Knowledge gaps with respect to the effects of genetically modified crops on the functioning of soil ecosystems

COGEM research 2003-03

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Dit rapport is in opdracht van de Commissie Genetische Modificatie (COGEM) samengesteld. De meningen die in het rapport worden weergegeven zijn die van de auteurs en weerspiegelen niet noodzakelijkerwijs de mening van de COGEM.

Contents

Executive Summary

This report presents the results of an inventory of needs and knowledge gaps with respect to the effects of genetically modified (GM) plants on soil ecosystems, with the goal of defining areas in need of future research attention. This inventory consisted of a questionnaire and related interviews that were presented to a variety of interested parties, including growers, horticultural companies and scientists. Results of this questionnaire were compiled together with knowledge gained through a number of workshops and scientific meetings and past research efforts dedicated to this topic.

Given the huge impact that plants have on soil systems, and the importance of these systems for sustainable agricultural practices, the question of whether GM crops have harmful effects on soil systems has received increased attention in recent years. In the agro-ecosystem context covered in this report, this refers to effects within the scope of standard agricultural practices in the Netherlands, wherein a healthy soil is defined as one that supports sustained crop yield over a range of environmental stresses, without requiring escalating levels of input or affecting surrounding habitats. Within this framework, the most important general functions of agricultural soil systems were determined to be 1.) cycling of nutrients and organic matter, 2.) buffering against biotic and abiotic stresses, and 3.) maintaining proper soil structure. Soil biota are for the most part responsible for these functions, and risk assessment efforts seek to determine if unintended perturbation of soil-borne communities via GM crop cultivation might disturb the procurement of these key soil functions.

Our survey results revealed the following necessities for research focus:

i. Better understanding of plant-soil systems

Agricultural practices have a tremendous impact on soil systems, and it was generally agreed that research on GM crop effects should consider existing farm practice, changes to farm practice associated with GM crop implementation, and the context of total farming businesses.

Key to the detection of GM crop-induced effects is to distinguish them from the baseline defined by the dynamics of soil-borne communities and processes. Soil ecosystems are highly dynamic, being exposed to numerous natural and anthropogenic fluctuations, including seasons, weather patterns and varying agricultural practices. This baseline is as yet poorly understood and crucial to our ability to assess change and risk. Proper controls, especially including the non-GM parental crop lines and appropriate management practices were seen as essential to proper risk assessment efforts.

ii. Developing and validating (better) tools to test for GM crop-induced effects

Due to the enormous biological complexity in the soil, practical studies of GM crop

effects on soil ecosystems must focus on specific indicators that provide information with respect to the functional status of the soil system. General soil characteristics and process measurements were determined not to be responsive or predictive enough, but rather only reveal the consequences of functional failure. However, a number of functional groups of soil organisms have been proposed as indicators of system perturbation including several microbial groups, nematodes and other soil animals, and some of these have recently become more accessible with the advent of culture-independent, molecular ecological methods. Unfortunately, it has been difficult to relate detected changes to changes in functionality of the system, and greater knowledge of the functional consequences of soil community changes is necessary to make this leap from detected change to functional consequence, the latter being essential to our interpretation of whether detected effects are harmful. A critical need for the further development and validation of existing indicators was identified, as well as a concentrated effort for the identification of new indicator tools, especially those that can be translated into effects on functional aspects of the system. Also key to these efforts will be an increased knowledge about the role of biodiversity within soil-borne communities, and greater knowledge concerning the strengths of interactions within interactive soil food webs.

iii. **Integrating knowledge and methods into workable and meaningful implementation strategies**
It was agreed that the impact of increased knowledge of measures of GM plant effects will be marginal if it cannot be implemented within feasible testing procedures. The standardisation and streamlining of (molecular) methods for systematic implementation was therefore suggested as a spearhead of research efforts. Also, study systems that decrease the necessary effort and investment of time, money and personnel for adequate testing will be important. Such measures include proper experimental designs with the necessary power, methods for concentrating testing within a short period of time, ways to automate and standardise sampling and analysis procedures, as well as methods for integrating data into total farming practices and over environmentally relevant spatial and temporal scales.

In summary, the current state of knowledge should allow us to make some important first steps in testing the impact of GM crops on soil systems. However, more comprehensive assessments should be facilitated by further progress with respect to the development and validation of (new) indicators of soil functional quality, methods to make these indicators routinely measurable, and systems to implement these methods in a structured scheme that leads to knowledge that is directly useful to regulatory agencies charged with assessing GM crop release applications.

1. Introduction

1.1 Goals

I. To identify areas of research need and demand for the implementation of a dedicated call for grants to the Dutch Research Foundation (NWO).

II. Within the framework of the tools and results of a research program, to assist the COGEM in the evaluation of requests for the cultivation of genetically modified plants. Help identify what needs to be known about GM crop effects on soil ecosystems and steps that are necessary to achieve this knowledge.

1.2 Mandate from the COGEM

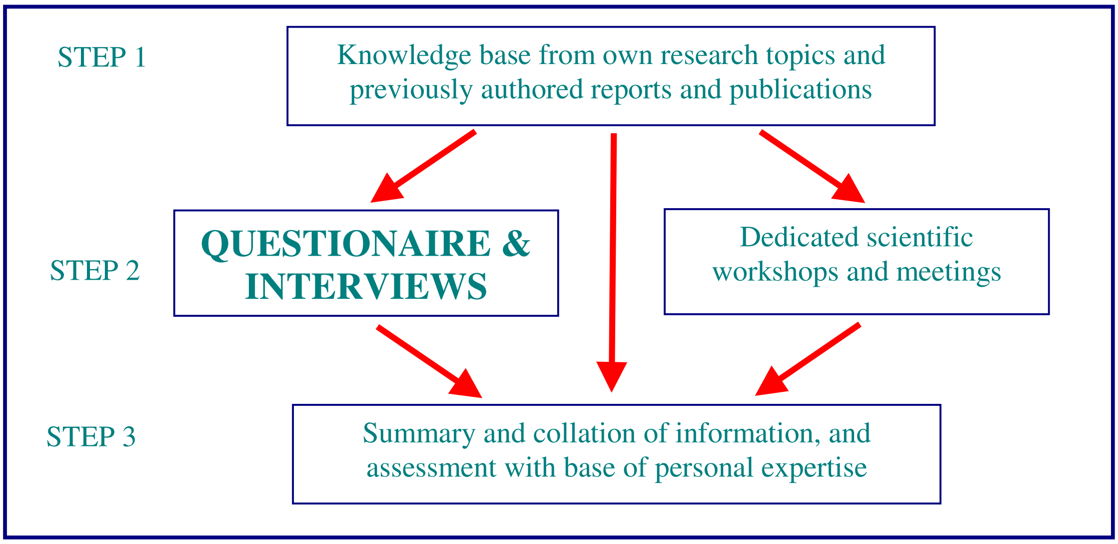
The COGEM's task is to advise the Minister of Housing, Spatial Planning and the Environment about the allocation to risk groups of procedures for the production and handling of Genetically Modified Organisms (GMOs). The COGEM also advises about the risks for people and the environment of the uses of GMOs, and about the security precautions required to protect people and the environment. Specifically, the Agriculture subcommittee of the COGEM advises on the safety for people and the environment of making, or working with, genetically modified plants or animals. This subcommittee has identified the potential effects of GM plants on soil ecosystems as an area for which few standards are present and for which they currently lack the necessary references to make sufficiently informed decisions.

While it might be of scientific interest to understand all aspects of GM crop effects on soil ecosystems, the mandate from the Netherlands Committee on Genetic Modification is clearly, “What do we need to know?” to make accurate risk assessment judgements when scrutinising applications for GM crop release. From this applied perspective, the COGEM seeks to gather the necessary information to answer the following specific questions:

- **What are the expected effects specifically due to the genetic modification?**
- **Are the potential effects hazardous for the functioning of the soil?**
- **How can the effects be documented?**
- **What is the normal operating range of plant-soil systems, which forms the point of reference for judging the extent of GMP-induced effects?**
- **Are GMP-induced effects reversible and, if so, over what timescale?**

1.3 Overview of Report Strategy

This report is based upon past research, the opinions of scientists and stakeholders, and our interpretation of these within the context of the mandate outlined by the COGEM: The major contributing steps in this procedure are given below:



Step 1.) Start from broad knowledge base and expertise on plant-microbe interactions and study of potential effects of GM plants on soil micro-organisms.

