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General Surveillance for effects of GM crops on the soil ecosystem

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Abstract

General Surveillance for effects of GM crops on the soil ecosystem

RIVM has investigated which indicators would be most suitable to detect unanticipated effects of genetically modified (GM) crops on the soil system. A limited number of processes and groups of organisms that provide information about the state of the soil appeared to be affected by GM crops. Processes and organisms such as the amount and degradation rate of organic matter, the presence of nematodes and certain soil fungi can be used as indicators.

EU legislation prescribes that genetically modified (GM) crops approved for cultivation should be submitted to General Surveillance (GS) in order to detect unanticipated adverse environmental effects. However, the modalities of GS are not clear. In this study issues regarding the development of a GS system for the soil ecosystem, are discussed.

Only very few and small effects of GM crops on the soil ecosystem were found. In all cases, effects of normal agricultural practice, such as tillage and the cultivation of other crop species, were larger than those caused by the GM crops. Based on the outcome of effects observed for GM crops in field studies, only a limited number of indicators could be proposed, such as breakdown of organic material and changes in the nematode community. We suggest the incorporation of these indicators in a GS system. Depending on the development of tools to study arbuscular mycorrhizal (AM) fungi adequately in a GS system, this group could be an additional indicator for a future GS system. Based on the complexity of detecting unanticipated effects of GM cultivation, we propose to combine data obtained with these indicators with information from existing monitoring networks and the use of other tools for surveillance.

Key words:
general surveillance, genetically modified crops, soil, indicators

Rapport in het kort

General Surveillance van effecten van genetisch gemodificeerde gewassen op het bodem ecosysteem

Het RIVM heeft onderzocht welke indicatoren het meest geschikt zijn om onverwachte effecten van genetisch gemodificeerde (GM) gewassen op de bodem aan te geven. Daaruit blijkt dat een beperkt aantal processen en organismen dat iets kan zeggen over de gesteldheid van de bodem wordt beïnvloed door GM-gewassen. Processen als het gehalte aan organisch stof en de mate waarin dat wordt afgebroken, de aanwezigheid van een bepaalde groep aaltjes en bodemschimmels kunnen daardoor als indicator worden gebruikt.

Europese regelgeving stelt General Surveillance (GS), een nog te ontwikkelen systeem om het milieu te monitoren, verplicht als GM-gewassen tot de markt worden toegelaten. Het is echter nog niet duidelijk hoe een dergelijk General Surveillance systeem dient te worden opgezet en welke indicatoren het beste kunnen worden gekozen.

Om geschikte indicatoren voor de bodem te vinden zijn in de literatuur de effecten van GM-gewassen op het bodemsysteem onderzocht. De gerapporteerde effecten bleken vaak beperkt en van korte duur. Bovendien kan niet worden beoordeeld of deze effecten negatief zijn. In alle gevallen bleken de effecten van de gewone landbouwpraktijk, zoals ploegen en andere gewassoorten op de bodem telen, groter dan de effecten van GM-gewassen. Op basis van deze bevindingen zijn de bovengenoemde indicatoren voor de bodem aanbevolen voor General Surveillance.

Trefwoorden:
general surveillance, genetisch gemodificeerde planten, bodem, indicatoren

Contents

1 Introduction

The area cultivated with genetically modified (GM) crops in the world is increasing rapidly. In 2008 the total area amounted to 134 million hectares in 25 countries (James, 2009). The major part of these GM crops consists of herbicide tolerant soybean, corn, canola, cotton and alfalfa and insect resistant crops that produce an insecticidal toxin from the soil bacterium *Bacillus thuringiensis* (Bt). Additionally, GM crops with stacked traits, combining herbicide tolerance and insect resistance, are being allowed on the market. In Europe, public concern regarding the cultivation of GM crops is considerable. Therefore, the European Union has decided that approved GM events should be submitted to post-market monitoring. Post-market monitoring consists of two types of monitoring; 1) Case-Specific Monitoring (CSM) and 2) General Surveillance (GS). CSM is focused on specific uncertainties identified in the risk assessment that may still exist after approval for cultivation, or is meant to further confirm assumptions made in the risk assessment of a specific GM event. An example of CSM for Bt corn is monitoring the potential development of resistance against Bt in target insects. CSM is not required for each GM event that is approved for cultivation. In contrast, each GM event requires GS. GS is defined as 'to detect unanticipated adverse environmental effects which were not identified in the environmental risk assessment'.

As GS is directed at observing unanticipated effects of GM events that are not expected based on the risk assessment of the GM event, GS is in principle unfocused and not related to the specific GM crop combination (Bartsch et al., 2006; Sanvido et al., 2005, 2007, 2009). Effects may become manifest on the long term or short term, inside or – as a result of spreading of the GMO – also outside the GM cultivation area and may be observed in the 'above ground' ecosystem or in the 'below ground' soil ecosystem. The modalities of GS are not clear. The EU directive 2001/18/EC, which prescribes GS, does not provide clear guidance how GS should be set up and which indicators should be selected which may lead to different approaches in the various member states. While some initiatives have been taken to develop guidance for GS of the above-ground parts of the ecosystems, GS of the soil ecosystem is so far an unexplored area.

