkable considering that they have very different modes of action, and a very different expected penetration depth of the effect. With the rapid onset of recovery no permanent damage is to be expected. The tendencies of all 3 test approaches are consistent in themselves for the sample.

Outlook

In order to better understand the effects of Electroherb, trials will be carried out in 2018 in which samples are to be taken in a stratification layer as well as at different distances from contacted plants (e. g. dock). This should help to clarify whether the effects shown here are real-life effects or could be caused by cross-treatment test effects.

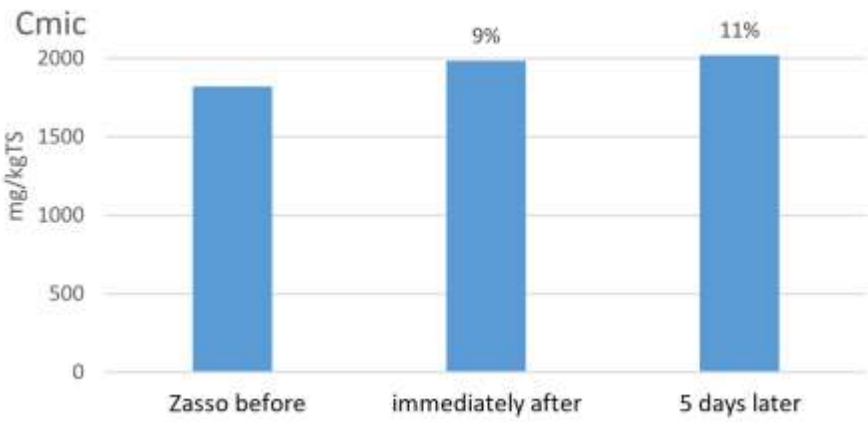
7.5 Microbiology – wet, extensively farmed meadow

Test objective: Analysis of microbiological effects under wet soil conditions

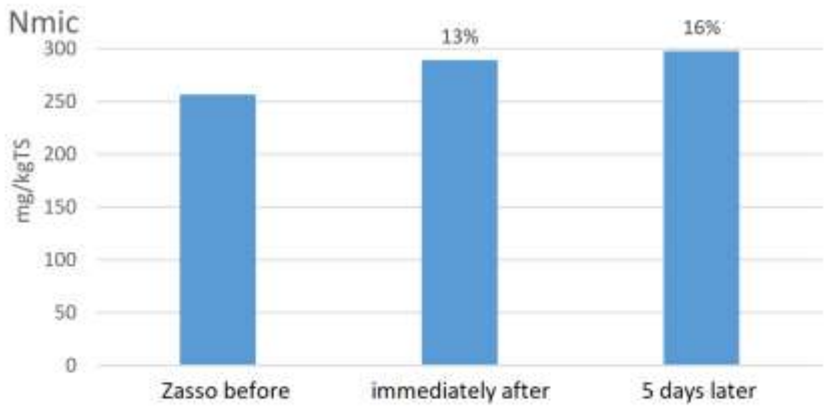
Overview table

Test title	Wet, extensively farmed meadow
Test species	Microorganisms
Test year	2017
Testing facility	Not published, test facility with long experience commissioned by Zasso
Test design	Acute screening
Ecotox. end point	Cmic, Nmic, basal respiration $C0_2$
Applied norms	
Deviation from standard	
Application area	Wet, extensively farmed meadow
Device specification Electroherb	Non-selective applicators for total area treatment of all plants (2 loop rows), approx. 200 kWh/ha
Material and methods	• Mixed sample of 10–15 sample rings (triple measurement per sample) • Soil samples are stored at 2 °C until analysis • Measurement of basal respiration respectively C-mineralization performed according to standard method • Determine microbial biomass according to CFE-method (Chloroform-Fumigation-Extraction)
Execution	• Samples are drawn immediately before and after the treatment • Further soil samples are drawn several days after the treatment

Results

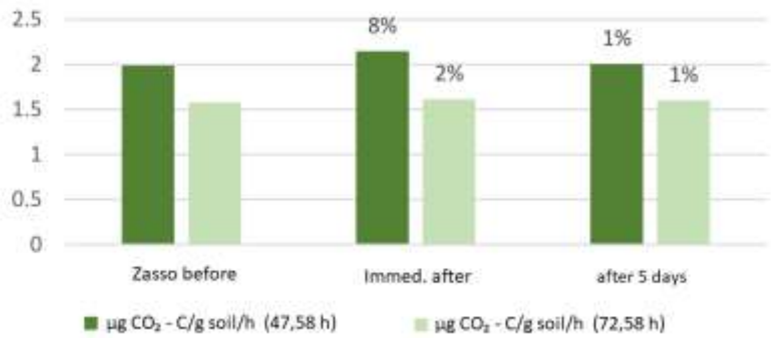


The measured microbial carbon contents increases after treatment and remains 11% higher even after 5 days.



