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Soil organisms as an essential element of a monitoring plan to identify the effects of GMO cultivation. Requirements – Methodology – Standardisation

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Abstract

After a release of genetically modified organisms, monitoring of potential adverse effects on the environment is mandatory. The protocol used for monitoring should be previously tested in practical studies and must be standardised. Moreover, sampling methods and the evaluation of results must meet current scientific and technical standards. Due to their particular role in maintaining soil quality and in a multitude of ecological processes in agro-ecosystems, soil organisms belong to those groups for which VDI guidelines are being developed. The guideline 4331 Part 1 describes fundamental criteria for the selection and sampling of soil organisms for GMO monitoring and gives guidance for sampling design, sampling strategy and statistical evaluation. In the guideline three approaches are followed: (1) a compilation of previously known effects and exposure pathways, (2) a documentation of ecological functions of soil organisms (ecosystem services) as well as (3) a description of characteristic species compositions in the soil. The aim was to develop a selection matrix that helps to choose the appropriate animal groups to be sampled. Besides the habitat type and the ecological relevance, the selection matrix also considers the suitability of animal groups in terms of practical issues and, in specific cases, anticipated effects. Further parts of the guideline 4331 will describe sampling methods for relevant soil animal groups.

Keywords

Soil Organism, Monitoring, Genetically Modified Organism, Standardisation, Sampling

Introduction

European directive 2001/18/EC on the deliberate release of genetically modified organisms (GMOs) into the environment prescribes compulsory post-market monitoring as a means of identifying adverse effects of GMOs and their use on the environment [EC 2001]. Consequently, the prevention of the potential occurrence of adverse effects on the environment attributable to the cultivation of authorised genetically modified organisms and the retrospective documentation of any such effects is a mandatory requirement. This calls for the implementation of a robust programme of case-specific monitoring and general surveillance to identify negative environmental impacts [GenTG 2010].

To ensure that the data obtained from GMO monitoring is comparable, reproducible and interpretable, it is essential that field-tested, standardised sampling and evaluation methods are used, which are available prior to the commencement of monitoring programmes [EC 2002]. Appropriate monitoring methods must be described in a manner that is both practical and understandable, e.g. in the form of guidelines or concrete instructions [Nobel et al. 2005]. Established methods used in existing environmental monitoring programmes and concepts may form the basis for developing a suitable and specific methodological repertoire for GMO monitoring [UPB 1996, Barth et al. 2000]. It may be necessary to develop additional methods to address new issues specific to genetic engineering.

Potential environmental impacts resulting from the cultivation of GMOs can affect any ecosystem. For this reason it is important to have access to a comprehensive methodological repertoire from which a suitable monitoring method can be selected according to the type and characteristics of the GMO. The results of a scientific review showed, however, that there is still a need for standardised GMO monitoring methods for a significant number of relevant animal groups [Lang 2007]. Soil organisms, wild honeybees and amphibians are of particular priority. Although a wide range of species-monitoring programmes exists at regional and national levels, the methods used are rarely standardised and not always suitable for characterising the effects of GMOs.

Due to their particular role in maintaining soil quality and in a multitude of ecological processes in agro-ecosystems, soil organisms belong to those groups for which draft guidelines are being developed (VDI 4331 guidelines series). Guideline 4331 Part 1 provides the framework for this series: "Monitoring the Effects of Genetically Modified Organisms (GMOs) – the Effects of GMO Cultivation on Soil Organisms". This guideline describes fundamental criteria for the selection and sampling of soil organisms for GMO monitoring and gives guidance on sampling design, sampling strategy and statistical evaluation. The guideline is intended to summarise and further develop existing individual standards [e.g. ISO 2006a, ISO 2006b, ISO 2007a, ISO 2007 b, ISO 2010a, ISO 2010b]. It can also be used for other soil monitoring objectives.

Monitoring the potential effects of GMO cultivation on soil organisms

Opportunities and challenges relating to the sampling of soil organisms

A programme to monitor the effects of GMOs in the field must aim to determine not only monocausal effects on individual species, as with laboratory studies, but also the consequences of direct and indirect cause-effect chains on organisms, their ecological communities (biocenoses) and their functions. The VDI monitoring guideline for soil organisms is thus based on three assessment levels (approaches): (1) effects and exposure pathways previously identified in laboratory tests and experimental investigations, (2) ecosystem services provided by soil organisms and (3) soil biodiversity.