A GS system of the soil ecosystem should be able to detect potential unforeseen effects of GM crops that may become harmful to the functioning of the soil ecosystem. In this context we define harmful effects as effects that cause an irreversible or long-term disturbance of key soil ecosystem functions that may result in changes in biodiversity or may have adverse effects on above-ground plant growth or development. The rationale behind the modalities for a GS system should ideally be based on scientific evidence and the system should also be practical, cost effective and targeted towards important soil processes or organisms. The most difficult issue of the concept of GS is that the system should be able to detect any unanticipated effect, without a specific focus. This is exactly the opposite of the scientific approach based on the formulation of a hypothesis, which is experimentally tested using the proper controls.

The major questions in setting up a GS system are: what should be monitored, can proper controls be defined for this approach and how to interpret the data. Since there is no experience with GS of the soil ecosystem, there are two options to achieve progress. Either one could base GS on an extended version of the Dutch Soil Quality Network, in which currently some 30 parameters are monitored on 300, mainly agricultural, locations once every six years. However, it will be a huge task to analyse such a large number of parameters on a country-wide scale, incorporating all different types of ecosystems with the appropriate frequency. Experts have stated that a limited number of indicators should be identified and that GS should be set up in a proportionate and cost effective manner (FAO, 2005; EFSA, 2004). On the other hand, it may be efficient to base the selection of indicators on published scientific data on the effects of GM crops. This approach is followed in the current study. Scientific evidence regarding effects of GM crops on the soil ecosystem was compiled and assessed based on three major questions: 1) 'can we find unanticipated adverse effect of GM crops in literature?'; 2) 'can we use these to select indicators for GS?'; 3) 'what can we learn from these studies for the development of GS of the soil?'. As such, this study is in fact a 'general surveillance' of recorded effects of GM crops in scientific literature. In our assessment the experimental design and ecological relevance of the studies is critically evaluated because they determine the value of the

data, but the main criterion in assessing the effects is to compare them to effects caused by common agricultural practice. The impact on the soil ecosystem of tillage, the use of plant protection agents or the cultivation of different crops are generally accepted and should therefore be used as baseline for assessing effects of GM-crops. It does not make sense to use more stringent criteria for the acceptability of effects of GM-crops.
For practical reasons, this study focuses on GM crops that may be cultivated in the future in the Netherlands such as corn, potato, sugar beet and oil seed rape and on the potential effects of these GM crops on soil micro-organisms and invertebrates living in or on the soil surface.

2 Assessing effects of GM crops on the soil ecosystem

When scientific literature for effects of GM crops on the soil ecosystem is reviewed, it is essential to have a good understanding of the limiting factors for comparing the results in a useful way. Proper assessment of data from ecosystem studies is hampered by the enormous diversity and variability of the microbial community, the lack of understanding of the relationship between structure and functioning, the high redundancy of functions related to the breakdown of organic matter and the limitations of the current methods to investigate the microbial community as a whole, (Buckley and Schmidt 2001; Smit et al., 2001; Clegg et al., 2003; Bruinsma et al., 2003; Fierer et al., 2007; Kowalchuk et al., 2003; Prosser et al., 2007). Molecular based methods for the analysis of microbial community structure have provided a wealth of new data and insights; however, the structure-function paradigm has not been solved. Moreover, the results generated by these methods usually can only be compared to controls within the experiment and are difficult to compare quantitatively with other experiments. Since not everyone may be familiar with the abbreviations commonly used for the molecular based methods, a short description is provided in Box 1. Performing meta analysis by constructing databases with quantitative information on non-target effects of GM may help us in the future to asses the data, but at this time, we do not have the methodology for such an analysis for soil microbial communities (Marvier et al., 2007; Wolfenbarger et al., 2008). A proper experimental set-up is also of key importance for a thorough assessment of the data (Birch, et al., 2007). Since most studies differ in experimental design and applied methodologies the data are often difficult to compare (Dolezel et al., 2005; Icoz and Stotzky, 2008). The results of this study will be evaluated in relation to the experimental set-up. Ecological relevance is our main criterion for assessing experimental results. For this purpose we recognise the following four categories of experiments: 1) single species laboratory tests; 2) laboratory (or greenhouse) microcosm studies; 3) field trials at a single location; 4) field trails at multiple locations, including different crops and/or agricultural management. Results of the experiments will be assessed accordingly. To derive indicators for GS from this study our main criterion was that a long–term, statistically significant and ecologically relevant effect of a GM crop on this indicator was found in field experiments.

Box 1. Short explanation of the methods used to analyse the soil microbial community.

CLPP Community Level Physiological Profiling determines which substrates the community can degrade. Multi-well plates with different substrates are used to which an extract of the soil microbial community is added. Data are presented in a principal component diagram, depicting differences in the physiological ability of the microbial community.

ARDRA Amplified Ribosomal DNA Restriction Analysis is a DNA-based method to generate a banding pattern. Ribosomal gene sequences are PCR amplified and form DNA extracted from the soil. The differences in sequence are visualised gel electrophoresis of DNA fragments cut with restriction enzymes. The banding patterns are representative of the genetic diversity of the community.

DGGE Denaturing Gradient Gel Electrophoresis is a DNA analysis method providing a banding pattern. Ribosomal gene sequences are PCR amplified from DNA extracts from soil. The differences between the sequences are visualised directly using a specific gel-based technique called DGGE. The banding pattern is visualized in the form of a dendrogram and depicts the similarities between microbial communities.

PLFA Phospholipid fatty Acid Analysis is based on the extraction of fatty acids from soil. Analysis of the diversity of the fatty acids yields a diagram representing the chemical diversity of the community.

