

Eric Smit, Hans Bergmans, Boet Glandorf

General Surveillance for Effects of GM Crops on the Soil Ecosystem (Poster Presentation at the 4th International Workshop on PMEM of Genetically Modified Plants, Quedlinburg, Germany 2010)

Allgemeine Beobachtung der Effekte von gentechnisch veränderten Pflanzen auf das Ökosystem des Bodens (Poster-Beitrag zum 4. internationalen Workshop zum Anbaubegleitenden Monitoring von gentechnisch veränderten Pflanzen, Quedlinburg, Deutschland 2010)

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Abstract

EU Directive 2001/18/EC 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 and the Directive does not provide sufficient guidance. In the Netherlands, possibilities for setting up so-called above- and below-ground GS systems are explored. In this study issues regarding the development of a GS program for the soil ecosystem, are discussed. As a first step, the currently available scientific literature on the impact of GM crops was analyzed for potential unanticipated adverse effects on the soil ecosystem. The idea behind this is that the soil processes and/or taxa that are sensitive to GM crops will be useful indicators for GS.

Given the currently available methodological tools and the necessary knowledge of the normal variability of the soil ecosystem the development of a functional GS system for the soil ecosystem provides major challenges. Our surveillance of scientific literature revealed only very few and small unexpected effects of GM crops on the soil ecosystem. 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 unexpected effects of GM cultivation, we propose to combine data obtained with these indicators with information of existing monitoring networks and the use of other tools for surveillance.

Key words: Genetically modified organisms (GMO), GM crops, post market monitoring, general surveillance

Zusammenfassung

Die EU-Richtlinie 2001/18/EG schreibt vor, dass der Anbau von transgenen Pflanzen durch eine *Allgemeine Beobachtung* der Auswirkungen begleitet wird, um etwaige, unvorhergesehene schädliche Effekte aufzudecken. Allerdings ist der erforderliche Umfang unklar, und auch die Vorgaben

Institute

GMO office, Expertise Center for Substances, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

Correspondence

Eric Smit, GMO office, Expertise Centre for Substances, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands, E-Mail: eric.smit@rivm.nl

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der Richtlinie bieten nur allgemeine Anhaltspunkte für die Planung. In den Niederlanden werden deshalb die Möglichkeiten für eine sogenannte „oberirdische“ und „unterirdische“ Allgemeine Beobachtung untersucht. Im Rahmen der hier vorgestellten Studie wird die Allgemeine Beobachtung mit Bezug zum Ökosystem des Bodens diskutiert. In einem ersten Schritt wurde dazu die wissenschaftliche Literatur zur Wirkung von gentechnisch veränderten Kulturpflanzen dahingehend untersucht, ob etwaige, unvorhergesehene Effekte dokumentiert wurden. Dahinter stand die These, dass sensitive Organismen(gruppen) oder Prozesse im Boden nützliche Indikatoren im Rahmen der Allgemeinen Beobachtung darstellen könnten. Vor dem Hintergrund der derzeit verfügbaren Methoden und des aktuellen Kenntnisstandes stellt die Entwicklung eines effizienten Programms für die Allgemeinen Beobachtung des Bodens eine große Herausforderung dar. Die analysierte Literatur bietet nur wenige Anhaltspunkte für unerwartete Auswirkungen des Anbaus von GV Kulturpflanzen auf den Boden. Daher lassen sich derzeit nur die Zersetzung organischen Materials und Änderungen der Nematoden-Populationen als mögliche, geeignete Indikatoren identifizieren. Deshalb schlagen wir vor, diese im Rahmen der Allgemeinen Beobachtung zu berücksichtigen. In Abhängigkeit von der weiteren methodischen Entwicklung könnten auch Untersuchungen an arbuskulären Mykorrhiza-Pilzen eine Option darstellen. Aufgrund der Komplexität der Aufgabe „unvorhergesehene Effekte“ des Anbaus transgener Pflanzen zu erfassen, schlagen wir vor, die Erhebungen der genannten Indikatoren mit den verfügbaren Informationen bestehender Umweltbeobachtungs-Programme und weiteren Beobachtungen zu kombinieren.