Genetically modified organisms can directly affect soil biodiversity as well as the natural functioning of soil as a habitat for soil organisms (§2 (2) 1.a BBodSchG 1998 - German Federal Soil Protection Act) [BBodSchG 1998]. As a result, this can adversely affect the ecosystem services provided by these organisms as a whole. Targeted monitoring of selected animal groups can also provide information about the decomposition, balancing, buffering and restoration properties of soil. Besides the determination of direct effects of GMOs, soil organisms can also be used to monitor landscape changes caused indirectly by GMO cultivation, in particular due to changes in soil cultivation methods and crop rotation.

Only regarding the diversity or services of soil organisms, observed at a particular site during the course of GMO monitoring, does not provide sufficient information about the ecological condition of the soil community (including potential effects of GMOs on this community). A set of assessment criteria (i.e. a reference system from which target values can be derived) must be additionally established for each measurement parameter in order to determine whether a specific observation is negative or positive. Such references must be defined in terms of "good ecological status" [UBA 2008], as this is the only way to identify and evaluate a deviation [Toschki 2008, Kowarik et al. 2006, Züghart and Breckling 2003a, Züghart and Breckling 2003b]. Assessment of biodiversity would be either impossible or highly inaccurate without an understanding of natural fluctuations in species incidence at the respective sites [Heissenberger et al. 2003]. Furthermore, when defining a reference system, thresholds must be established, exceedance of which indicates a significant or harmful deviation [Potthast 2004]. In concrete terms, a reference system for the diversity of soil organisms may consist of lists of species, which are expected to be present at a specific site associated with specific conditions (e.g. climate, soil factors, region etc.). The reference sites for biological soil quality defined in the Netherlands are one example of such a reference system [Rutgers et al. 2008, Rutgers et al. 2009]. Proposals for a Europe-wide monitoring of soil quality put forward by the EU ENVASSO (Environmental Assessment of Soil for Monitoring) project also call for the development of a reference system [Beylich and Graefe 2009, Bispo et al. 2009, Cluzeau et al. 2012, Römbke et al. 2012].

How a reference system can be adapted to different sites (in terms of feasibility, differentiated according to site types) remains to be clarified. Standardised and systematic investigation of species, communities and site conditions at as many sites as possible is required to provide a basis for assessing species diversity and their ecosystem services, including their variability [Toschki 2008, Römbke and Breure 2005]. Whilst literature provides this information for many groups of organisms (i.e. plants and vertebrates) and regions, there is a dearth of data available for the majority of invertebrates, in particular those which live in the soil. There are two different approaches, which may (and in some cases must) be used in combination, and which can be described as follows for the purpose of evaluating the effects of GMPs (genetically modified plants) [VDI 4330 Part 1 2006]:

Temporal comparison: comparison of the environmental condition prior to the cultivation of GMPs with the condition following the cultivation of GMPs.

Spatial comparison (differentiated by region): comparison of GMP and GMP-free monitoring areas at the same time.

In the case of temporal comparisons, recording the conditions prior to the cultivation of GM crops must be performed using selected sampling points. The reliability of the reference data is thereby dependent on the time at which monitoring of the conditions prior to cultivation takes place. Where appropriate, existing data may be used that describe the fluctuation of measurement parameters that are not associated with GMPs.

A spatial comparison requires reference areas where no GMPs have been grown and that differ as little as possible from the GMP sites.

GMP and reference areas may also be subjected to changes which are not attributable to the GM crop [VDI 4330 Part 1 2006]. The biodiversity and ecosystem services of soil organisms are generally influenced by a multitude of factors (e.g. climate, chemical pollution etc.), which in individual cases makes it difficult to distinguish between GMP-related effects and other effects [Toschki 2008]. To identify causal relationships, further complimentary and reciprocal studies are needed (weight of evidence approach) [Linkov et al. 2009]. This may involve laboratory tests or studies using terrestrial model ecosystems [see Schäffer et al. 2010], although ideally field experiments with soil organisms should be conducted on sites which have been partially planted with GM crops. Isogenic plants are then cultivated in parallel on control plots on the same site to ensure that both plots differ by only a single factor. In other words, this type of spatially and temporally adjacent control can be considered as a special type of reference area.