ARISA Automated Ribosomal ITS Sequence Analysis is an automated DNA-based method providing a standardised genetic diversity profile of the community, comparable to DGGE. The advantage over DGGE is that a standardised set of peaks is produced that can be easily compared with other experiments.

SSCP Single Strand Conformation Polymorphism is a DNA-based method to generate a banding pattern representative of the community. Ribosomal DNA sequences are amplified and single strand fragments are separated on a gel, based on differences in size and structure.

2.1 Effects on soil microbial communities

2.1.1 Effects of Bt crops on soil microbes

The impact of Bt crops on soil micro-organisms during the growing season has been investigated in several studies. A number of studies provide evidence that Bt crops do not affect bacterial communities in soil (Saxena and Stotzky, 2001; Baumgarte and Tebbe, 2005; Griffiths et al., 2005, 2006; Devare et al., 2004; Philippot et al., 2006; Naef and Defago, 2006; Vaufleury et al., 2007; Liu et al., 2008; Icoz et al., 2008). Other studies, however, indicate that Bt crops did affect soil microbial communities (Blackwood and Buyer, 2004; Brusetti et al., 2004; Turrini et al., 2004; Castaldini et al., 2005; Lamarche and Hamelin, 2007). Table 1 presents an overview of the results of these studies.

Experimental design seems to be one of the major factors determining the possibility to detect differences between microbial communities influenced by Bt and non-Bt crops. The differences which were found were very small and the experiments were all conducted in growth chambers or greenhouses. In one study, the effects of two Bt corn lines, one expressing Cry1Ab and the other

Cry1F, were only detected in one of the three different soil types (Blackwood and Buyer, 2004) while in another study, differences in bacterial community structure were caused by differences in plant exudates (Brusetti et al., 2004). Laboratory and greenhouse microcosm experiments are less subject to varying environmental conditions and ecological variation and may be more sensitive to detect small differences compared to field studies. In field studies, small differences between the bacterial communities in soils planted with GM crops compared to soils planted with their near isogenic lines were found, but these effects were outweighed by the differences in community structure caused by soil type, plant cultivar and plant growth stage (Saxena and Stotzky, 2001; Baumgarte and Tebbe, 2005; Griffiths et al., 2005, 2006; Devare et al., 2004; Philippot et al., 2006; Naef and Defago, 2006; Vaufleury et al., 2007; Liu et al., 2008; Icoz et al., 2008). Small and short-term effects of Bt crops on microbial community structure were only detected in laboratory or greenhouse experiments. In our opinion, these results have limited ecological relevance because in field studies it was shown that the effects of Bt crops were smaller than the differences caused by normal agricultural practice.

In a number of studies, potential effects of Bt corn on the soil fungal community have been investigated. Several publications have provided evidence that Bt crops do not affect soil fungal communities (Naef and Defago, 2006; Liu et al., 2005; Bianciotto et al., 2005; Valfleury et al., 2007). In a single-species test and a laboratory study, effects of Bt crops on AM fungi were detected (Castaldini et al., 2005; Turrini et al., 2004). Castaldini and co-workers (2005) investigated the effects of two Bt corn lines modified with *cry1Ab* on the AM fungus *Glomus mosseae* and the soil bacterial community, using a genetic fingerprinting method. Their results revealed differences in microbial community structure between the different Bt corn lines and significant lower colonisation of the Bt 176 and Bt 11 by the AM fungus *Glomus mosseae*. Turrini and co-workers (2004) found that pre-symbiotic hyphal growth of the AM fungus *Glomus mossae* was reduced in Bt 176 but not in Bt11, although both corn lines had similar modifications (*cry1Ab)*. Their data suggests that not the Bt toxin but another factor affected *G. mosseae*. Feirrera and co-workers (2003) found that AM colonisation of soybean plants inoculated with *B. thuringiensis* strains was not affected by the Bt toxin but by the bacterial cells themselves.

Considering the different results and the limited number of studies investigating effects of Bt crops on AM fungi, it is difficult to properly assess the results for this specific taxon. Results suggest that differences in corn lines and secondary effects of the modification may also affect AM colonisation. Mycorrhizal symbiosis can increase the plant's ability to take up nutrients and is important for plant growth. Therefore, Icoz and Stotzky (2008) advocate to take a precautionary approach and state that AM fungi should be monitored in relation to GM crop cultivation. The great uncertainty regarding possible effects of GM crops on AM fungi stresses the need for more research on the presence and diversity of AM fungi in different corn lines.

2.1.2 Effect of glufosinate tolerant crops on soil microbes

Non-target effects on the soil microbial ecosystem of glufosinate tolerant transgenic plants have been found, but the impact was shown to be small and transient (Gyamphi et al., 2002; Sessitsch et al., 2004; Schmalenberger and Tebbe, 2002; 2003; Fang et al., 2005), see Table 1. Sessitsch and co-workers (2004) studied the effects of glufosinate tolerant oilseed rape on the rhizosphere bacterial community and found that the community was affected both by the genetic modification and by the herbicide application. Statistical analysis showed that the effects were minor compared to the effect caused by plant growth stage.

Several field trials were done that did record small effects of the GM plants in combination with the herbicide but in all cases the effect caused by plant growth stage appeared larger than that of the combination GM crop and herbicide application (Schmalenberger and Tebbe, 2002, 2003). Schmalenberger and Tebbe (2002, 2003) used a genetic profiling method to analyse the composition of the bacterial community in the rhizosphere of glufosinate tolerant corn and sugar beet. While they could detect differences between the soil bacterial communities of corn and sugar beet they did not detect differences caused by the modified plants in combination with glufosinate application.