Stichwörter: Gentechnisch veränderte Organismen (GVO), GV Pflanzen, Monitoring, Allgemeine Beobachtung

1 Introduction

The area cultivated with genetically modified (GM) crops in the world is increasing rapidly. In 2009, the total area amounted to 134 million hectares in 25 countries (JAMES, 2009). The major part of these GM crops consists of herbicide tolerant crops and insect resistant crops that produce an insecticidal toxin from *Bacillus thuringiensis* (Bt). 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 the 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 potential 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 guidance how GS should be set up and which indicators should be selected. Therefore each EU country has to decide how to implement and design its own GS system. 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 that 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 major questions in setting up a GS system are: “what should be monitored and how can the data be interpreted?”. This study focuses on the first question, “what should be monitored” and more specifically “what could be useful indicators”. GS should be designed in a cost effective way with a limited number of indicators (FAO, 2005; EFSA, 2004). The indicators ideally are based on processes or taxa sensitive to GM crops. Therefore in this study literature on effects of GM crops on the soil ecosystem is reviewed to find suitable indicators for GS.

2 Assessing effects of GM crops on the soil ecosystem

In assessing scientific literature for effects of GM crops on the soil ecosystem it is essential to have a good understanding of the factors that limit our capability to compare 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 and our 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; FIERER et al., 2007; KOWALCHUK et al., 2003). MARVIER et al. (2007) and WOLFENBARGER et al. (2008) set up databases

with quantitative information on non-target effects of GM crops on invertebrates in the soil ecosystem, but at this moment we do not have the methodology to create such a database for micro organisms. Besides the methodology, experimental set-up is of key importance for a thorough assessment of effects of GM crops (BIRCH et al., 2007). Therefore, we will evaluate the results in relation to the experimental set-up.

2.1 Effects of Bt crops

For more than two decades plants are being modified with various genes from *B. thuringiensis* such as *cry1Ab*, *cry1Ac*, *Cry1F* and *cry3Bb* coding for different insecticidal proteins to combat lepidopteran pests such as the European corn borer (*Ostrinia nubilalis*). The Cry1 proteins are used to control Lepidoptera, and the Cry3 proteins are used against Coleoptera.

2.1.1 Effects of Bt crops on the soil microbial community.

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 did not affect bacterial communities in soil (SAXENA and STOTZKY, 2000; 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).

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 that were found were very small and the experiments were all conducted in growth chambers or greenhouses. In one study, 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, greenhouse or 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 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, 2000; 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).

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;

BIANCOTTO et al., 2005; VAUFLEURY 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 et al. (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 colonization of the Bt 176 and Bt 11 by the AM fungus *Glomus mosseae*. TURRINI et al. (2004) found that pre-symbiotic hyphal growth of the AM fungus *Glomus mosseae* was reduced in Bt 176 but not in Bt11 although both corn lines had similar modifications (*cry1Ab*). Their data suggest that not the Bt toxin but another factor affected *G. mosseae*. FEIRREIRA et al. (2003) found that AM colonization of soybean plants inoculated with *B. thuringiensis* strains was not affected by the Bt toxin but by the bacterial cells themselves.

The large uncertainty regarding possible effects of GM crops on AM fungi stresses the need for more knowledge on the presence and diversity of AM fungi in different corn lines and may warrant monitoring of AM fungi (ICOZ and STOTZKY, 2008).

2.1.2 Degradation of Bt plant residues after harvest.

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 results that show 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).

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. Results revealed similar trends, as on all three locations decomposition of Bt-corn was greater than non-Bt corn. However, this difference was only statistically significant at one site. Data from a laboratory study by MULDER et al. (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 soil degraded significant slower than that of their near isogenic lines (FLORES et al., 2005; SAXENA and STOTZKY, 2000). It was hypothesized 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, 2000). 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 differences in plant composition and microbial communities between Bt corn and their near isogenic lines causing difference in mineralization rates (POERSCHMANN et al., 2005; RAUBUCH et al., 2007). Interactions between plant, soil and the microbial com-

munity may be responsible for these differences in plant constituents and thus affect mineralisation. (CELLINI et al., 2004; ROESSNER et al., 2000).

Although the results are ambiguous, these differences in organic matter degradation require attention, not in the least because degradation of organic matter is a key function of the soil ecosystem.

2.1.3 Impact 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) and they are thus readily exposed to Bt crops. Nevertheless, most invertebrates are not affected by Bt crops. Several laboratory studies revealed that Collembola were not affected by Bt crops (BAKONYI et al., 2006; EPA, 2000; YU et al., 1997; SIMS and REAM, 1997; HECKMANN et al., 2006). A field study confirmed this (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, 2000; GRIFFITHS et al., 2005; GRIFFITHS et al., 2007). GRIFFITHS et al. (2005) analyzed 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 (VERCESI et al., 2006; CLARK and COATS, 2006; SCHRADER et al., 2008; SAXENA and STOTZKY, 2000; O'GALLAGHAN et al., 2005). ZWAHLEN et al. (2003) performed a long term field study on 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, 2000; ARNDT, 2006; WEI et al., 2002; GRIFFITHS et al., 2006). In four other studies small effects were detected. MEADOWS et al. (1990) performed laboratory experiments with purified Cry1Ab protein and found negative effects on eggs and juveniles. HÖSS et al. (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 Bt countries.

