MicroSoil – Correctly assessing the risks for microorganisms in agricultural soils – identifying meaningful endpoints under field-relevant exposures of biocides, pharmaceuticals and plant protection products

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Introduction and aims

Over the next three years, the project "MicroSoil" aims to identify meaningful endpoints for microorganisms under field-relevant exposure of chemicals in soils (e.g. plant protection products (PPP), biocides or pharmaceuticals). Within five work packages (Figure 1), it will be investigated whether additional endpoints should be proposed for the environmental risk assessment, in order to correctly address the risks for soil microorganisms exposed to chemicals in soils. In addition, uncertainties in the risk assessment due to the current consideration of exposure to single substances will also be investigated by determining the influence of repeated applications of e.g. PPPs on processes driven by soil microorganisms.

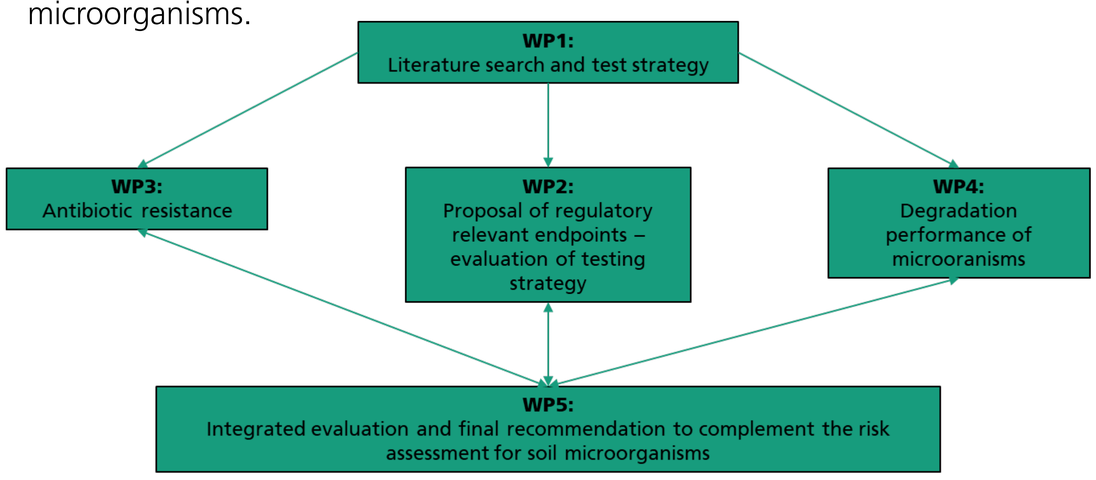


Figure 1: MicroSoil – general structure and work packages.

Work package 1 "Literature search and test strategy"

Step 1: Literature search

- Aim: Identification of test methods addressing changes of functional and structural diversity of microbial community (especially bacteria and fungi) in soils.
- The search focused on the following three different areas:
  - *General terms:* Literature regarding the measurement of soil functions, soil microbial structure and their assessment.
  - *Methods assays:* Literature regarding i) specificity of selected, A appropriate test methods and ii) determination as well as assessment of relevant soil processes.
  - *Effect studies*: Literature on the effect of PPP or other chemicals on soil microorganisms

Results of Step 1

- 23 methods (Table 1) were identified and divided into six groups.
- Groups include methods for determining (i) the activity of aerobic and anaerobic heterotrophic microorganisms, (ii) nitrifying and denitrifying bacteria, (iii) the activity of exo- and endoenzymes, (iv) the effect on arbuscular mycorrhizal fungi, (v) the determination of functional genes and structural changes in soils and (vi) carbon cycling and sequestration.

Step 2: Assessment of the methods

- Five assessment parameters were determined: practicability, estimated costs, replicability/reproducibility, feasibility depending on soil type and relevance for regulatory purposes.
- A traffic light system including green (well suited), yellow (suited) and red (barely suited) was used to classify the individual test methods.
- The individual parameters (see above) were assigned with scores from 1 (red) over 2 (yellow) to 3 (green) and finally summed up.
- Firstly, all five parameters were treated equally. Secondly, the parameters were prioritized differently, depending on their relevance for risk assessment.
- Both approaches were considered for the identification of possibly suitable tests methods.

Table 1: Compilation of the test methods found by the literature search.