See - 2001 report "Effects of genetically modified plants on soil ecosystems" http://www.nioo.knaw.nl/CTE/pmi/cogem.pdf

- Kowalchuk GA, Bruinsma M, Van Veen JA. 2003. Assessing Soil Ecosystem Responses to GM Plants. Trends in Ecology & Evolution, 18: 403-410.

- Bruinsma M, Kowalchuk GA, Van Veen JA. 2003. Effects of genetically modified plants on microbial communities and processes in soil. Biology & Fertility of Soils, 37: 329-337.

Step 2.) Identify knowledge gaps and research needs via symposia, international meetings and survey of scientists, farmers and other stakeholders.

<u>List of relevant international meetings attended</u>

- The effects of genetically modified plants on soil ecosystems. Rhenen, the Netherlands. January 20-22, 2002.

- Impact of genetically modified organisms on soil microbiology and nutrient cycling (IGMO). Vienna, Austria. November 3-6, 2002. (http://www.boku.ac.at/boden/igmo/home%5B1%5D.htm)

- The impact of genetically modified plants (GMPs) on microbial communities (AIGM-2003); Trosmo, Norway. May 26-29, 2003 (http://www.farmasi.uit.no/~knielsen/AESF5_1.htm)

- FAO Expert Consultation on Environmental Effects of Genetically Modified crops. Rome Italy. June 16-18, 2003. (see http://www.fao.org/)

Step 3.) Collate information to identify needs and knowledge gaps and provide recommendations for future research based upon personal expertise, input from questionnaire, interviews and scientific discussion, and direction of COGEM needs and interests.

1.4 Problem Description

Among the most important issues related to the use of genetically modified (GM) crops, is the potential for undesirable environmental impacts of GM crop cultivation. Although GM crops may offer great potential to advance agricultural, there is also a keen desire and need for well-defined environmental risk assessment. A key element of risk assessment procedures for genetically modified (GM) plant cultivation involves the potential for unwanted effects on non-target organisms. To date, the vast majority of research and risk assessment regarding potential for such non-target effects has focussed on aboveground effects. However, up to half of a plant's biomass can be belowground and over 80% of the world's terrestrial organic matter is present in soil. Plants influence numerous aspects of soil biology via *e.g.* exudation of organic compounds, turnover of roots, and by providing a solid substrate for attachment. Soil-borne communities are extremely complex and are known to be responsible for numerous life-sustaining functions of soil. Via their input of energy and carbon, plants are major drivers of below-ground communities.

Due to this link between plants and soil communities and their functions, risk assessment concerns with regard to GM crop cultivation have expanded to encompass potential non-target effects belowground. Thus, while GM crops may have specific desired effects on soil biota and their functions, they may also induce undesired side-effects that may influence soil functions, plant growth, or the growth of future crops (Fig. I.1). However, until recently, very few tools have been available to address issues concerning belowground communities and their functions.

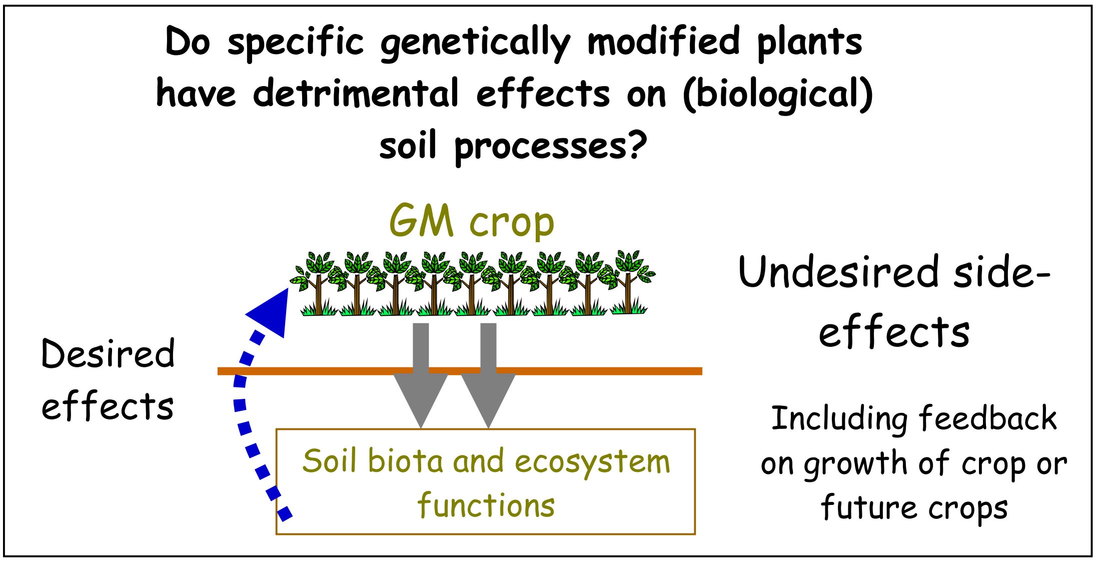


Figure I.1 *Schematic drawing of potential GM crop effects on soil systems*

Recent advances in soil ecology have spurred a wave of research on the impact of (GM) crops on soil-borne biota and their functions. Studies performed to date have only found minor effects, but have typically been restricted in their scope. Effects found thus far usually pale in comparison with 'normal' sources of variation. Most importantly, we still do not have a good handle on the functional consequences (if any) of any detected effects. Also, as illustrated by figure I.2, plant-soil systems are highly complex, with high levels of heterogeneity. Soil-borne microorganisms are the greatest source of biological and genetic diversity on the planet. Numerous functional groups of soil animals also exhibit very high levels of biodiversity. All these soil components fit into complex food and energy webs, and we still lack a full understanding of the interrelation-ships between these many players. Such systems are also highly dynamic in space and time, adding to the difficulty of detecting and interpreting the significance of changes caused by perturbations such as GM crop introductions.

Our knowledge of well-studied plant-soil systems has been increasing rapidly, but is still far from complete. While we do possess some reference information on how some important crops and farm practices affect soil systems, we have few points of reference to assess potential risks related to non-target belowground effects of novel types of GM crops, such as those that may be involved in the mining of pharmaceutical products. Also, each GM crop introduction is unique with respect potential interactions with soil-borne communities and processes.

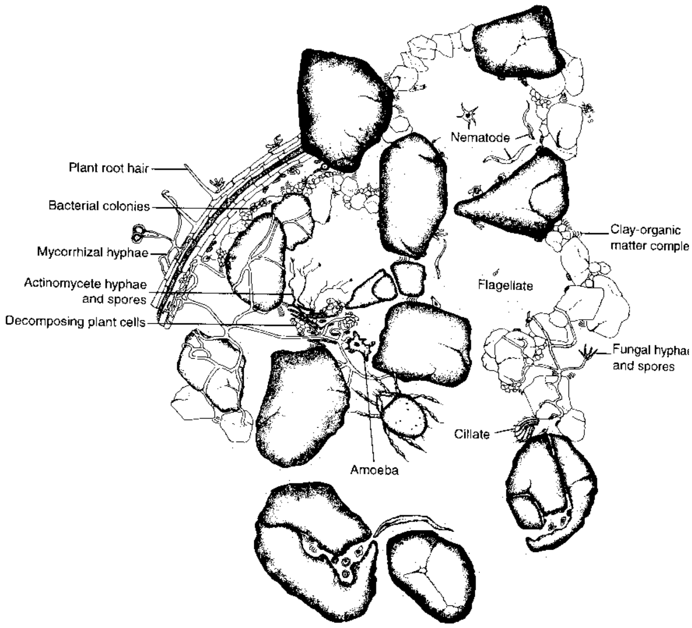


Figure I.2 *Drawing of highly heterogeneous nature of the soil environment; S. Rose and E.T. Elliott*

The goals of this report were therefore based upon the current state of the art with respect to the needs and knowledge concerning the potential of GM crops to exert harmful influence of plant-soil systems. The current state of affairs can be summarised in the following statements:

1.) The effect of GM plants on non-target soil organisms and processes has become a major issue in the risk assessment of GM plant cultivation.