The effects of Bt cotton and Bt corn on (non soil) invertebrates were recently evaluated in two meta studies involving data from various publications (MARVIER et al., 2007; WOLFENBARGER et al., 2008). In their study MARVIER et al. (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. WOLFENBARGER et al. (2008) did not include the target species and their parasitoids and did not find effects of Bt cotton, corn and potato on non-target arthropods. Insecticide effects were much larger than those of Bt crops. This confirms that experimental design and the proper selection of treatments and controls are essential for the interpretation of such data.

2.2 Effects of herbicide tolerant crops

To improve weed control, plants modified with genes providing tolerance to non-selective herbicides, such as glyphosate and glufosinate (trade names resp. Roundup and Basta) can facilitate weed control. Herbicide tolerant plants do not produce themselves any substance to control plant pests or pathogens. Thus, potential non-target effects may be caused by either the herbicide application itself, the different crop management, or by the modification affecting plant composition or exudation patterns.

In several studies non-target effects on the soil microbial ecosystem of herbicide 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; SICILIANO et al., 1998; SICILIANO and GERMIDA, 1999; DUNFIELD and GERMIDA, 2001; ZABLOTOWICZ and REDDY, 2004). In several field studies glyphosate tolerant oilseed rape, used in combination with glyphosate caused small differences in the composition of the rhizosphere community (SICILIANO and GERMIDA, 1999; DUNFIELD and GERMIDA, 2001). SICILIANO et al. (1998) found differences in the culturable microbial community and the carbon utilization patterns. DUNFIELD and GERMIDA (2001) showed that the field site influenced the microbial community composition, since the effect of plant variety on a particular location differed from the effect of the same plant on 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 minor in comparison to other factors such as plant growth stage and field site.

Other field trials recorded small effects of glufosinate tolerant crops 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 analyze 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.

From 2000 to 2002 in the UK a Farm Scale Evaluation (FSE) of genetically modified herbicide tolerant (GMHT) crops were conducted. More than 60 fields on 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 abundance and diversity of arable plants and invertebrates (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 FSE, BROOKS et al. (2003) investigated the impact of several herbicide tolerant GM crops on soil surface active invertebrates. GMHT crop management affected 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 detritivore counts were consistently higher in the fields with GMHT crops. BROOKS et al. (2005) reanalyzed 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 (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. Negative effects were found on butterflies and bees which can be explained by the superior control of weeds by glyphosate (DEWAR et al., 2005). However, the assessment of the effect was done on the level of crop species by comparing data from the GM plants with its near isogenic line. Differences between the four different crops were much larger than those caused by the GM variety. If an overall analysis had been done, including beet, maize and the spring and winter oil seed rape, effects of the GM crops would have been smaller than the effects of the different species.

2.3 GM crops with other traits

Three studies in which effects of GM potatoes were investigated have been reviewed. In one study no effects on the soil microbial community of T4 lysozyme-expressing potatoes were found (HEUER et al., 2002). A field study by COWGILL et al. (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, the variation in bacterial and fungal community due to plant development was larger.

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 and are unlikely to have 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. Analysis of pseudomonads showed some differences between the communities in the rhizosphere of transgenic and non-transgenic potatoes. The authors suggest that these differences may have been caused by different exudation patterns of the transgenic plants.

3 Assessment of the data in relation to GS

No major effects of GM crops on the soil ecosystem have been found; in all cases the effects were smaller than the normal environmental variability. This is in line with previous findings (SANVIDO et al., 2007; WIDMER, 2007; ICOZ and STOTZKY, 2008; EFSA, 2008).

3.1 Microbial community structure and diversity as indicators

Effects of GM crops on soil microbial populations were observed, but these effects were either small or only detected in laboratory studies. Statistically significant differences appeared to be transient and smaller than 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 minor compared to the impact of other factors appears valid. 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 or the impact of plant age or weather conditions. The use of molecular or physiologically based methods has clearly enhanced the researchers' 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 from a decision making perspective. 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. We are only beginning to comprehend the natural variability because the soil ecosystem was never studied in such detail before. 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 normal agricultural practice. GRIFFITHS et al. (2007) for instance showed that tillage and the type of crop had the largest effect on microbial community structure in soil. Therefore we strongly advocate that the impact of GM crops will be assessed in relation to this variability.