Potential effects of GMO cultivation on soil organisms

Toxins such as the insecticidal Bt maize protein can damage organisms directly (due to death or reduced fertility), or indirectly by affecting prey organisms, predators, competitors or mutualists. Changes in land management practices caused by the cultivation of GM crops, which affect soil cultivation, application of organic material and soil coverage, have a significant impact on soil organisms. In this context, the following research findings are particularly relevant to the design of monitoring programmes:

1) Many Bt toxins are able to persist in soil or in the droppings of different herbivorous soil invertebrates for more than one growing season [Zurbrügg and Nentwig 2009, Escher et al. 2000]. Sublethal effects can lead to long-term population changes [Hönemann and Nentwig 2009, Escher et al. 2000, Vercesi et al. 2006, Xin et al. 2004, Zwahlen et al. 2003, Höss et al. 2008, Höss et al. 2011]. Moreover, biological systems show delayed direct or indirect responses to toxins depending on the generation and age of the organism (see Zurbrügg and Nentwig 2009, Saxena et al. 2002, Donegan and Seidler 1999]. For this reason, a long-term monitoring plan should be implemented which extends beyond the period of GMO cultivation.
2) GMO-related changes of the structure and function of soil microflora, particularly in the rhizosphere or litter layer, can alter carbon transformation rates, and as a result, modify supplies of organic material in the long-term [Donegan and Seidler 1999, Griffith et al. 2005, Motavalli et al. 2004]. In addition, changes of nutrient resources [Escher et al. 2000, Motavalli et al. 2004, Cortet et al. 2006] can cause changes in the soil food web, which may affect taxa particularly at lower trophic levels (saprophages, microbivores).
3) The soil food web can also be affected by the transfer of toxins across several trophic levels [Hilbeck et al. 1998, Rossi et al. 2007]. This means that taxa in higher trophic levels should be studied simultaneously to gain information about food web structures.
4) Since positive and negative effects were found to be species-dependent in both laboratory and field studies (e.g. in the case of Protozoa, Nematoda, Collembola, Acari, Lumbricidae, Isopoda, Carabidae, Araneae [Xin et al. 2004, Höss et al. 2011, Donegan et al. 1999, Bitzer et al. 2005, Brooks et al. 2003, Clark et al. 2006, Griffith et al. 2006, Manchini and Lozzia 2002]), it is vital to record more than just cumulative parameters (e.g. total abundance) for whole animal groups. Studies should be performed at species level.
5) GMO studies have shown that the multitude of environmental factors (e.g. soil type, pH, temperature, year, season, region etc.) makes it difficult to demonstrate the effects of GMOs [Pagel-Wieder et al. 2004, Icozand and Stotzky 2008]. These factors contribute to the variability of biological systems and have an impact on the potential disturbance value of the GMO (e.g. degradation or bioavailability of Bt toxins). Proper evaluation of data thus requires extensive reference studies and adapted approaches to statistical evaluation (e.g. at landscape level).

Animal groups as indicators of functions and services

In the literature on soil ecology and soil protection, the term "function" has two meanings: Firstly, it refers to soil functions both in terms of the importance of soil for the ecosystem and for human use. Secondly, it is used to describe the activities and services of soil organisms. The list of soil functions in the German Federal Soil Protection Act is an example of the first meaning [BBodSchG 1998]. According to this law, soil is expected to fulfil:

1. "natural" functions
   a) as a basis for life and a habitat for people, animals, plants and soil organisms,
   b) as a part of natural systems, especially regarding its water and nutrient cycles,
   c) as a medium for decomposition, balance and restoration as a result of its filtering, buffering and substance-converting properties, and especially for groundwater protection,
2. functions as an archive of natural and cultural history and
3. functions linked to human activities such as a) a source of raw materials, b) land for settlement and recreation, c) land for use in agricultural and silvicultural use, d) land for other economic and public uses, for transport, and for supply, provision and disposal."

Soil can fulfil many of these functions only with the aid of a multitude of soil organisms, which help maintain nutrient cycles, for example. These interactions become particularly clear in the case of the habitat function, since the role of soil as a habitat for plants, animals and microorganisms is especially emphasized.

In biological terms the aforementioned functions (hereafter referred to as "services") of soil are driven soil organisms (which become functions in some processes). Table 1 contains a summary of some of these services, processes, relevant soil organisms and the respective measurement parameters [Turbé et al. 2010]. The examples used in this document mostly relate to soil invertebrates. See [VDI 4331 Part 7 in prep.] for more information about the services provided by soil microorganisms.