2.) Until recently it has been difficult if not impossible to look into the black-box of soil communities.

3.) Some important steps have been taken recently to provide the tools and the knowledge necessary to understand the influence of GM crops in soil systems, but our knowledge base and toolbox are far from complete.

4.) Further research and knowledge is necessary to allow for full assessments of GM crop effects on soil communities and processes, and it is critical that we target research efforts properly so as to maximise the gain provided by investments in research.

2. Recommended Research Foci

Research priorities regarding potentially harmful effects of GM crop cultivation for soil-borne communities and processes

The knowledge gaps identified during this study dictate areas for which future research should improve our ability to assess the potential risks of GM crop cultivation on soil agro-ecosystems. Although they are based upon the outcome of the questionnaire, related interviews and discussions with research experts, we have also relied on the guidance of the COGEM and our personal knowledge of the subject to provide the necessary focus and to crystallise areas of interest into defined research topics.

These gaps and their related research foci can be grouped into three major areas, each of which contains several related points that deserve research attention.

The three major areas determined to deserve special research attention are:

1.) Establishing a better understanding of plant-soil systems

2.) Developing and validating (better) tools to test for GM crop-induced effects

3.) Integrating knowledge and methods into workable and meaningful implementation strategies

1.) Establishing a better understanding of plant-soil systems:
A certain level of understanding of plant-soil systems is necessary before meaningful conclusions can be draw concerning the effects that GM crops might have on them. While the importance of research in this area is not restricted to the applied topic of GM crop effects, the evaluation of GM crop effects is impossible without knowledge in the following areas of soil agro-ecosystem functioning:

- **Knowledge of keystone species, groups and processes and their interactions:** While considerable progress has been made in the area of defining the key players in soil functioning, this information is not complete, and current knowledge and concepts are often difficult to apply and generalise outside of a specific study soil.
- **Understanding how plants influence soil-borne communities:** We are only just beginning to understand how plants influence organisms and properties in the soil. Large gaps are still present with respect to the topics of how plant influence in the rhizosphere translates to changes in overall (bulk) soil properties, how plants differ in their influence on soil communities, and how soil properties and plants interact in steering belowground

communities. Also lacking in most studies on GM crop effects is information on the presence of actual GM products in the soil, which is key to our understanding of how soil systems respond.

- **Knowledge of the 'normal operating range' or 'baseline'** of soil communities and processes. To date, the majority of studies focus on single snapshots of plant-soil systems in space and time. However, plant-soil systems are highly heterogeneous and dynamic, and there is insufficient data concerning the 'normal' range of soil-community properties in response to non-GM plant-induced variables (such as season, crop, weather, management, etc.).
- **Establishing links between changes in soil-borne community structure and with soil functions** (including impacts at the food-web and ecosystem levels). It is one thing to be able to detect some kind of effects, and another to assign a functional significance to the detected change. In other words, "So what if a change is detected, should we care?" Answering this question requires a strengthening of the link between a community's structure and it functions. Topics of particular importance in the area are knowledge of the level of functional redundancy of functions within soil-borne communities, and methods that allow simultaneous identification and functional assignments to soil (micro) organisms. It is also not known how quickly soil functions can recover, and research into the resiliency of soil functions requires attention.
- **Understanding the relationships between diversity and function** within and among key functional groups of soil-borne organisms. Should we be concerned with losses in soil-borne biodiversity within and among functional groups? In order to answer this question, we must understand how and to what extent particular functions respond to losses in biodiversity.

2.) Developing and validating (better) tools to test for GM crop-induced effects:

Despite the tremendous progress that has been made in methods for the assessment of (changes within) soil-borne communities, there are still many omissions in the current toolbox:

- **Molecular screening procedures for fungal groups** need to be improved. The use of molecular community analysis techniques was introduced into fungal ecology later that for bacterial community analysis. Therefore, techniques in this area are less developed and require further examination and validation. Also, fungal taxonomy and the utility of molecular community approaches would be greatly improved with a more detailed and systematic analysis of the vast amount of well-described reference material in culture collections.
- **Development in structural-gene based assays**, such as those targeting key activities in nitrogen cycling, antagonism, and degradation. In addition to improved biological assays for such activities, functional gene targets for such activities show great promise for providing improved *in situ* information concerning key soil functions.

- **Identification and assessment of novel microbial indicators** is a top priority, as our list of important indicators is no doubt not complete or optimal. Investigation of possible surrogate indicators (properties that may not be of importance themselves, but correlate well with key functions) might also prove beneficial, but first require identification and validation.
- **Improving the experimental accessibility of relevant indicators** (including keystone species, groups and processes) of soil functioning. Groups that may deserve special attention include soil fungi (including AMF and degraders of recalcitrant organic matter), soil animals (including nematodes), and bacterial groups that can be assessed by phylogenetic or functional markers.
- **Better methods to assess soil-borne community structure and function:** The toolbox for the analysis of soil-borne communities is improving rapidly, but improvement is desired in the reproducibility, systematic application (simplification), statistical analysis and interpretation of such methods. Efforts might include the development of ready to use 'kits' that have been tested across a wide variety of situations. Numerous recent breakthroughs allow for a direct coupling between identity and function in soil community analysis, but these are not yet available for routine use, and increased development and application of molecular function-linked strategies will be an area of research focus in the years to come.
- **Development of rapid and less expert-dependent methods for key groups of soil organisms, such as fungi, nematodes and other soil animals.** This is necessary to reduce reliance on laborious efforts of highly trained experts in taxonomy. More simplified and direct (molecular) methods for assessing such groups are urgently needed. Molecular strategies may offer attractive and more feasible alternatives for rapid analysis of multiple samples, and this is an area that demands future development.

3.) Integrating knowledge and methods into workable and meaningful implementation strategies.

Although many methods have been developed to assess changes in the structure and function of soil-borne communities, many of these are not yet available for routine use or implementation in integrated risk assessment programs. Also, a tremendous amount of information can presently be generated with respect to soil-borne communities, but this does not necessarily lead to a better understanding of (changes in) the system. In order to fully utilise available and developing methods, the following areas of research deserve special attention:

- **Methods to ensure power of testing procedures:** Testing procedures must have the necessary statistical power and experimental robustness both to assign significance to detected effects, as well as to specify the limits of detection where no effects are found. A framework of explicit standards in experimental design and statistical power should be developed and implemented.
- **Development of integrated experimental set-ups to examine effects within the context of total agronomic strategies.** GM crop cultivation would typically occur within

the scheme of complex farming businesses. Thus, study of GM crop effects may not make sense in isolation, and strategies to integrate testing into realistic full business models is desirable.

- **Testing of methods to accelerate the testing procedure** to shed light on potential long-term or multiple exposure effects within a relatively short period of time. Some effects of GM crops may only become apparent over an extended period of cultivation. However, it is unrealistic to have extremely long pre-release testing programs. Methods to help examine multiple and prolonged expose to GM crops should help in the acquisition of as much ‘long- term’ data as possible without delaying release unduly. Also highly desirable are methods to reduce the scale of initial testing procedures in time and space without sacrificing validity, thereby reducing costs and effort.
- **Advancement in data analysis** (including bioinformatics) to determine patterns within (large) datasets on changes in soil communities and functions. Such pattern recognition must also deal with the natural variation present in agro-ecosystems.
- **Extending knowledge across different ecological scales:** Some effects of GM crop cultivation may only become apparent at geographical and temporal scales that cannot reasonably be addressed via pre-release assessment efforts. Thus, methods that can help extend available knowledge beyond what is experimentally accessible may prove very helpful.
- **Facilitating post-release monitoring efforts:** Post-release monitoring at some level may be desired to track potential long-term effects and ensure sustainability. Advancements that may aid such efforts include methods for simple on-site sample collection, preliminary handling and storage, identification of the necessary frequency, breadth and intensity of monitoring procedures, and streamlined procedures suitable for routine, long-term testing.