We conclude that the way in which the soil microbial community structure is being studied today prevents that the results of these studies can be used for decision making purposes. Currently, microbial community structure is not a useful indicator for GS. 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, differences in standards, controls, data processing and incompatibility of formats. Moreover, if microbial community structure or diversity would be chosen as indicator for GS it would be difficult to select a robust, easy to use monitoring method that can generate data in a standardized 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 mineralization (ANDERSON, 2003). Future developments along the line of micro-arrays for determining microbial diversity could lead to the development of a standardized methodology for assessing community changes (ZHANG et al., 2007). However, if such micro-array based technology would be routinely applicable, analysis and interpretation of huge datasets on soil microbial diversity will become the next challenge (FIERER et al., 2007). Data assessment and interpretation will become the future challenge for microbial ecology.

3.2 Soil functions as potential indicators

Soil functions, chosen for their relevance for the soil ecosystem, may be more useful indicators for GS. Surveillance of effects of Bt crops on the degradation of plant residues, revealed small differences between the degradation rates of Bt crops versus non-Bt crops. Different publications reveal that degradation of Bt crops is sometimes slower than that of the non-modified line, and sometimes faster. 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, we hope that it might be possible to make any judgment 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 would be outside this range, it is not clear if faster or slower degradation rates should be regarded as negative effect.

Function based measurements such as respiration or nitrogen mineralization may currently be more useful as indicators for GS than microbial community structure since they provide direct data relevant for 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 et al.,

2006; ANDERSON, 2003). According to ANDERSON (2003) soil quality is more likely correlated to soil microbial biomass and microbial respiration than to microbial diversity and community structure and 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. Total organic C or microbial C, soil respiration, organic matter decomposition and the ratio between microbial C and total C are good indicators for GS.

3.3 Soil invertebrates as potential indicators

Literature on the effects of Bt crops on soil organisms does not provide clear clues for selecting indicators (soil invertebrates) for GS. Several studies on collembola and earthworms did not reveal any effects while others provide indications that the nematode communities are slightly affected and some sub-acute effects were detected. Nematodes is the only taxon that revealed small differences between soils planted with GM crops compared to the isogenic lines and could therefore be a useful indicator for GS. In our opinion both the FSE and the study by WOLFENBARGER et al. (2008) do not reveal specific taxa are affected by herbicide tolerant crops.

3.4 Toward a General Surveillance system

This study shows that we do not have the proper tools and knowledge to set up a GS system for the soil ecosystem yet since we lack knowledge on what should be measured and how the data should be assessed and interpreted for the specific purpose of GS. Literature provides some indications on what to measure. Function based parameters for organic matter degradation and the nematode communities were shown to respond to GM crops and could therefore be useful indicators for GS. Moreover, the given uncertainty of the effects of GM crops on AM fungi and their importance for the ecosystem, AM fungi may also be selected as indicator for GS. In the future it may become useful to incorporate micro-array based analysis as well, but currently this technique is not sufficiently developed for routine purposes. However, it would appear very limited to design a GS system solely based on these indicators. Therefore incorporation of selected parameters from existing monitoring programs could be a valuable addition. For example in the Netherlands the parameters monitored by the Dutch Soil Quality Network could be integrated in a future GS system (RUTGERS et al., 2009). Since the Dutch Soil Quality Network is not designed for GS it may be necessary to adapt the sampling strategy and locations based on, *inter alia*, statistical considerations.

From a technical point of view, sampling and analysis will not be a major problem for parameters indicative for organic matter degradation and nematodes. Currently, no easy to use methodology exists for detecting AM fungi, once the methodology is developed sufficiently this group can be included in a GS system.

Data assessment and interpretation still remains a challenge and cannot be solved easily. It is necessary to increase knowledge on the temporal and spatial variability. We therefore propose to start sampling and collect data. Integration of data from various monitoring programs can be used to create and extend baseline data. In our opinion, for GS effects of GM crops should be assessed by taking the variability such as plant development stage, crop type, agricultural practice and soil type into account. It will be necessary to develop a framework to assess data properly and to determine possible acceptable or unacceptable levels of disturbances.

Alternative approaches for GS may be explored based on the linkage between above-ground and below-ground ecology. There is evidence for a relationship between above-ground and below-ground communities (BARDGETT et al., 2005; VAN DER HELDEN et al., 2006). Above-ground plant health could be a useful indicator for the quality of the soil ecosystem and may be monitored by earth observation systems that can cover large areas (BÄR and LÖFFLER, 2007). These kind of systems can be used to observe unexpected effects on an (inter)national scale and potentially locate effects originating from GM cultivation areas. Other sources of information to be used for GS could be existing systems involved in monitoring fluctuations of flora and fauna (ecological monitoring) and the farmers questionnaires in which growers of GM crops indicate unexpected 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 of various different sources in a GS system.

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