Table 1. Effects of GM crops on the microbial community in soil or rhizosphere

GM crop	Effect	Method	Experimental design	Reference
Bt	Difference in microbial community in one soil	CLPP	Microcosm Study / three soils	Blackwood & Buyer, 2004
Bt	Differences in microbial community (not stat) and in mycorrhizal colonisation	DGGE / bioassay	Microcosm Study / greenhouse	Castaldini et al., 2005
Bt	Differences in microbial community	ARISA	Microcosm Study /greenhouse	Brusetti et al., 2004
Bt	Negative effect on AM fungi	Myc. development	Single species laboratory test	Turrini et al., 2004
Bt	No difference in microbial community	cultivation	Microcosm Study	Saxena & Stotzky, 2001
Bt	No difference in microbial community	SSCP	Multi Location Field trial	Baumgarte & Tebbe, 2005
Bt	No difference in microbial communities	CLPP/PLFA	Multi Location Field trial including different crops	Griffiths et al., 2005
Bt	No difference between microbial communities	CLPP/PLFA	Microcosm Study incl. different soils and insecticide treatments	Griffiths et al., 2006
Bt	No difference between microbial communities	biomass/nitrification / TRFLP	Single Location Field trial, insecticide treatment	Devare et al., 2004
Bt	No effect on nitrate reductase act.	Nitrate reductase, narG diversity	Single Location Field trial, long term, three cultivars	Philippott et al., 2006
Bt	No effect on microbial population	counts, DGGE, Enzyme act	Single location field trial with four varieties, 4 years	Icoz et al., 2008
Glyphosate	Differences in root associated bacteria	CLPP / FAME	Field trial	Siciliano et al., 1998
Glyphosate	Differences in root associated bacteria	FAME	Two site field trials	Siciliano and Germida, 1999

Glyphosate	Transient differences in rhizo. community	CLPP / FAME	Two-year multiple site field trial	Dunfield and Germida, 2001
Glyphosate	Transient inhibition nitrogenase activity	nitrogenase activity	microcosm	Zablotowicz and Reddy, 2004
Glyphosate	Minor transient differences	SIR / CLPP	Multiple site field trial	Lupwayi et al., 2007
Glyphosate	no change in rhizosphere community	FAME	Field trial	Weaver et al., 2007
Glufosinate	Change in rhizosphere community smaller than plant growth stage	DGGE	Microcosm study	Gyamfi et al., 2002
Glufosinate	Change in rhizosphere community smaller then plant growth stage	DGGE / probes	Microcosm study	Sessitsch et al., 2004
Glufosinate	no differences in rhizosphere community	SSCP	Field trial, single location, normal agricultural practice	Schmalenberger and Tebbe, 2002
Glufosinate	no differences in rhizosphere community	SSCP	Field trial, single location, normal agricultural practice	Schmalenberger and Tebbe, 2003
Glufosinate	no differences in rhizosphere communities	CLPP / DGGE	microcosm	Fang et al., 2005

2.1.3 Effect of glyphosate tolerant plants on soil microbes

A number of studies report transient effects of glyphosate tolerant crops on the soil microbial community (Siciliano et al., 1998; Siciliano and Germida, 1999; Dunfield and Germida, 2001; Zablotowicz and Reddy, 2004) while others do not report any effects (Bennet et al., 2004; Lupiwayi et al., 2007), see Table 1. In several field studies glyphosate tolerant oilseed rape, used in combination with glyphosate caused differences in the composition of the rhizosphere community (Siciliano and Germida, 1999; Dunfield and Germida, 2001). Siciliano and co-workers (1998) analysed the effects of herbicide tolerant canola on the microbial community and found differences in the culturable microbial community and the carbon utilisation patterns. Dunfield and Germida (2001) also showed that the field site influenced the microbial community composition, since the effect of plant variety in a particular location differed from the effect of the same plant in another location. Dunfield and Germida (2003) investigated the rhizosphere microbial community of transgenic canola (Quest) using molecular-based methods and found transient differences between microbial communities; however, these changes could no longer be observed after winter. In their review, Dunfield and Germida (2004) state that although the herbicide tolerant plants and the herbicide application may affect the rhizosphere microbial community, these changes are not permanent and are minor in comparison to other factors, such as plant growth stage and field site. Zablotowicz and Reddy (2004) found a transient inhibition of nitrogenase activity when studying the impact of glyphosate in combination with a glyphosate tolerant soybean.

2.1.4 Effects of other GM crops on microbial community structure

As stated in the introduction, this review is limited to discussing the soil ecosystem effects of genetically modified corn, potato, sugar beet and oil seed rape, since only these crops are of relevance for the Netherlands. For more information regarding other crops and modifications, readers are referred to the review by Widmer (2007).

Three studies in which the effects of GM potatoes were investigated have been reviewed. In one study no effects on the soil microbial community of T4 lyzozyme-expressing potatoes were found (Heuer et al., 2002). A field study by Cowgill and co-workers (2002) showed that transgenic potato plants expressing cysteine proteinase inhibitors did not affect the microbial community in the first year, but in the second year, reduced bacterial and fungal abundance was detected. However, careful analysis of the data showed that the variation in the bacterial and fungal community due to plant development was larger. Moreover, no differences in plant decomposition rates were found.