3. Additional Recommendations

This report has attempted to summarise the perspectives of scientists, end users, and other interested parties and thereby identify knowledge gaps, which in turn define areas ripe for future research. In doing so, we have identified a number additional issues that are highly relevant to research efforts in this area, and offer the following additional recommendations:

1. Increased cooperation between different research organisations throughout Europe would be highly desirable. For instance, similar interests in soil-borne effects have been voiced in parallel efforts within most notably the UK (ACRE's subcommittee on soil ecosystems) and Germany (Zentrale Kommission für die Biologische Sicherheit [ZKBS]). More concerted research would also help stimulate more uniform legislation throughout Europe.

2. Countries that have a long history of release of GM crops should be used as sources of information, and future research within Europe should stimulate relevant research links with such countries (*e.g.* USA, Canada). The knowledge that can be gleaned from shared experience and critical analysis of results produced to date cannot be achieved through simple literature searches, since not all relevant materials are published in some cases, due to proprietary considerations. Direct contact with research organisations and the researchers involved is highly recommended.

3. Europe is a major importer and exporter of crops to and from developing countries. These countries are also dealing with similar issues with respect to GM crop cultivation, but often lack the proper infrastructure, expertise and funding for the evaluation of GM crop safety. Therefore, research efforts should also focus on these mutual interests and relevant contacts should be maintained with organisations key to 3rd world agricultural efforts (such as The Food & Agricultural Organisation of the UN (FAO) and the Agricultural Science and Resource Management in the Tropics and Subtropics (ARTS)).

4. Regulatory procedures must allow room for responsible research into the effects of GM crops on soil functions. In the past, some research projects designed to address effects of GM crops have not been approved because they involve GM plants with undefined potential risks to the environment. This circular argument can only lead to a stagnation of research. In turn, where appropriate and possible, researchers should take all reasonable precautions in the design and execution of experiments. Also, efforts to minimise vandalism of experimental sites may prove necessary.

5. European legislature has been highly sceptical of GM crop safety. The demand for highly stringent standards for market release of GM crops should be appeaseable by powerful and valid scientific approaches. However, if GM crop use is rejected out of hand, than research money can be better invested in topics that remain more relevant to current societal issues.

4. Questionnaire & Results

4.1 Content and interpretation of questionnaire regarding the effects of genetically modified crops on the functioning of soil ecosystems

Within the questionnaire presented below, which was used as a guideline in the interviewing process, some questions were intentional posed in an overly simplified or leading manner in order to elicit a response.

A number of the questions are interrelated and therefore have led to compound answers. We have attempted to group answers according the questions posed, but cross-referencing of answers reflects the overlap in subject matter between certain questions.

Some questions address more complex issues that others, and the length of our summary of questions is relative to the complexity of the questions and the corresponding responses.

We have attempted to summarise the most important points from the answers received in an effort to arrive at a consensus wherever possible. Although it is probably impossible not to interject some of our own opinions and perspectives during this process, we have tried to preserve the content of answers as well as possible, while keeping the discussion focussed to the topic of this report.

The questionnaire focuses specifically on the potential effects of GM crops on (agro-)soil systems, but given the nature of the subject and the expertise of the expert panel, some overlap with other issues was expected. Answers depend strongly on the perspective and experience of the interviewees, and we have therefore attempted to interview a broad range of individuals from different fields related to this topic.

We have limited our analysis to answers that pertain directly to the issue of GM crop cultivation, within the framework of standard agricultural practices in the Netherlands. Thus, some soil functions that are not particularly pertinent to this setting are not discussed. A healthy soil in this context has been defined as one that supports sustained crop yield over a range of environmental stresses, without requiring escalating levels of input or overly affecting surrounding habitats.

4.2 Questionnaire regarding the effects of genetically modified crops on the functioning of soil ecosystems

1.) What are the most important soil functions in your opinion and which would you expect to be most detrimentally affected by genetically modified plants? Are such detrimental effects always unacceptable, and if so, why? If not, under what conditions are detrimental effects acceptable?

2.) What do we need to know to be able to assess potential detrimental effects of GM plants on soil systems? What knowledge is missing in order to make such assessments?

3.) Are there certain soil organisms or functions that might act as indicators of negative effects of GM plants on soil systems? What are the characteristics of a good indicator of negative effects of GM plants on soil systems?

4.) How should the effects of GM plants be interpreted in the light of the natural dynamics (in space and time) of biological soil processes? What are the proper controls? What is the reference point – the so-called baseline?

5.) For how long should monitoring occur and what are your arguments for this duration?

6.) Are there certain methodological developments that would offer better opportunities to assess if a GM plant has negative effects on soil functions?

7.) Which tests could be used to examine the effects of GM crops on soil functions? Are such test available or do they still have to be developed?

8.) If a research program were initiated for the study of GM crop-induced effects on soil systems, what points of focus should receive attention? Why specifically these points?

9.) Do you have anything else to add or additional comments?

4.3 Results of questionnaire

Question 1a: What are the most important soil functions?

The identification of key soil functions clearly depends upon the viewpoint and goal of the user. From an agricultural viewpoint, soil is the substrate used to sustain crop growth, and key necessary functions are geared toward this goal. From an ecological viewpoint, however, other values are important, such as providing a pool of biological diversity, sustaining belowground food-webs, and contributing to regional and global nutrient cycles. From the standpoint of biotechnology, soils are most important as a source of novel products and useful activities.

Despite these differences, a clear set of core functions could be identified:

- **Cycling of nutrients and organic matter** (see Figure IV.1)
  - To allow efficient use and delivery of nutrients to plants (especially in low input agricultural systems
  - To prevent soil leaching of nutrients to surrounding habitats
  - To reduce the amount of nutrient input necessary
  - Decompose organic matter to make it biologically accessible

- **Buffering**
  - Against shifts in chemical gradients, pH, water availability, temp. etc.
  - Against plant pathogens

- **Maintenance of soil structure**
  - Provide proper substrate for plant growth
  - Avoid erosion, desiccation
  - Proper grain size and clumping of soil

Many other soil functions were identified, but were deemed to fall outside the main scope of agro-ecosystems and their impact on the environment.

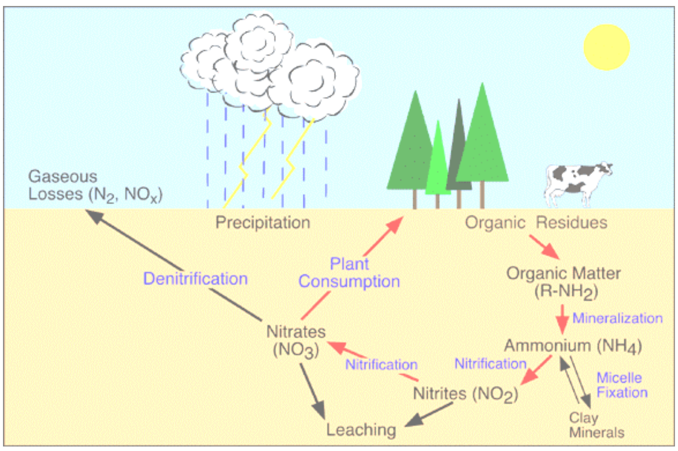


Figure IV.1 *The importance of soil systems in nutrient cycling is exemplified by this cartoon of the nitrogen cycle*

Question 1b: What are potential influences GM-plants on important soil functions?

Clearly, intensive agricultural practice is highly disturbing to soil systems. It was unanimously agreed that an intensification of agricultural practices will have a more detrimental effect on soil-borne biotic functions than nearly any imaginable genetic change introduced into a crop via genetic engineering.