Due to their complexity, most services provided by individual organisms or groups of organisms cannot be directly quantified: for example we would have to measure the potential activity of all soil organisms to determine the maintenance of nutrient cycles. Since this is unfeasible, three approaches remain which complement one another to a large extent: (1) direct measurement of clearly definable soil organism services (for selected species and/or entire communities), (2) indirect measurement of such services by determining the structural characteristics of soil organism communities or (3) direct measurement of abiotic soil properties. In the latter two cases the indicator value for individual services is used. This involves assigning to each service a measurement parameter that does not necessarily depict the service directly, but serves as an indicator. For example, the abundance of deep-burrowing earthworms can be correlated with the process of litter decomposition as part of the service nutrient recycling. More specifically, changes in the measurement parameters indicate whether or not the respective service can be maintained.

Table 1. Selection of soil organism ecosystem services (MEA 2005) with a description of the organism groups which act as indicators of these services and of suitable monitoring methods [Lavelle et al. 2006]. The functional division used in the table is based on the definition of soil functions in the German Federal Soil Protection Act [BBodSchG 1998].

Service	Process	Indicator variable / taxonomic group	Potential measurement parameters
Soil as part of natural systems, especially due to its water and nutrient cycles			
Nutrient cycling (Supporting Service)	Decomposition of organic material	Earthworms, Enchytraeus, Woodlice, Diplopods, Oribatid Mites, Springtails, wood-decaying Fungi	Species spectrum and abundance of relevant soil organism taxa
	Metabolisation of organic material	Bacterial decomposition pathways Plant litter and dung fungi	Enzyme activities
	Stimulation of microbial decomposition	Nematodes	Functional groups (channel index)
Climate regulation (Regulating Service)	Storage of organic substances, esp. Carbon	Formation of stable humic substances	Corg/Cmic
Fresh water supply (Provisioning Service)	Storage of water in the soil pore system	Burrowing and tube-forming organisms (earthworms)	Species spectrum and abundance
Soil for agricultural and forestry use			
Soil formation (Supporting Service)	Tube and aggregate formation (development of soil structure)	Earthworms, Microorganisms	Species spectrum and abundance
Disease control (i.e. harmful organisms) (Regulating Service)	Direct and indirect competition, predation, parasitism	Predators: Spiders, Ground Beetles, Gamasina, Nematodes	Diversity, species spectrum and abundance

Selection of relevant animal groups

For practical reasons alone, it is clearly impossible to include every animal group occurring in an ecosystem in any given monitoring programme. Over 1000 species of invertebrates can be expected only in Germany's grassy field margins, which represent a relatively homogeneous biotope type. These species are spread across numerous animal groups at different taxonomic levels [Roß-Nickoll et al. 2004]. Due to the large numbers involved, it is important to identify which animal groups are relevant to monitoring using the criteria listed below [Dunger 1998, Römbke et al. 1997]:

- important ecological function in the respective ecosystem;
- close association with the respective sub-compartment (i.e. organisms dwelling in the mineral soil or litter layer);
- sufficiently large number of species per group to enable differentiation between sites;
- good taxonomic or ecological understanding of the relevant group and availability of experts capable of putting this knowledge into practice;
- broad distribution (here: in Central Europe);

- availability of standardised monitoring methods;
- potential for routine application (in particular opportunities to simplify determination);
- sensitivity to anthropogenic stress factors;
- representative of one trophic level (especially microbivors, saprophages and predators);
- habitat on or in the soil (epigeic or endogeic);
- representatives of a specific size class (micro-, meso- or macrofauna) and thus indirectly associated with a specific exposure pathway (e.g. the smaller the organism, the greater the likelihood of exposure via pore water).

The last three criteria specifically address the potential exposure of epi- and endogeic soil organisms towards genetically modified organisms (GMOs). Since not only living or dead GMO material but also other stressors connected with their cultivation may affect soil organisms, a broad spectrum of exposure pathways have to be covered. In addition it has to be checked whether the selected organism group contains key species such as ecosystem engineers (e.g. the anecic earthworm Lumbricus terrestris [Jones et al. 1994; Lavelle et al. 1997]). Since epigeic organism groups have already been used in nature protection activities (including monitoring programs) Plachter et al. (2002) have recommended the following taxa: Araneae, Carabidae, Chilopoda, Diplopoda, Gastropoda, Isopoda, Opiliones, and Staphylinidae. In parallel, the suitability endogeic soil invertebrate groups for monitoring purposes was studied by Römbke et al. (1997), using the same criteria as listed above. These authors recommended Collembola, Enchytraeidae, Gamasina, Lumbricidae, Nematoda and Oribatida. The VDI Working Group, using own experiences, added carabid and dipteran larvae to this list.