Potatoes with altered starch composition are likely candidates to be grown in the Netherlands in the near future. Such plants do not produce any specific compound to combat plant pathogens or pests, so it seems less likely that such plants could have any negative effects on the soil ecosystem. The study of Milling et al. (2004) on the effects of potatoes genetically modified to have an altered starch composition confirmed this. They found no obvious effect on the total bacterial community; both the alpha- and beta- proteobacterial communities were highly similar while the actinobacteria revealed small differences.

2.2 Effects on degradation of GM plant residues after harvest

2.2.1 Degradation of Bt crop plant material

Degradation of plant residues of Bt crops was reported to be slower than the degradation rate of non-Bt plant remains (Saxena and Stotzky, 2000; Dinel et al., 2003; Stotzky, 2004; Flores et al., 2005; Castaldini et al., 2005; Raubuch et al., 2007). However, other publications provide evidence for the opposite (Cortet et al., 2006 ; Mulder et al., 2006) and there are publications revealing similar degradation rates of Bt and non-Bt crops (Accinelli et al., 2006 ; Hopkins and Gregorich, 2003). See Table 2.

At harvest, large quantities of plant residue enter the soil, this material decomposes during autumn and winter (Saxena et al., 1999; Saxena and Stotzky, 2000). Hopkins and Gregorich (2003, 2005) studied the decomposition of plant material from Bt and non-Bt corn in laboratory experiments. Under these optimised conditions, about 40% of the plant C was decomposed after 43 days. These results are comparable to those of Zwahlen and co-workers (2003a), Palm and co-workers (1994) and Sims and Ream (1997).

The breakdown of plant material was studied in the field by using litterbags (Cortet et al., 2006). The litterbags, containing either Bt corn or non-Bt corn material, were buried in soil in experimental fields representing different climates in Foulum (Jutland, Denmark), Varois (Bourgogne, France) and Narbons (Midi-Pyrénées, France). Results revealed similar trends, as in all three locations decomposition of Bt-corn was greater than non-Bt corn. However, this difference was only statistically significant at one site (Cortet et al., 2006). Data from a laboratory study by Mulder and co-workers (2006) also indicated that Bt crop residues were degraded faster than non-Bt residues. However, others found that plant material from several different Bt crops added to the soil degraded significant slower than their near isogenic lines (Flores et al., 2005; Saxena and Stotzky, 2001). It was hypothesised that the effects were not caused by the Bt toxin but by changes in plant composition as a result of the genetic modification (Donegan et al., 1995; Saxena and Stotzky, 2001). The lower degradation rate was explained by the observation that Bt corn had a higher lignin content than non-Bt corn. More recently, evidence was found for the fact that differences in plant composition and microbial communities between Bt corn and their near isogenic lines maybe responsible for the differences in mineralisation rates (Poerschmann et al., 2005; Raubuch et al., 2007; Naef et al., 2006). Interactions between plant, soil and the microbial community may result in differences in plant constituents and thus affect mineralisation. (Watanabe and co-workers, 2007; Cellini et al. 2004; Roessner and co-workers 2000; Liu et al., 2005; Raubuch et al., 2007).

2.2.2 Degradation of herbicide tolerant plant material

There are hardly any studies evaluating the effect of herbicide tolerant crops on plant litter decomposition (Powell et al., 2009). Glyphosate was shown to have no effect on microbial biomass and respiration even after long-term repeated applications to soil, and the variation in microbial characteristics was shown to be a function of vegetation development (Busse et al., 2001). A recent field study showed that the cultivation of glyphosate tolerant soybean and maize had little effect on plant litter composition (Powell et al., 2009).

Although the results are ambiguous, the reported differences in organic matter degradation in relation to GM cultivation require more research, not in the least because degradation of organic matter is a key function of the soil ecosystem.

Table 2 Potential differences between the degradation of Bt and non-Bt crop plant residues

Effect	Modification.	Method	Experimental design	Reference
Different degradation rates	Cry1Ab	CO_2 production	material collected from field	Dinel et al., 2003
Different degradation rates	Cry1Ab Ac, Cry3A	CO_2 production	in vitro laboratory test	Flores et al., 2005
Different degradation rates	Cry1Ab	CO_2 production		Castaldini et al., 2005
Different mineralisation rates	Cry1Ab	CO_2 production different fungal/bact. ratios	in vitro laboratory test	Raubuch et al., 2007
Equal degradation rates	Cry1Ac	glyphosate mineralisation	in vitro laboratory test with purified Bt toxin	Accinelli et al., 2006
Equal degradation rates	Cry1Ab	litter bags	field trial	Zwahlen et al., 2007
Equal degradation rates	Cry1Ab	CO_2 production	in vitro laboratory test	Hopkins and Gregorich, 2003
Different degradation rates	Cry1Ab	CO_2 production	in vitro laboratory test	Mulder et al., 2006
Different in degradation rates	Cry1Ab	CO_2 production and different Biolog profiles	in vitro laboratory test	Mulder et al., 2007
Faster degradation of Bt mat	Cry1Ab	litter bags	field trial/ three locations	Cortet et al., 2006