The measured microbial nitrogen contents increases after treatment and remains 16% higher even after 5 days.

The overall effects are small.



Basal respiration increases immediately after Electroherb treatment by 8% and normalizes to the initial value after just 5 days (deviation 1%). The tendency for the 48 and 73 hour measurements is practically identical.

Conclusions and evaluation

In this soil the increased basal respiration is connected to a growth of biomass.

At this location, basal respiration, Cmic and Nmic can be influenced in a very similar way, which is quite remarkable considering that they have very different modes of action and a very different expected penetration depth of the effect. With the rapid onset of recovery and the normalization of soil respiration after 5 days, no permanent damage is to be expected. This is particularly true as better current conduction and exposure of the microorganisms was to be expected in humid soil.

Outlook

In order to better understand the effects of Electroherb on different soils, further trials will be carried out in 2018 with various soil types and humidities, in which samples are to be taken in a stratification layer as well as at different distances from contacted plants (e. g. dock). This should help to clarify whether the stimulation effects shown here are real-life effects or could be caused by cross-treatment test effects.

7.6 Oribatid mites and collembola – extensively farmed meadow

Test objective: Clarify if collembola and oribatid mites are influenced by high-dosage effects of Electroherb

Overview table

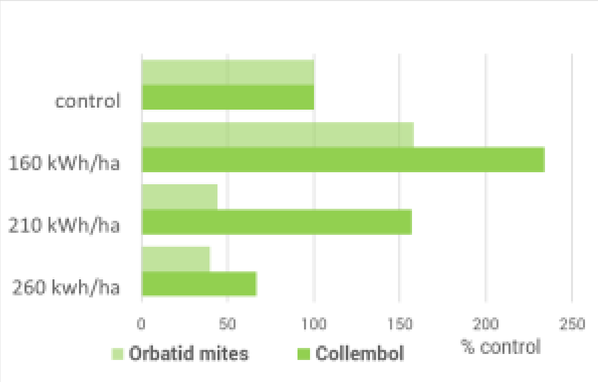
Test title	Maximum effects on an extensively farmed model meadow
Test species	Collembola and oribatid mites
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso
Test design	Acute screening before and after Electroherb treatment
Ecotox. end point	Abundance after expulsion
Applied norms	ISO-23611_2 soil sampling
Deviation from standard	none
Application area	Extensively farmed meadow, neglected for many years

Device specification Electroherb	3 kW unit with two 20 cm wide applicators, both driving over the test area and then placing a separate, adjacent track, power logging Applicators with full area contact and very good ground hugging
Material and methods	Sample rings 5 cm cylinders 3 replicates per treatment MacFadyen-Extractor Counting the organisms sorted into collembola and oribatid mites
Execution	Electroherb in 3 dosages (160, 210, 260 kWh/ha) Randomized small field design (approx. 40 x 40 cm) 10 controls, 5 treatments each On the same evening transfer to extractor for 2 weeks

Comments about the test

Patchiness and the resulting standard deviation is high at the test site. Options for the evaluation of smaller groups in the immediate vicinity have not changed the result and are therefore not followed up.

Results



A total of 548 collembola and 1651 oribatid mites were counted in the 25 plots overall.

With an energy density of 160 kWh/ha, which is sufficient for many areas, far more organisms were counted than in the controls. The cause of this is unknown.

With an energy density of 210 kWh/ha, which seems only to be necessary for very stubborn areas, there were still far more collembola found than in the control, whereas oribatid mite numbers decreased sharply. Whether this is due to escape or mortality cannot be determined.

With an energy density of 260 kWh/ha (which is generally above any planned application), the number of expelled collembola decreased to 60%.