Method/guideline		Endpoint
(i) Activity of aerobic (and anaerobic) heterotrophic microbial biomass	*OECD 217**	C-transformation*
	Biolog®	Nutrient cycles or turnover of C-, N-, P- and/or S
	MicroResp™	Nutrient cycles or turnover of C-, N-, P- and/or S
	Fe(III) reduction test	Soil microbial activity
(ii) Nitrifying and denitrifying bacteria	*OECD 216**	N-transformation*
	ISO 15685	Potential ammonium oxidation
	ISO 20131-1/2	Soil denitrifying enzyme activities
(iii) Enzymatic activity	ISO 20130	Enzyme activity patterns (colorimetric substrates)
	ISO 22939	Enzyme activity patterns (fluorogenic substrates)
	ISO 18187	Dehydrogenase activity of *Arthrobacter globiformis*
	ISO 23753-1/2	Dehydrogenase activity (TTC or INT)
	Urease	Urease activity
	ABTS	Phenol oxidase activity
	Peroxidase	Peroxidase activity
	Fluorescein diacetate	Total microbial activity
(iv) Fungal community	ISO 10832	Spore germination test (*Funneliformis mosseae*)
	Laccase	Enzyme activity based on laccase from fungi
(v) Functional genes and structural profile	ISO 17601	Abundance of selected microbial gene sequences
	ISO 29843-1/2	Soil microbial diversity (phospholipid fatty acids)
	DGGE	Denaturing gradient gel electrophoresis
	T-RFLP	Terminal Restriction Fragment Length Polymorphism
	ARISA	Automated rRNA intergenic spacer analysis
(vi) Carbon cycling and sequestration	OECD 56	Guidance document on the breakdown of organic matter in litterbags

* OECD 216/217 were only mentioned as reference. OECD 56 is mentioned but was not further considered (see comment below (step 3).

Step 3: Test strategy

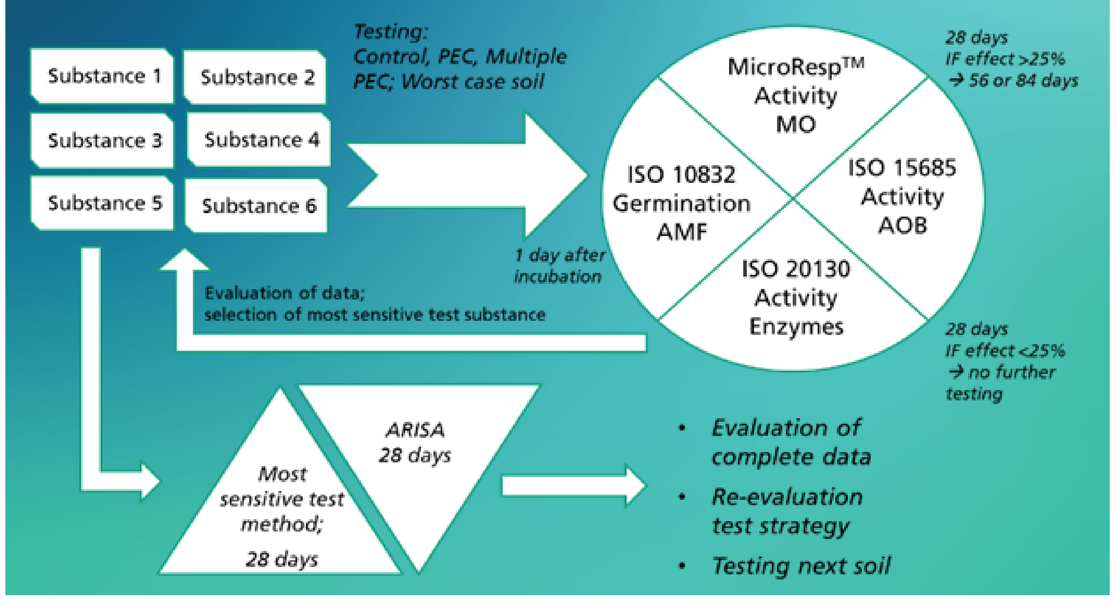
- Based on Step 2, five test methods with the highest score for each group were elaborated:

(i) Activity of aerobic, heterotrophic microorganisms: MicroResp™
(ii) Activity of nitrifying and denitrifying bacteria: ISO 15685
(iii) Enzymatic activity: ISO 20130
(iv) Fungal community: ISO 10832
(v) Functional genes and structural profile: Automated Approach for Ribosomal Intergenic Spacer Analysis (ARISA)

- OECD 56 (vi) is a long-term test conducted as field experiment, and a modified application in the laboratory might be possible. However, due to the low score and low relevance for environmental risk assessment it was not included in the experimental work.

Outlook

Sensitivity of selected test methods will be investigated for six model substances (PPPs, biocides and antibiotics) and three soils varying in their physico-chemical properties:



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