2.3 Effects of GM crops on soil invertebrates

2.3.1 Effects of Bt crops on soil invertebrates

Many soil dwelling invertebrates are involved in the decomposition of plant material after harvest (O'Gallaghan et al., 2005; Warburg et al., 1993) and they are thus readily exposed to Bt crops. Nevertheless, most invertebrates are not affected by Bt crops. For instance, several laboratory studies revealed that Collembola were not affected by Bt crops (Bakonyi et al., 2006; EPA, 2000; Yu et al., 1997; Sims et al., 1997; Heckmann et al., 2006). A field study confirmed that the collembola were not affected by Bt corn (Al-Deeb et al., 2003). Similarly, no effects of Bt crops on isopods have been found (Sims and Ream, 1997; Escher et al 2000; Pont and Nentwig, 2005; Clark et al., 2006). There is also no evidence for effects of Bt crops on protozoa (Saxena and Stotzky, 2001; Griffiths et al., 2005; Griffiths et al., 2007). Griffiths et al. (2005) analysed the effects of Bt corn expressing Cry1Ab on protozoa in the field. Despite reduced protozoan populations at two locations, an overall analysis revealed no statistically significant differences. To date, no evidence has been found that Bt crops have effects on earthworms. Tests of purified Bt toxin on earthworms revealed little impact (O'Gallaghan et al., 2005). Similarly, Bt crops did not have any negative effect on earthworms (Vercesi et al., 2006; Clark and Coates, 2006; Schrader et al., 2008; Saxena and Stotzky, 2001). Zwahlen and co-workers (2003b) performed a long-term field study on the effects of Bt corn on immature earthworms (*Lumbricus terrestris*) in cages burrowed in soil. No differences in weight between Bt corn and non-Bt corn fed earthworms were recorded.

Soil nematodes are known for the fact that they respond quickly to perturbations (O'Gallaghan et al., 2005) and are therefore considered useful tools to assess disturbances in terrestrial systems (Gupta and Yeates, 1997; Icoz and Stotzky, 2008). In four studies, no significant effects of Bt crops on nematodes were found (Saxena and Stotzky, 2001; Arndt, 2006; Wei et al., 2002; Griffiths et al., 2006). In four other studies, small effects were detected. Meadows and co-workers (1990) performed laboratory experiments with purified Cry1Ab protein and found negative effects on eggs and juveniles. Höss and co-workers (2004, 2008) found negative effects of Cry1Ab on the growth and reproduction of *Caenorhabditis elegans* in laboratory experiments. Manachini et al. (2005) found a statistically significant reduction of plant feeding nematodes under oil seed rape modified with *cryIAc.* Griffiths et al. (2005) found significant differences in nematode populations caused by Bt corn expressing Cry1Ab at three field sites in different countries.

Although not specifically focussed on soil, the effects of Bt cotton and Bt corn on invertebrates were recently evaluated in two meta studies involving data from various publications (Marvier et al., 2007; Wolfenbarger et al., 2008). These studies have a high statistical power and thus maybe able to pick effects that can not be detected in small individual experiments. In their study, Marvier and co-workers (2007) did not separate target from non-target insects and found that the mean abundance of invertebrates was significantly reduced in Cry1Ac cotton and Cry1Ab corn, compared to non-Bt cotton controls. However, when insecticide treatments were included in their analysis, the mean abundance of invertebrates was significantly higher in Bt versus the controls. However, Marvier and co-workers included the target pest insects and their parasitoids. Wolfenbarger and co-workers (2008) did not include the target species and their parasitoids and did not find any effects of Bt cotton, corn and potato on non-target arthropods. Insecticide effects were much larger that those of Bt crops. This confirms that experimental design and the proper selection of treatments and controls are essential for the interpretation of such studies.

2.3.2 Effects of glyphosate tolerant crops on soil invertebrates

From 2000 to 2002 in the UK a Farm Scale Evaluation (FSE) of genetically modified herbicide tolerant (GMHT) crops was conducted. More than 60 fields in various locations in the UK were selected together with various different crops such as beet, spring oilseed rape and forage corn. The null hypothesis was that there was no difference between the effect of the management of the GMHT varieties compared to the conventional varieties on the abundance and diversity of arable plants and invertebrates (Champion et al., 2003; Firbank et al., 2003). Most of the data generated in the FSE focus on effects on weed abundance and on non-target organisms (Heard et al., 2003; Haws et al., 2003). As part of the farm scale evaluations, Brooks et al. (2003) investigated the impact of several herbicide tolerant GM crops on soil surface active invertebrates. GMHT crop management affected the counts of many surface-active invertebrate species. Both higher and lower counts were encountered according to crop, ecology of the species and periods or location. Counts of carabids were smaller in GMHT beet and oilseed rape but higher in GMHT corn. Collembola detrivore counts were consistently higher in the fields with GMHT crops. Brooks et al. (2005) reanalysed the data, now excluding the atrazine treatment. Atrazine is a herbicide that is being withdrawn from the approved list of chemicals and was originally one of the controls (Dolezel et al., 2005; Brooks et al., 2005). Although the invertebrates had a mixed response, the vast majority was not affected. There was no effect on the total number of carabids and collembola except for maize, where 56% higher numbers were found in the herbicide tolerant variety. Effects were found on butterflies and bees, which can be explained by the superior control of weeds by glyphosate (Dewar et al, 2005). However, again experimental set-up and the method of analysis were essential with respect to data interpretation. In the FSE, the assessment of the effect was done on the level of crop species by comparing data from the GM plants with their near isogenic lines. Differences among the four different crops were much larger than those caused by the GM variety. An overall comparison including beet, maize and the spring and winter oil seed rape, reveals that effects of the GM crops are smaller than the effects of the different species.