Conclusions and evaluation

Due to the small-scale treatment, it cannot be ruled out that the reduced counts were not caused by mortality, but by escape behavior. However, it is certain that the application was carried out here as an ecotoxicological efficacy study and not as a simulation of real-life field conditions, since the meadow was treated over the entire area. It is evident that no inhibition has been found with a normal application energy density of 160 kWh/ha.

Outlook

Further tests with wider applicators need to show if the test results were caused by the escape of organisms, by activation or mortality. Application under realistic conditions with further optimized applicators in 2018 will lead to a better understanding of the observations. In the meadow area, Zasso will only focus on the height-selective application against dock etc. These applications are localized and therefore particularly beneficial for the survival of organisms in escape reactions.

7.7 Monitoring – collembola, oribatid mites, nematodes in chicory fields

Test objective: Monitoring on former chicory fields (only cultivated), which were treated 2 months beforehand with very high dosages to deactivate deep-set roots

Overview table

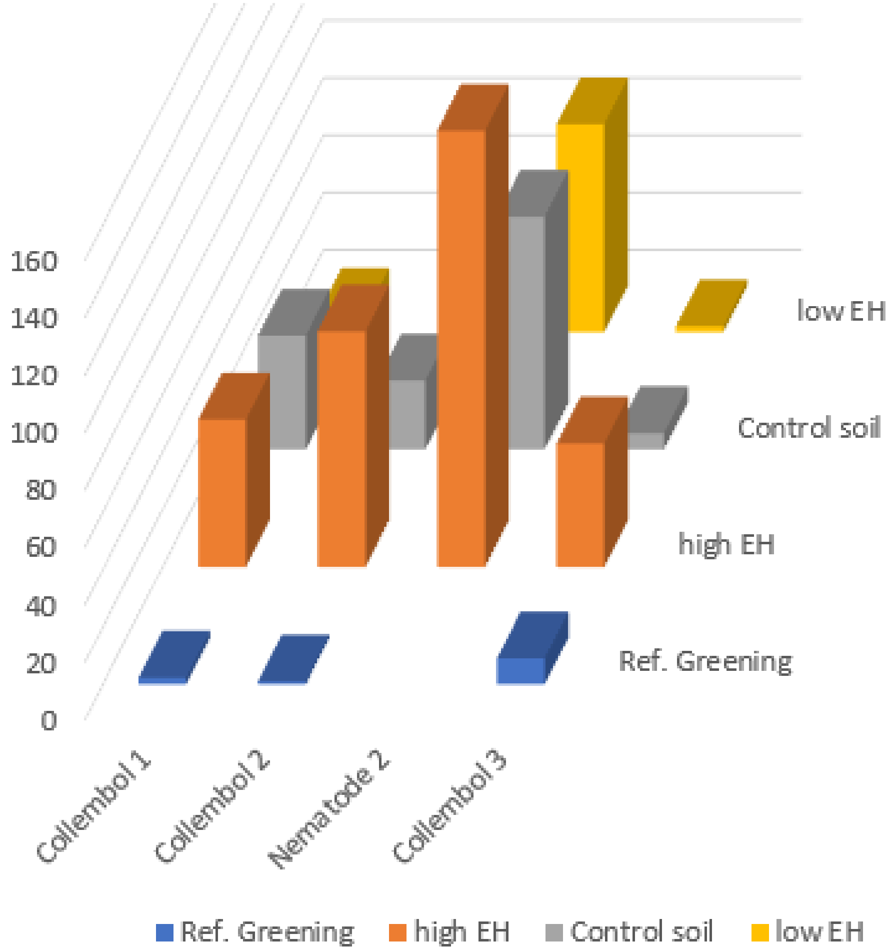
Test title	Monitoring chicory field
Test species	Collembola, oribatid mites and nematodes
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso
Test design	Long-term monitoring 2 months after treatment with Electroherb
Ecotox. end point	Abundance after expulsion or extraction
Applied norms	ISO-23611_2 soil sampling
Deviation from standard	
Application area	Chicory field then winter grain, treatment of the stubbles with chicory regrowth and volunteer grain
Device specification Electroherb	Tongue applicators at the front, with rubber edge to minimize stray currents into the soil, at the back full area loop applicators for full area treatment of all plants with approx. 200 kWh/ha per drive
Material and methods	Sampling ring 5 cm cylinder 3 replicates per treatment MacFadyen-Extractor Counting of the organisms sorted into collembola and oribatid mites
Execution	Electroherb 200 kWh/ha on one stripe 3 measurement points each in dense stubbles and in the bare soil

	On the same evening transfer to extractor for 2 weeks Samples from 5 sampling rings were mixed and partial samples from this extracted with Ludox to count all nematodes under the microscope

Comments about the test

The number of oribatid mites was so low that evaluation did not make sense.