It was generally agreed that the greatest impact of genetically modified crops would probably occur due to changes in farm practices associated with the cultivation of some GM crops, such as changes in tillage regime, pesticide use or herbicide application. It should be noted that some of these changes (for instance lower levels of pesticide application) may also have a positive effect on certain soil parameters. With respect to herbicide application, use of some GM-crops might allow for a more efficient use of herbicides, thereby reducing the amounts that need to be applied, however, it was noted that GM technologies may dictate the repeated use of a very narrow range of herbicides, which may not be desirable in the long term (*i.e.* potential development of resistant weeds and build-up of long-term application effects)

Key to understanding the impact of GM plants in soil systems is recognition of the routes of influence that plants may have on soil systems. While this mechanistic information may not be expressly necessary for the monitoring of potential GM-induced effects, it may be critical to outlining the soil system criteria that should be targeted.

GM plants can impact soil communities by introducing changes in the following parameters:

- Plant root exudation patterns (resulting in: selection of different microbial populations – different rates of degradation
- Degradation of plant residues (both roots and deposited material)
- Effects of specific (toxic) plant compounds
- Changes in root architecture that effect soil aeration and depth profiles
- Associated management practices (see above)

An additional function of some soil bacteria that might be affected by GM crops is the promotion of plant growth via the production of growth factors.

Also, many biological activities in soils are regulated by molecular signalling systems that can involve multiple organisms. Clearly disturbances within such signalling regimes may disturb the elicitation of important soil-borne activities.

Unfortunately, most studies to date have not attempted to address soil exposure to specific transgene products.

Question 1c: What soil effects might be expected to be specific to the cultivation of GM crops?

Based upon molecular and microbiological assessments, it is thought that most crops do not have a very large effect on soil properties outside the rhizosphere. However, the success of simple crop rotation measures in avoiding the build up of pathogen pressure highlights the fact that plants can indeed exert functional consequences upon the soils they inhabit (either not providing continuous selection for specific pathogens or by stimulating the diversity of potential antagonists disease agents). This paradox remains a topic of debate in soil ecology, and impedes our ability to predict the extent of GM crop impacts.

GM plants could in some cases lead to changes in pathogen pressure and the suppression of soil-borne plant pathogens, but again this is predicted to be more the result of reliance on a narrow range of cultivation practices (such as a single herbicide or ability to continue cultivation of a single crop for multiple years), rather than specific properties of the GM crop itself.

The one concern peculiar to GM crops is the potential for horizontal gene transfer (HGT). However, within the context of soil systems, it was agreed that HGT would probably play little or no role because:

(1) many transgenes are originate from organisms already present in the soil.
(2) HGT from plants to soil-borne organisms is expected to occur at extremely low frequencies.
(3) HGT events will only exert an impact if the acquired trait provides a selective advantage. In this respect, the use of antibiotic resistance markers within genetic constructs introduced into plants may be a concern in field situations where large antibiotic loads are present, such as via their use on livestock.

In general, there is little reason to believe that GM crops should have any peculiar impacts as compared to crops bred by other means. However, this may not hold for novel crops or crops that introduce totally novel traits for which we have no frame of reference.

Question 1d: What are acceptable and unacceptable GM-related changes in soil functions?

The most important factor in determining the acceptability of disturbance to soil functions is whether the changes are short-term or long-term and reversible or irreversible.

Irreversible changes can either occur via two mechanisms:
1.) Loss of key species or functional groups
2.) Creation of species with new traits such as via horizontal gene transfer (see 1d).

Transient changes that return back to normal prior to the subsequent growing season are generally perceived as acceptable in most cases.

One of the most important features of soil is its large buffering capacity against change, both in terms of physical characteristics, as well as biological functions. A large degree of functional redundancy resides within soil-borne communities. Thus, most soil functions can withstand a great deal of disturbance and display a higher degree of resiliency. However, little is known about the degree of diversity, keystone species, or biological thresholds necessary to maintain this buffering capacity and resilience (see question 2b).

The acceptability of soil systems disturbances also has to do with the perceived importance of the function in question. However, general agricultural practice most certainly imposes a large disturbance on soil ecosystems. As such, it is most reasonable to view further disturbance due to GM crop cultivation within the context of the functions necessary to maintain sustainable agricultural systems with minimal outside environmental impact. In this respect, the most important functions that should not be negatively affected, especially not for the long term, are those affecting soil fertility, pathogen pressure and nutrient retention (i.e. low leaching of nutrients to neighbouring areas or groundwater).

Question 2a: What do we need to know to be able to assess potential detrimental effects of GM plants on soil systems?

In general, an number of principles must be met in order to determine the harmful effects, or lack thereof, of GM crop cultivations on soil systems.

1.) What function(s) is affected, to what extent, and for how long?
2.) Can the observed effect indeed be ascribed to the introduction of the transgenic material into the plant?
3.) What is the sensitivity of detection? In other words, how large does an effect have to be before it is detected?

Agro-ecosystems are highly complex and vary from location to location and with the agricultural practices involved and the environmental parameters encountered. Also, each transgenic introduction is unique with respect to the combined factors of host genotype, genetic construct and method of introduction. Thus, meaningful risk assessment analyses with regard to the effects of GM crops on plant-soil systems, require an **integrated case-by-case analysis** that incorporates both the plant and the ecosystem of introduction.

1.) It is necessary to have a good understanding **of the agro-ecosystem of introduction**, including its **important soil functions** and their **normal operating ranges**.

2.) The **specific effect that can be assigned to the given transgenic construction** must be determined what the within this context.

Thus, effects of GM crop introductions must be assessed with respect to both the **properties of the transgenic crop itself** as well as the **properties of the environment of introduction.**

Key to assessing effects of GM crop introductions is the ability to compare against a suitable control involving the non-GM crop, and such controls must also include their associated changes in agricultural practices and environmental variables (see question #4a&b).

There is a long history of research pertaining to impacts on soil ecosystems within the field of ecotoxicology. One of the staples of this area of research is the theoretical analysis of the exposure to the agent in question. This "ecotoxicological recovery time" can be estimated by examination of the initial dose of an agent (chemical), its toxicity and its persistence. Similar principles can be applied to GM crop assessments by determining the **expected exposure to transgene products** (or differences compared to non-transgenic parent), the **level of impact of these products per unit**, and the **persistence** of these in the environment. Such analyses have already been applied with respect risk analysis of BT crops.

Question 2b: What knowledge is missing in order to make such assessments?

In most cases, little is known about how GM crops differ from their non-GM parental lines with respect to belowground attributes (see 1b).

While we do have a reasonable understanding of the key functions of soil ecosystems, we have a far poorer grasp of which organisms are responsible or necessary for these activities. This lack of information seriously hampers our ability to determine if observed changes in species composition have an actual impact on the functioning of the system as a whole. We also lack knowledge of how quickly soil functions can recover.

Diversity vs. Ecosystem functioning

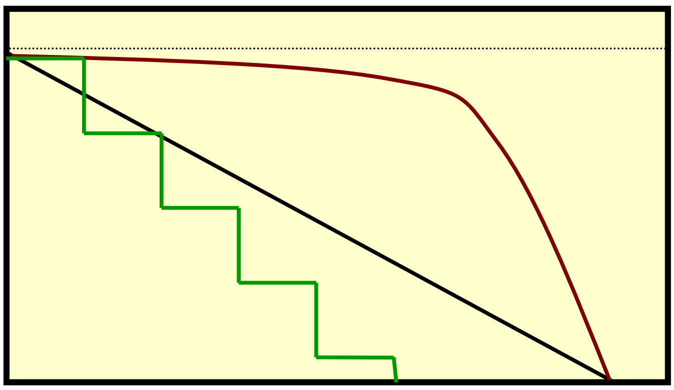


Figure IV.2: *Potential relationships between diversity and ecosystem function*

The relationship between soil-borne diversity and function is not well understood. We do not know when changes in diversity will lead to disturbance in function.

As pictured in Figure IV.2, function may decrease sharply at some threshold of minimal diversity (curve A), may be linear with respect to diversity (B) of may proceed in a punctuated fashion with the loss of key species (C). Diversity changes can be at the level of functional groups, species, and genetic diversity within a species, and the shape of the functional response curve may vary with respect to the group or taxonomic level examined. While research into affects of diversity at the species and functional group levels is proceeding with some progress, we still lack any information about genetic diversity within species and how this affects function, adaptability and resilience.