3 Assessment of the data in relation to GS

Our first question was; ‘can we find unanticipated adverse effect of GM crops in the literature?’ In our opinion, the answer is no. Although some effects of GM crops were detected, all effects were either small relative to other effects, only found in laboratory studies or transient. Nevertheless, it is difficult to qualify the effects as adverse or not because there are no useful reference data. Most studies compare the effect of a certain GM crop on its near isogenic line. Sometimes, differences were found but such information cannot be used for risk assessment or decision making because of a lack of baseline data for many different crops and soil types to do a proper assessment. Data from thoroughly executed scientific experiments on the effects of GM crops on the soil are often not very useful for decision-making purposes.

Our second question was: ‘can we use this information to select indicators for GS?’ In our opinion, the answer is yes. A number of soil processes and taxa responded to GM crops. Although the effects cannot be qualified as adverse, this information provides us with a small set of indicators that have been shown to be sensitive to GM crops. This is worked out in more detail in paragraphs 3.2 to 3.3.

Our third question was: ‘what can we learn from these studies for the development of GS of the soil?’ In our opinion, the answer is quite a lot. Literature data provided a wealth of information on various methodologies, different experimental set-ups and ecological relevance in relation to the effects of GM crops. This is discussed in more detail in paragraph 3.4.

3.1 Soil Microbial community structure as potential indicator

With Bt crops, small effects on soil microbial communities were observed but these effects were only found in studies that were performed in laboratories or greenhouses. Statistically significant differences could not be detected in field studies; probably because the effects caused by other environmental parameters were more extensive or variable and prevented the detection of the relative small impact of the GM crops. The use of molecular or physiologically based methods has clearly enhanced the researcher’s ability to detect changes in specific microbial communities. But the application of these methods has not really contributed to a better understanding of the soil microbial ecosystems and do not provide surveillance data that can be used for decision–making. Effects of GM crops were often shown to be surpassed by the effects of other parameters, such as differences between crop lines, different field locations and the use of biocides for crop protection, the impact of plant age or weather conditions. Interpretation of the results remains a challenge, since it is not known what changes in soil microbial community structure or diversity mean with respect to soil functions. Last but not least, there is a lack of knowledge of the natural variability of the parameters that are measured. The impact of agriculture on the soil ecosystem has never been studied in such detail. All studies on GM crop effects that included other crops or other agricultural practices show that the impact of GM plants is insignificant compared to the impact of other ‘natural’ influences. Griffiths and co-workers (2007) for instance, showed that tillage and the type of crop had the largest effect on microbial community structure in soil.

Literature data revealed considerable uncertainties regarding the effects of Bt crops on AM fungi and, because AM fungi are key species in the soil ecosystem, it is recommended that this group be selected as an indicator for GS. Although, currently there is no suitable method to survey AM fungi.

With herbicide tolerant crops, several researchers have reported differences between soil microbial communities in the rhizosphere of these crops compared to the non-modified isogenic lines. These data have probably limited relevance for GS since they are most likely caused by the herbicide application. Moreover, in all cases the differences appeared to be transient and smaller that the variations caused by, for instance, crop rotation or plant development stage. The conclusion of Dunfield and Germida (2004) that these differences are not permanent and are minor compared to the impact of other factors appears to be valid.

The way in which soil microbial community structure is being studied today prevents the results of these studies from being used for surveillance since they can not be used for decision-making purposes. The usefulness of the results may be enhanced by performing a meta-analysis (Marvier et al., 2007; Wolfenbarger et al., 2008), which could increase the statistical power to assess GM crop effects on the soil microbial community. However, the data from currently used molecular methods cannot be compiled in such a meta-analysis because of differences in experimental setup, standards, controls, data processing and incompatibility of formats. Moreover, if microbial community structure or diversity were chosen as an indicator for GS, it would be difficult to select a robust, easy to use monitoring method that can generate data in a standardised way. Interpretation of the data generated by modern molecular methods is difficult, if not impossible from a decision-making perspective. We do not actually know what these changes in community composition and diversity mean and what the normal variation is in soil. To date it is not possible to link microbial diversity to soil quality or functions such as respiration or N mineralisation (Anderson, 2003). Future developments along the line of micro-arrays for determining microbial diversity could lead to the development of a standardised methodology for assessing community changes (Zhang et al., 2007). However, if such micro-array based technology were routinely applicable, the analysis and interpretation of huge datasets on soil microbial diversity would become the next challenge (Fierer et al., 2007). Data assessment and interpretation will become the future challenge for microbial ecology. In our opinion, the generation of large data sets, given the enormous and constant fluctuating microbial diversity, may only create uncertainty if we do not have the proper methodology or models for assessing the data (Prosser et al., 2007).

We conclude that this study does not provide strong arguments for the incorporation of microbial community structure as an indicator for GS and we recommend that AM fungi will be included in GS as indicator.