Results



The number of collembola in the highest treatment, furthest away from the field edge, is the highest. Control and lower treatment have similar abundances. Nematodes show exactly the same trend. Interestingly in practically all cases the abundance is higher than in the adjacent greening area.

Conclusions and evaluation

It can be assumed that the position in the field has a considerable influence on the result. However, it is certain that even very high-voltage Electroherb treatments on an almost bare field do not obviously restrict the number of collembola.

The low abundance on greening areas has to be further clarified.

Outlook

Complete time series on sample treatment areas are planned for 2018 in order to better monitor the dynamics of organisms. This will also include various types of green manure.

There will also be tests carried out with deliberate overdoses in order to generate effects and to be able to better determine safety factors.

7.8 Collembola – post-harvest treatment of stubble fields

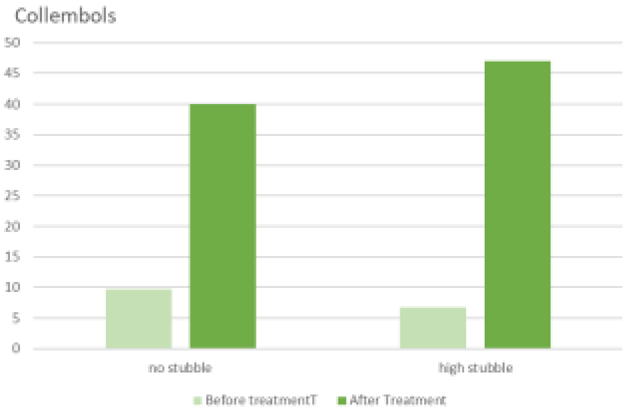
Test objective: Determine whether effects occur on stubble fields during post-harvest treatment and whether other effects occur in the stubble area (fewer contact of the applicators with the soil) compared to the open ground area (e. g. vehicle traces during harvesting)

Overview table

Test title	Stubble field post-harvest treatment
Test species	Collembola, oribatid mites
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso
Test design	Acute test
Ecotox. end point	Abundance after expulsion
Applied norms	ISO-23611_2 soil sampling
Deviation from standard	
Application area	Harvested grain field, conventional
Device specification Electroherb	Tongue applicators at the front, with rubber edge to minimize stray currents into the soil, at the back full area loop applicators with an overall width of 2.8 m, applicators for full area treatment of all plants with approx. 150–200 kWh/ha per drive
Material and methods	Sampling ring 5 cm cylinder 3 replicates per treatment MacFadyen-Extractor Counting of the organisms sorted into collembola and oribatid mites
Execution	Electroherb 200 kWh/ha on one stripe, double dosage with second drive-over on second stripe, in between untreated control stripe 3 measurement points each on a slope + fourth one in the directly adjacent green manure (approx. 30 cm high yellow mustard/fodder reddish) On the same evening transfer to extractor for 2 weeks

Comments about the test

The number of oribatid mites was too low for relevant evaluation.



Results

Regardless of the position on the field, the use on the stubble area or bare areas, significantly more collembola were counted after the treatment than before the treatment. The second sampling took place a few minutes after the treatment. Inhibitions are by no means noticeable, even if the soil has always been touched directly, at least with the second applicator.

Conclusions and evaluation

The treated zones were each over 1 m wide and sampling was carried out in the center. This makes escape reactions to the applicators relatively improbable. The effect of the higher counter values after treatment, which has already been observed in the meadow area, is repeated. Inhibitions are unlikely. However, the much lower abundance of all organisms compared to the no-till chicory field is alarming, despite sampling on the same day and at a distance of only 20 km on soils not dissimilar.

Outlook

Further trials in 2018 will try to clarify these phenomena.