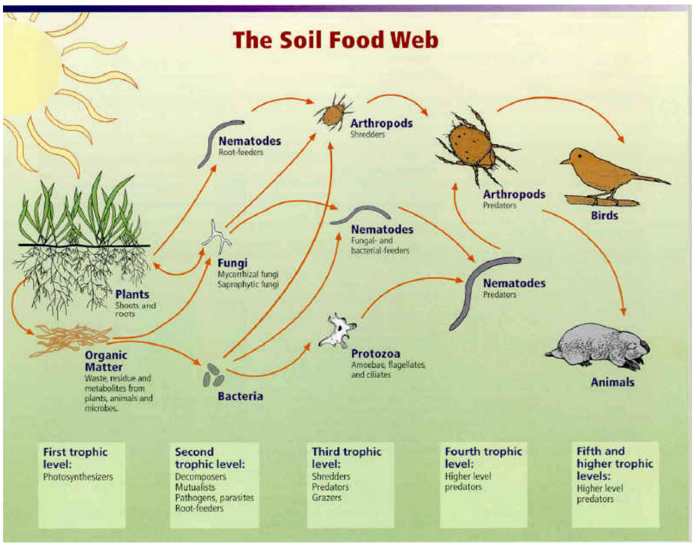


Figure IV.3 *Drawing of a typical soil food web (by Elaine R. Ingram)*

Also, individual groups and functions make up part of a complex food-web structure with numerous interactions. While many keystone species and functional groups have been identified in soil systems (see Figure IV.3), it is less clear how changes in one group affect other links in the soil food web and, in turn, soil functions.

Question 3a: What are characteristics of a good indicator of soil function?

As indicated previously, for the definition of indicator criteria for studying the potential harmful effects of GM crops on soil systems, much can be borrowed from the field of ecotoxicology, where some similar assessments have been necessary for the introduction of chemical agents.

It was agreed that the most important general characteristics of a good indicator include:

- <u>Predictive power</u> (includes reproducibility, link to relevant functional components, and availability of suitable controls)
- <u>Susceptibility to change or vulnerability</u> (includes non-redundancy, i.e. that the function cannot be taken over by other organisms)
- <u>Measurability</u> (includes experimental accessibility, cost effectiveness, and manageable experimental protocols)

Furthermore, in order to be relevant, soil function indicators should address key soil functions as outlined in the response to question #1. While this is may be the ideal situation, it has also been suggested that indicators themselves need not necessarily address meaningful properties as long as they are strongly correlated with more relevant (less accessible) properties. Thus, as long as this correlation has been established, simplified surrogate indicators may be useful predictors of meaningful parameters.

Question 3b: What are good indicators of soil function?

Simple functional assays can be employed to access cursory functions of system, such as **harvest quantity and quality, decomposition of complex organic matter, N-mineralization & fixation, general enzyme activities (*e.g.* dehydrogenase, protease, phosphatase, ß-glucosidase, & sulphatase), pH, and soil aggregation**. However, such measurements give no insight into whether systems are approaching critical diversity thresholds within key functional groups (see question 2b). Furthermore, such measurements **are not proactive in predicting dysfunction**, but rather simply describe existing problems, which may be too late for the implementation of successful mitigation strategies. Also, measurable effects in some simple parameters, might only given indications of drastically damaged systems or might not address meaningful ecosystem functions.

Alternatively, one could attempt to use the specific components of the soil-borne community, if these relate to key functions of the system. Changes in the structure, diversity, size and activity within functional groups may provide insight into the disturbance level relative to the function in question. Numerous soil-borne organisms have been proposed as possible indicators, based upon their adherence to the indicator qualities outlined in 3a.

Below is a list of indicator groups that have been discussed in previous assessments of potential microbial indicators of GM crop-induced soil effects. There may be other groups that might provide additional or better information but to our knowledge, given the input of those interviewed, the list below represents the state of the art with respect to the best key microbial functional group indicators:

- Mycorrhizae (Arbuscular Mycorrhizal Fungi - AMF)
- Symbiotic N2-fixing bacteria
- Degraders of recalcitrant organic matter
- Antagonists to plant pathogens
- Nitrifying bacteria (particularly ammonium-oxidisers)

It may also be possible to use specific (microbial) biosensors engineered to detect specific GM plant products. Although this may be a promising route for some applications, most such sensors are themselves genetically modified and thus are subject to their own regulations.

The general structure of microbial communities may also give insight into the level of change induced in soil systems under influence of GM crops. Numerous molecular tools are available for such assessments, most notably those targeting ribosomal RNA genes by polymerase chain reaction (PCR) for subsequent community profiling of this marker via techniques such as denaturing gradient gel electrophoresis, (DGGE), single-strand conformational polymorphism (SSCP) and terminal restriction fragment length polymorphism (T-RFLP). These general methods do not address specific functions, but can be adjusted to target specific microbial groups or give information about the identities of affected populations. Important steps can still be made in the better validation of such strategies and the application of statistical methods that can score the significance of observed differences.

Nematodes have also been used extensively as indicators of soil disturbances. Parameters such as the Maturity Index (MI) have been highly useful in categorising levels of soil disturbance. It is however, not yet clear how nematode characteristics would respond to various GM crop traits, or if parameters such as the MI are relevant to this end.

The species composition or population structure of several other groups of soil animals (i.e. mites, springtails, earthworms, insect larvae, etc.) have also been illustrative in tracking the effects of agents in soil systems. Similar to the case with nematodes, however, it is not clear yet how such potential indicator organisms might respond to specific traits of GM crops.

Question 4a: How should the effects of GM plants be interpreted in the light of the natural dynamics (in space and time) of biological soil processes? What are the proper controls?

It was generally agreed that the proper controls rely on an **experimental set-up that takes both the specific genetic modification as well as the system of introduction into account.**

Isolating the effects of a specific genetic modification within a cultivar requires **comparison against the non-modified parental line**. This can sometimes be problematic as isogenic or near isogenic lines may not be available. A wide **range of cultivars** might also be examined in order to determine the variability between non-GM lines.

Each area of introduction might present a unique set of circumstances in terms of soil biology and abiotic structure. One might not be able to generalise from a single test site across a wide range of potential release locations.

Cultivation of GM crops may result in changes **in agricultural land management**. Such changes must be **included in assessments of GM crop cultivations**. Some effects may only be evident upon wide-scale implementation of GM crop or after extended periods of time (see question #5).

Key to determinations of GM crop-induced effects is the statistical power of the experiments being conducted. A **power analysis** should be performed to determine how large effects must be before they become significant. Also, showing a negative result remains less convincing than display of a positive one. Thus, demonstrations of experimental sensitivity are essential to show that effects fall below a certain tangible level. Negative controls are often easily available, but there is often a lack of positive controls (i.e. experimental situations that do provide a measurable change in the parameter under study).

Question 4b: What is the reference point – the so-called baseline?

In order to determine the extent to which a GM crop may influence soil communities and processes, it is essential that the many 'normal' sources of variation within plant-soil systems be considered. Sources of such variation include the following:

- agricultural practice (including crop rotation, tillage, herbicides, insecticides, etc.)
- variation within crop cultivars
- seasonal variation (plant developmental stage)
- short term environmental fluctuations (rain, etc.)
- environmental variation (from micro through macro scales) including climate & soil properties

This variability must be taken into account in the design and interpretation of tests related to potential harmful effects of GM-crop cultivation.

The expected fluctuations in plant-soil systems due to the factors listed above denote the **baseline** of values against which GM crop-induced changes must be judged. The assessment of the baseline requires a determination of the **'normal operating range'** (NOR) for soil-borne communities and processes. Without knowledge of normal fluctuations within agricultural systems, it is impossible to make the necessary comparisons for the assessment of effects of GM crops. There exists a good knowledge base on how to track effects in light of environmental variation from the field of ecotoxicology, but such work is far from complete and often deals with less dynamic systems (i.e. without cultivation and management variables). Key to dealing with sources of variation within plant-soils systems is the design of proper experiments and use of appropriate statistics to allow the power to detect trends within the context of special and temporal variation (see also question 4a).
GM crop cultivation is typically integrated into a total farming strategy, and baseline measurements should be placed within the context of total agronomy practices.