However, it is neither practical nor necessary to use all organism groups listed above when monitoring potential effects of GMOs at a specific site. Therefore, a decision matrix has been developed for VDI Guideline 4331 Part 1 which enables the most suitable animal groups to be selected for GMO monitoring on the basis of three criteria (Table 2). First of all, the landuse form of the monitoring site (crop site, grassland, or forest including hedgerow strips) has to be determined. Afterwards, three criteria are used:

1. the practicality of the animal groups (input values: current knowledge, availability of standard methods and handling).
2. the information value an organism group affords for a specific habitat type. The information value is defined as "a measure of the ability to organize a community by ecological groupings and species differentiation in order to indicate habitat conditions and changes" [Plachter et al. 2002].
3. the functional feeding type.

Therefore, various combinations of animal groups are possible, depending on the specific ecological conditions of the monitoring site and the properties of the GMO to be monitored.

Table 2. Matrix for the selection of the most suitable organism groups for the monitoring of potential effects of GMOs in three different land use forms (crop sites, grassland and forest / hedge). The numbers behind the names of the organism groups refer to their information content (see VDI 4331 Part 1 for further definition): 3 = very high, 2 = high, 1 = high, but only for individual species, 0 = rather low.

	Organism group	Crop site			Grassland			Forest / Hedge			Pract.
		pred	sapro	mic	pred	sapro	mic	pred	sapro	mic	
epigeic	Carabidae	3	3		3	3		3	1		good
	Araneae	3			3			3			
	Gastropoda		0			3			3		
	Opiliones	0			1			1			
	Staphylinidae	2	1		2	1		2	0		rather good
	Diplopoda		1			2			2		rather bad
	Isopoda		1	0		1	0		2	1	
	Chilopoda	0			1			2			
endogeic	Lumbricidae		1			2			2		good
	Collembola			3			3			3	
	Nematoda	2	2	2	2	2	2	2	0	2	rather good
	Oribatida		1	1		2	2		3	3	
	Gamasina	0			0			3			
	Enchytraeidae		2	2		3	3		3	3	rather bad
	Dipltera larvae		0			0			0		bad
	Coleoptera larvae	0	0		0	0		2	2		

The following rules have to be obeyed regarding the outcome of the selection process:

1. Four taxa have to be selected out of those listed in Table 2.
2. All three trophic levels have to be represented.
3. For each biotope type, at least two taxa must have a high information value.
4. Two epigeic and two endogeic taxa have to be selected.

In order to secure a high efficiency and sensitivity of the monitoring, the following information has to be considered when selecting the organism groups:

- In case it is known that the GMO (or one of its components, e.g. a Cry-protein) has a specific efficacy for one specific taxonomic group, this group must be included.
- In case one trophic level is specifically exposed towards one specific taxonomic group, this group must be included.
- At least two of the selected groups should be easy to handle (i.e. show a high practicality).

Details of the sampling design or the statistical evaluation of the monitoring results go beyond the scope of this paper (see the VDI Guideline 4331 Part 1). In the guideline there are also case studies presented, focusing on the selection process as described above. In addition, the use of the reference system is described more in detail.

Conclusion

Soil, just like water and air, is vital for life. Therefore it is important to monitor threats to soil structures, species communities, functions and services. Soil organisms provide wide-ranging opportunities for monitoring the effects of GMO cultivation. They are extremely species rich, sensitive to changes in their environment, involved in global material cycles and can be monitored using standard methods. The huge diversity of soil organism groups facilitates the establishment of an exceptionally sensitive monitoring system. In the future, modern molecular methods could help to achieve monitoring at the species level [Holterman et al. 2008; Pérez-Losada et al. 2012]. Furthermore, reference systems based on the occurrence and diversity of soil invertebrate communities have been proposed [Beylich and Graefe 2009; Cluzeau et al. 2012; Roembke et al. 2012; Rutgers et al. 2008]. However, there is still room for improvement; mainly due to a lack of data for specific land use types (i.e. grassland and forests have been studied more intensively than agricultural land. This approach of specifying a standardised monitoring framework for the selection of animal groups provides a suitable basis for the identification of potential side-effects of GMOs in the field. Standardized methods ensure that studies are comparable and that the data pool for deriving reference values continuously expands.

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