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7.9 Collembola, nematodes – greening

Test objective: Determine whether Electroherb treatment in regrown greening prior to flowering leads to ecotoxicological effects

Overview table

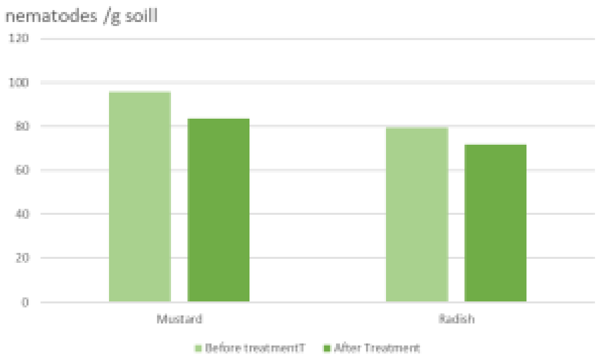
Test title	Greening area
Test species	Collembola, oribatid mites and nematodes
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso
Test design	Acute test
Ecotox. end point	Abundance after expulsion or extraction
Applied norms	ISO-23611_2 soil sampling
Deviation from standard	
Application area	Greening after winter grain, volunteer grain removed by multiple cultivating
Device specification Electroherb	Tongue applicators at the front, with rubber edge to minimize stray currents into the soil, at the back full area loop applicators for full area treatment of all plants with approx. 200 kWh/ha per drive
Material and methods	Sampling ring 5 cm cylinder 3 replicates per treatment MacFadyen-Extractor Counting of the organisms sorted into collembola and oribatid mites Nematodes from partial sampling of 5 sampling rings counted after LUDOX extraction
Execution	Electroherb 200 kWh/ha on one stripe 1 measurement point each in both green manure areas (mostly fodder reddish or mostly yellow mustard) On the same evening transfer to extractor for 2 weeks

	Collected samples from 5 sampling rings were mixed and partial samples from this extracted with Ludox to count all nematodes under the microscope.

Comments about the test

The collembola and oribatid mite numbers found in all samples of the green manure were for the most part between 0 and 3 per sample ring, which makes any relevant evaluation impossible. This is a continuation of the trend shown in the chicory experiment, where hardly any collembola and oribatid mites were found in the green manure.

Results



There was a slight reduction of nematode numbers observable in the yellow mustard as well as in the fodder reddish.

Conclusions and evaluation

As this was a first screening test about the applicability of nematode tests as such, no further differentiation into useful and potentially harmful nematodes has been made.

Outlook

Further tests in 2018 will clarify whether one nematode type was particularly badly harmed, which should be suppressed if necessary. If it turns out to be a general rule that very few collembola and oribatid mites can be found in green manure (or some mixtures), intensive tests with regard to their damage become unnecessary.

7.10 Collembola, nematodes – ryegrass

Test objective: Determine whether Electroherb treatment of annual pasture grass has a similar effect as on extensively farmed meadows

Overview table

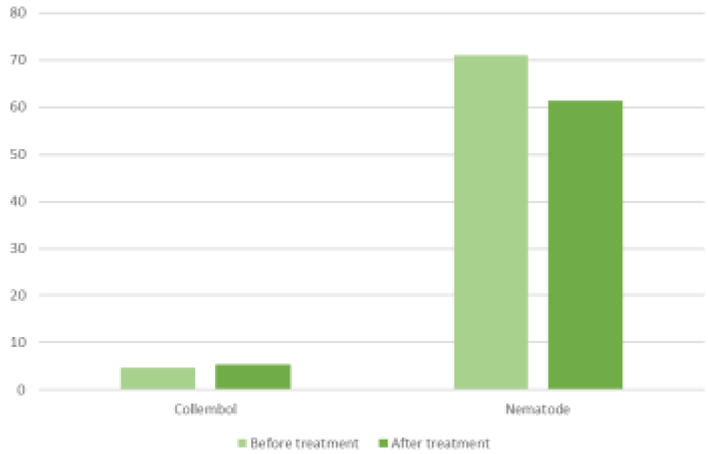
Test title	Ryegrass, 1 year old
Test species	Collembola, oribatid mites and nematodes
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso
Test design	Acute test
Ecotox. end point	Abundance after expulsion or extraction
Applied norms	ISO-23611_2 soil sampling
Deviation from standard	
Application area	Ryegrass, 1 year old, mowed
Device specification Electroherb	Tongue applicators at the front, with rubber edge to minimize stray currents into the soil, at the back full area loop applicators for full area treatment of all plants with approx. 200 kWh/ha per drive
Material and methods	Sampling ring 5 cm cylinder 3 replicates per treatment MacFadyen-Extractor Counting of the organisms sorted into collembola and oribatid mites Nematodes from partial sampling of 5 sampling rings counted after LUDOX extraction
Execution	Electroherb 200 kWh/ha on one stripe 1 measurement point each in both green manure areas (mostly fodder reddish or mostly yellow mustard) On the same evening transfer to extractor for 2 weeks

	Collected samples from 5 sampling rings were mixed and partial samples from this extracted with Ludox to count all nematodes under the microscope.