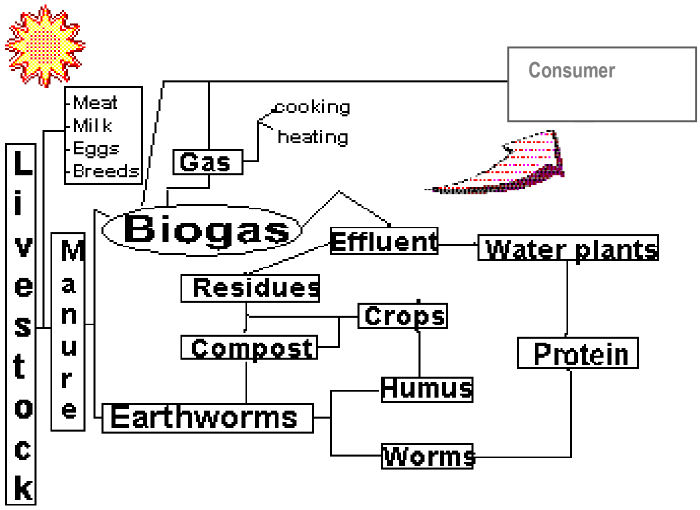


Figure IV.4 Cartoon illustrating how crop cultivation often is integrated in to a total farm operation

Question 5: For how long should monitoring occur and what are your arguments for this duration?

The length of time necessary for successful assessment of GM crop effects on soil systems will eventually be influenced by the following three issues:

1.) Temporal variability: A number of the most important factors that contribute to variation within plant-soil systems are functions of time. These include seasonal and annual variations in weather and agronomic practices.

2.) The time necessary to register an effect: While some effects might be expected to occur over the short term, some may, such as those due to a gradual build up of products or litter may require an extended period of time to occur.

3.) Practically: In principle the longer and more thorough the monitoring scheme, the greater the chance of providing an accurate assessment of effects. However, time and resources are limited. Clearly, monitoring duration should consider the time needed for typical rotation schemes; however, extensive, long-term testing prior to release may be unreasonable in some cases, making post-release monitoring essential.

Related to these is the effect of scale on the assessment of effects. Microorganisms act on a micro-scale, whereas some properties may only manifest themselves at the landscape or ecosystem scale. This stresses the importance of scaling up to ecologically relevant levels, which in some cases may only be ultimately possible at the stage of post-release monitoring. It should be noted that some soil-borne organisms may be more limited that aboveground organisms in dispersal. This may influence the study area size in some cases.

Question 6: Which methodological developments would offer better opportunities to assess if a GM plant has negative effects on soil functions?

The identified methodological developments that would significantly add to our toolbox for the assessment of GM crop effects on soil systems can be grouped into advances in five major categories:

1. Better indicators and better access to indicators:

In general, many of the advances that would help the assessment of GM crops involve the **better definition and measurement of good indicators of soil function**. These may often involve the validation and application of molecular strategies. This includes the development and testing of (better) oligonucleotide **primers and probes to target specific functional groups or specific functional genes.** Some of the indicators given in question #3 are already reasonably accessible, whereas others (including new, potentially better ones) still lack robust evaluation procedures. Also, the assessment of some groups of soil organisms, such as fungi, nematodes and other soil animals, often still requires laborious efforts of highly trained experts in taxonomy, and simplified, more direct (molecular) methods for assessing such groups would be highly desirable.

2. Standardising and simplifying procedures:

Key to success of measurement strategies is the ability to apply them in a timely, routine and cost-efficient manner. **Adapting complex experimental strategies to feasible tests** is a major hurdle. Furthermore, the ability to standardise tests from the field to the final result is important. The development and testing of standardised sampling, sample storage, nucleic acid extraction, and molecular diagnostic procedures are all key steps in producing protocols that can be applied widely for comparative analyses. The development of standardised kits for the analysis of specific aspects of soil communities is a high priority, as is the testing of such procedures for their applicability to the range of the experimental and field conditions that are likely to be encountered.

3. Translating detected effects into functional significance:

Of a more fundamental nature, advances in our understanding of soil ecosystem functionality are seriously lacking (see questions #2b & 8). Methods that allow us to **determine the functional significance of changes in soil-borne communities** will be valuable in this effort.

4. Experimental design:

Several advances in experimental design would also facilitate assessment possibilities. These include: methods to allow **greater extrapolation** from limited experiments, methods to **speed up the process** of looking at long-term effects, procedures for **integrating GM crop evaluations into total farming strategies** and procedures for **assuring the correct level of power** within assessment experiments.

5. New technologies for assessing soil communities:

Certainly, **novel technological breakthroughs** will continue to advance our possibilities to soil-borne microbial communities. A number of techniques, all with certain limitations, are available for the analysis of soil-borne microbial communities (see Figure IV.5 for example of microbial community analysis methods. However, advances in **micro-array and high-throughput sequencing technologies** will no doubt be an important part of this process, but **such technologies must become practical and feasible** (see Figure IV.5) for widespread implementation before they can be truly useful in a monitoring context. Especially attractive will be methods that can address the *in situ* situation in a highly detailed fashion at a reasonable cost and level of necessary technical expertise.

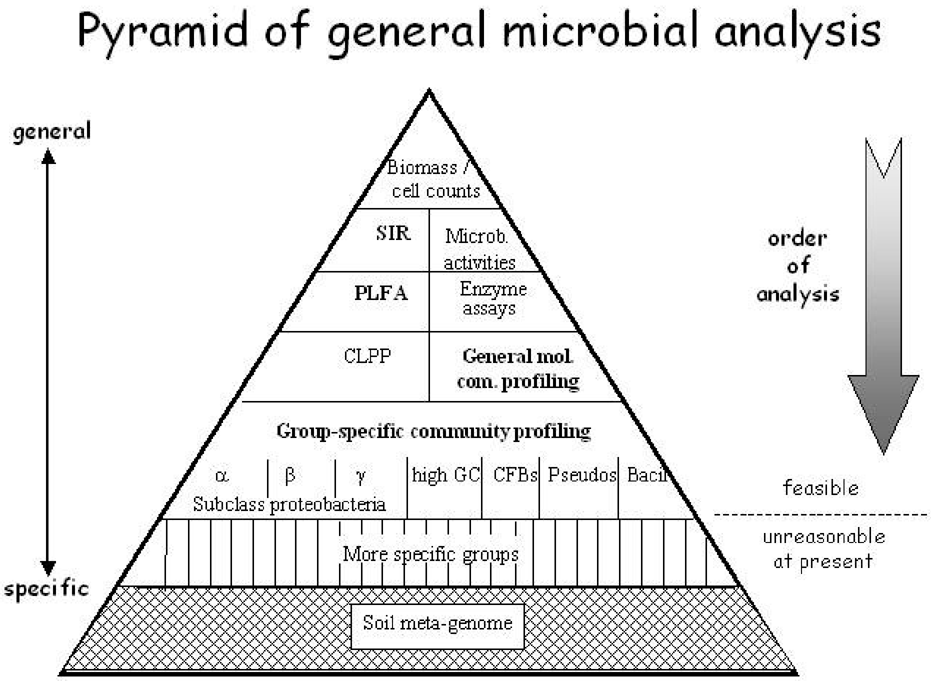


Figure IV.5 Scheme of microbial community analysis techniques, including those that are currently beyond the scope of routine use.

Question 7: Which tests could be used to examine the effects of GM crops on soil functions?
Are such test available or do they still have to be developed?

It was agreed that it is unreasonable to expect that all aspects of soil systems be examined in all cases. Clearly, there needs to be a reliance on indicator groups of processes or general tests that give relatively fast, yet detailed and responsive results. Even if numerous good indicators are available, it should not be necessary to test all of them in all cases, and it makes sense to have a critical evaluation of which indicators are most relevant for a particular case.