3.2 Soil functions as potential indicators

Data from the literature suggest that soil functions, such as the organic matter content and degradation, are suitable indicators for GS.
Surveillance of the effects of Bt crops on the degradation of plant residues revealed small differences between the degradation rates of Bt crops versus non-Bt crops (See Table 2). Different publications reveal that the degradation of Bt crops is sometimes slower than that of the non-modified line and sometimes faster. The literature provides some indications that differences in degradation rates could be caused by differences in concentrations of plant constituents such as lignin, glucosamine and ergosterol (Poerschmann et al., 2005; Raubuch et al., 2007). There are no reliable baseline data on the range of degradation rates of different crops in different soils and under different climatic conditions. Only when baseline data become available, may it be possible to make judgments regarding these effects from a decision-making perspective. The different degradation rates observed in Bt crops could well fall within a normal range; and even if they were outside this range, it is not clear if faster or slower degradation rates should be regarded as a negative or unwanted effect.
Function-based measurements, such as respiration or nitrogen mineralisation, may currently be more useful as indicators for GS than microbial community structure, since they provide direct data relevant to soil quality (Joergensen and Emmerling, 2006; Anderson, 2003). Total organic C or microbial C, soil respiration, organic matter decomposition or the ratio between microbial C and total C are indicators often used to assess soil quality (Bloem, Hopkins and Benedetti, 2006; Anderson, 2003). Soil organic matter content is generally considered as an indicator of soil quality and organic carbon content is closely related to soil organic matter content (Burns et al., 2006). According to Anderson (2003), soil quality is more likely correlated to soil microbial biomass and microbial respiration than to microbial diversity and community structure. Anderson (2003) reasons that any environmental impact that affects

the microbial community should be detectable by a change in metabolic activity, which depends on available carbon sources. The close relationship between microbial carbon and total soil carbon may be used to define baseline values for different soils.

Considering the ecological importance of organic matter degradation as an indicator for soil health, we strongly recommend the incorporation of the following parameters in GS: total organic C, microbial C, soil respiration, organic matter decomposition and the ratio between microbial C and total C.

3.3 Soil invertebrates as potential indicators

Literature on the effects of Bt crops on soil invertebrates revealed that many taxa are not affected and are therefore not likely candidates to select as indicators for GS. Several studies on collembola and earthworms did not reveal any effects, while others provide indications that the nematode communities can be affected by Bt crops. Nematodes is the only taxon in which small differences between soils planted with Bt crops compared to the isogenic lines in community structure were detected. We therefore recommend nematodes as a useful indicator for GS.
Regarding effects on invertebrates by herbicide tolerant crops, the farm scale evaluations (Brooks et al., 2003, 2005) provide the most statistically sound results. The farm scale evaluations have been designed in a robust way and provide statistically and ecologically sound data. However, the analysis revealed that the vast majority of the soil invertebrates were not affected. Moreover, if an overall statistical comparison had been made, GM effects on other invertebrates would have been smaller that the effect of crop species.

We conclude that based on current literature, nematodes is the only invertebrate taxon that should be selected as an indicator for GS.

3.4 Towards a General Surveillance system

We have identified that indicators for AM fungi, organic matter content and nematodes should be incorporated in a future General Surveillance system of the soil. From a technical point of view, sampling and analysis will not be a major problem for parameters indicative for organic matter degradation and nematodes. In the future, the incorporation of micro-array based analysis in GS should be seriously considered; however, currently this technique is not sufficiently developed for routine purposes. Currently, no easy to use methodology exists for detecting AM fungi. Once such a methodology is developed sufficiently, this group can be included in a GS system.
Developing a nationwide GS system will prove to be difficult. For that reason, we recommend making use of existing soil monitoring initiatives. In the Netherlands, GS can be integrated into the Dutch Soil Quality Network (DSQN). Within the DSQN, samples are taken at 300 carefully chosen locations once every 6 years (Rutgers et al., 2009). The suggested indicators will have to be analysed in addition to the standard set of indicators or parameters of the DSQN. Since the DSQN is not designed for GS, the sampling strategy and locations will also have to be adapted based on, *inter alia*, conceptual and statistical considerations. This will be the subject of further study.
Data analysis and interpretation will also be a challenge. Currently, the limited knowledge of the range of values of the parameters within Dutch soils prevents us from doing a proper assessment. We cannot simply set upper and lower limits for the parameters or indicators. The wide range of soil types and land uses makes it impossible to devise one set of limit values for the whole country. Data assessment and interpretation cannot easily be solved. Knowledge of the temporal and spatial variability, important for data assessment for decision-making purposes, will only be gained when the database and experience is being built up. We therefore propose to start sampling and data collection for GS as soon

as possible. Integration with data from DSQN can be used to create and extend baseline knowledge. Data assessment may be assisted by making comparisons of farms with GM crops with related reference farms without GM crops but with a comparable agricultural practice or management regime. Care should be taken to avoid comparing the effects of GM crops with their near isogenic lines, as would be done in case-specific studies. For GS, an overall assessment should be done at the farm level. Many studies on GM crop effects lack a thorough ecological assessment of the GM crop by integrating common agricultural practice, as was done by Griffiths and co-workers (2005, 2006) and Schmalenberger and Tebbe (2002, 2003). Effects of GM crops should be put into perspective by comparing them with other crop species and the effects of agricultural practice. Therefore, we recommend that for GS, the data should be analysed at the farm level. This still does not solve the problem of making judgements as to what levels of effects are considered unwanted. We will need more reference data and will have to develop a framework that facilitates decision making based on soil surveillance data in order to solve this issue.

Information on certain soil parameters maybe complemented with data from other sources, such as that from existing programmes involved in monitoring the flora and fauna (ecological monitoring) and the farmers’ questionnaires in which growers of GM crops indicate unanticipated effects as a result of cultivation.

Given the complexity and dynamics of the soil ecosystem and the small effects of GM crops found so far, it will prove necessary to use and integrate data and information from various sources in a GS system.

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