Comments about the test

The numbers of oribatid mites found in all samples were usually between 0 and 2 per sample ring, which makes any relevant evaluation impossible. At least the low numbers of collembola were consistent in themselves.

Results



For the collembola, the mean number of organisms indicated per sample ring here was consistently low before and after treatment.

As already observed in other tests, the nematode numbers decreased in a range of between 10 and 15%.

Conclusions and evaluation

At least the investigated ryegrass area clearly behaves more like a field than a meadow. Relevant damage could not be found.

Outlook

Further trials in 2018 should clarify whether effects or their absence can be better determined for example on no-till grassland areas with a slightly higher organism density.

7.11 Collembola – meadow with selective thistle control

Test objective: Determine whether selective thistle control affects surrounding soil areas

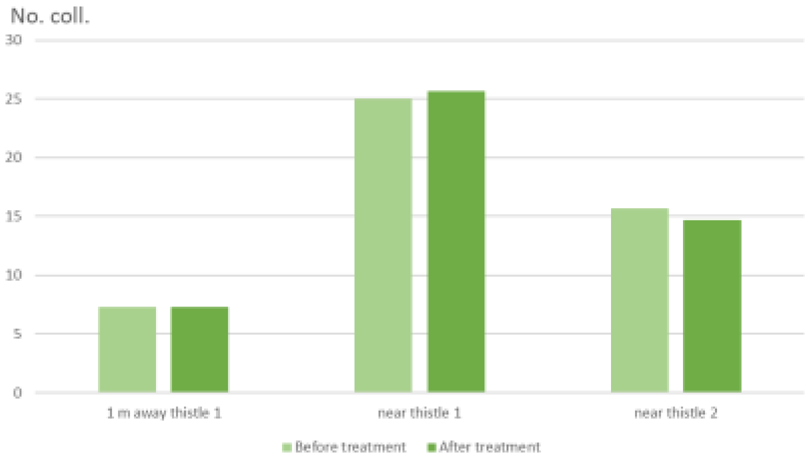
Overview table

Test title	Conventional cow meadow thistles
Test species	Collembola
Test year	2017
Testing facility	[redacted] Not published, test facility with long experience commissioned by Zasso
Test design	Acute test
Ecotox. end point	Abundance after expulsion
Applied norms	ISO-23611_2 soil sampling
Deviation from standard	
Application area	Cow meadow, grazed down to thistle, conventional
Device specification Electroherb	Tongue applicators at the front, with rubber edge employed as height-selective, at the back full area loop applicators not driven over the area to simulate height selectivity of all plants with approx. 200 kWh/ha per drive
Material and methods	Sampling ring 5 cm cylinder 3 replicates per treatment MacFadyen-Extractor Counting of the organisms sorted into collembola and oribatid mites
Execution	Electroherb 200 kWh/ha on one stripe 1 measurement point each in 1 m distance to thistles and then close to thistle foot, 3 replicates each On the same evening transfer to extractor for 2 weeks

Comments about the test

Only collembola could be evaluated because of the low density of oribatid mites.

Results



As expected, the number of collembola near the thistles is higher than it is 1 m away in the grass. The overall density is low. However, the values do not change in any relevant way as a result of the treatment of the thistles, causing them to die.

Conclusions and evaluation

The concept of minimized impact with height-selective devices works. In contrast to other tests, the number of collembola remained the same and did also not increase. This suggests that the collembola did not come into contact with any attractive voltages and currents. The grass was not damaged by the amount of applied energy, which was kept low by the height-selective applicators.

Outlook

Height-selective trials on meadows are going to be continued and intensified into 2018 as they can help to avoid a lot of work and soil disturbance in the grassland.