Status, improvement and expansion of indicators:

- **General soil quality indicators:** (such as organic matter, pH, general enzyme activities, etc. see Question 3b) are well established but provide little information on soil system status, and do not provide the necessary sensitivity to detect change.
- **Specific microbial indicators:** Although all of the microbial functional group indicators given in Question 3b (Mycorrhizal Fungi, Symbiotic N2-fixing bacteria, Degraders of recalcitrant organic matter, Antagonists to plant pathogens, & Nitrifying bacteria) are accessible via some functional or genetic screening, not all are equally accessible. Molecular screening procedures for fungal groups need to be improved, and there needs to be additional effort in development in structural-gene based assays, such as those targeting key activities in nitrogen cycling, antagonism, and degradation. In addition, other microbial groups may act as important indicators, and the identification and assessment of novel microbial indicators is top priority.
- **General microbial indicators:** Recent advances in the molecular analysis of microbial communities, has introduced quite standardised tools for assessments of general microbial community structure. These may be highly useful to get rather detailed pictures of total community structure, which may detect general disturbances to community structure as a result of GM crop cultivations, outside of range of available specific indicators. Methods to introduce statistical significance tests into such approaches have also been introduced recently, and this remains an important topic of development, necessary for the detection of meaningful patterns. Such analyses still provide no information on functionality (and perhaps never will). It has been agued that diversity *per se* is an important property of biological systems. Definitions of microbial diversity have been problematic, as have been attempts to measure microbial diversity in soil. Methods that explicitly address microbial diversity (either within the total community or within functional groups) require additional attention, as do studies that relate diversity to function.
- **Soil animal indicators:** There is a good level of acceptance of some soil animal indicators (*i.e.* nematode maturity index). However, these require extended inventories by highly specialised personnel. It would highly beneficial to have more rapid and less expert-dependent evaluations of soil animal species compositions. Molecular strategies

may offer attractive and more feasible alternatives for rapid analysis of multiple samples, and this is an area that demands future development.

In addition to the question, “What should you measure?”, it is important to know the answer to the question “At what system or at what scale should you measure it”? Due to limited resources and time, and desire to begin conservatively, it is clear that scaled down tests at the laboratory level are often highly desirable as an initial step. Although initial tests may be done at the small scale (laboratory and glasshouse), larger field-based trials will be essential in many cases to provide a representative picture of real situation. The **importance of field trials cannot be underestimated**, as these provide the conditions that are closest to those that will be encountered in actual practice. These should include post-release trials to assess long-term and location-dependent effects (aspects that are difficult or impossible to test at the experimental scale).

Question 8: If a research program were initiated for the study of GM crop-induced effects on soil systems, what points of focus should receive attention? Why specifically these points?

A number of topics were clearly identified as deserving of special attention is future research programs on GM-crop induced effects on soil systems. The major areas identified are listed as follows:

- Identification and experimental accessibility of relevant indicators (including keystone species, groups and processes) of soil functioning. Groups that may deserve special attention include soil fungi (including AMF and degraders of recalcitrant organic matter), soil animals (including nematodes), and bacterial groups that can be assessed by phylogenetic or functional markers.
- Knowledge of the ‘normal operating range’ of ‘baseline’ of soil communities and processes
- Advancement in data analysis (including bioinformatics) to determine patterns within data on changes in soil communities and functions
- Development of methods for more detailed and rapid assessment of soil-borne community structure and diversity
- Establishing links between changes in soil-borne community structure and diversity with soil functions (including impacts at the food-web and ecosystem levels)
- Determining the recovery potential of soil communities and processes, and the influence that biodiversity has on resilience
- Development of integrated experimental set-ups to examine effects within the context of total agronomic strategies

- Testing of methods to accelerate the testing procedure to shed light on potential long-term or multiple exposure effects within a relatively short period of time

The reasoning given for the specification of these research foci is that these are areas for which critical knowledge is still lacking. Thus, it is not surprising that these topics reflect to a large extent the issues raised in question 2b as topics for which necessary knowledge is still lacking.

Question 9: Do you have additional comments or perspectives to add?

While a whole range of additional comments were given, it was striking that the vast majority fell within two major topics:

A. The majority of additional comments dealt with the relevance of assessment procedures given the general political climate against the cultivation of GM crops, as summarised below:

- It remains noteworthy that plants engineered by other means also produce novel gene combinations, which may involve many unknown and foreign genes, yet these are not subject to any tests for undesirable non-target effects.
- The whole discussion of testing for potential undesirable effects of GM crop cultivation is mute if GM crop cultivation and product use are rejected out of hand by political and public institutions.
- In order to assess GM crop effects, experiments to test these would of course first have to be approved by regulatory agencies.

B. The other major topic of additional comments focused on the importance of general farming practices in affecting soil systems, as exemplified below:

- An important function of soil is its maintenance of biodiversity. However, in our choice to expose a soil to intensive agricultural practices, we have already made the decision to offer up soil systems to this purpose.
- The primary goal of agro-soil systems is the sustainable production of high quality crops with a minimal environmental impact outside the cropping area. Other, intrinsic properties such as food webs, and diversity become irrelevant unless they affect this primary goal.
- The use of a genetic modified crop is but one of many factors that affect soil systems, and some changes due to GM crop use may be positive.

4.3 Notable Quotes

The results given above are meant to form a consensus of the various opinions that were encountered during the interviewing and information gathering activities for this report. Unfortunately, the process of summarising and ordering responses does not leave room to reflect the full range of colour of the answers received. We therefore have included a number of the most poignant and provocative quotes below.

"The soil is just the farmer's glass-wool."

"The worst thing you can do is to start farming. GM-effects will almost certainly be only minor compared to the overall effects of agricultural practice."

"There has never been a significant effect of GM plants found on soil microbial communities."

"We should protect microbial diversity in soil, as this is the world's greatest source of biodiversity and new products."

"Sole reliance on a single technology will always lead to eventual failure of that technology – farmers know this better than anyone."

"We need to lift the focus from the gene to species to ecosystems."

"It will always remain difficult to prove a negative (in reference to proving that there is no detrimental effect of GM crops on soil systems)."

"The precautionary principle has its limits. If we always followed a strict precautionary principle, we wouldn't allow the introduction of anything new."

"Studies of GMP effects must look at the technology as integrated into total farming systems – it must be viewed in the context of the farm business."

"It is ironic that studies designed to investigate the potential impact of GMPs are denied permission because they themselves have an as yet undefined risk due to the GMP under study – it's the ultimate catch 22."

4.4 List of Interviewees (in alphabetic order)

This list has been restricted to those who were provided input via our dedicated questionnaire / interviewing process. A total of 30 persons were approached, of which approximately two-thirds provided responses. Although it was not possible to provide a complete list of the numerous scientists who provided valuable input via informal conversations at conferences and meetings, these people also helped to form the content of this document and their insight is deeply appreciated.

List of participant interviewees:

- Peter A.H.M. Bakker (Utrecht University - Dept. of Phytopathology)
- E. Brommer (farmer)
- Lijbert Brussard (Wageningen University and Research Centre - Sub-dept. Soil Quality)
- Yves Dessaux (Centre National de la Recherche)
- C. de Visser (teamleader PPO Cultivation & Nutrition)
- Gert I. de Vries (Plant Industrial Platform)
- E. Greve (scientific coordinator National Agricultural Counsel (HPA))
- Gerard Korthals (PPO Arable Farming, Field Production of Vegetables and Multifunctional Agriculture)
- Jos Raaijmakers (Wageningen University and Research Centre - Molecular Ecology and Biological Control)
- Phillip Sayler (Environmental Protection Agency, USA)
- Hans Schneider (IRS-Bergen op Zoom)
- Ruud Scheffers
- E. Smit (The National Institute of Public Health and the Environment (RIVM-MGB)
- O. Smit (farmer)
- P. Spoorenberg (teamleader PPO Crop Health)
- Nico M. van Straalen (Vrije Universiteit Amsterdam - Animal Ecology)
- Bert Uijtewaal (Bayer Crop Science)
- Ariena H.C. van Bruggen (Wageningen University and Research Centre - Microbial ecology, Soil science, Plant pathology)
- Richard G.F. Visser (Wageningen University and Research Centre - Laboratory of Plant Breeding)