SOIL ECOLOGICAL AND ECONOMIC EVALUATION OF GENETICALLY MODIFIED CROPS – ECOGEN

The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: A review of the ECOGEN project

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Summary

The interactions of genetically modified (GM) crops with soil species and ecosystems is complex, requiring both specific and broad spectrum assessments. In the ECOGEN project we undertook experiments at three scales of increasing complexity, using *Bt* maize expressing the Cry1Ab protein from *Bacillus thuringiensis* as an example. Test species were selected for laboratory-scale experiments to represent taxonomic groups that we could also monitor at glasshouse and field scales (e.g., nematodes, protozoa, micro-arthropods, earthworms, and snails). In the laboratory, single species were exposed to purified Cry1Ab protein or to *Bt* maize leaf powder incorporated into simplified diets under controlled conditions. In the glasshouse, multiple test species and soil microbial communities taken from ECOGEN's field sites were exposed to *Bt* maize plants growing under glasshouse or mesocosm conditions. In the field, evaluations were conducted on our selected indicator groups over multiple sites and growing seasons. Field evaluation included assessment of effects due to the local environment, crop type, seasonal variation and conventional crop management practice (tillage and pesticide use), which cannot be assessed in the glasshouse. No direct effects of Cry1Ab protein or *Bt* leaf residues were detected on

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our laboratory test organisms, but some significant effects were detected in the glasshouse. Total nematode and protozoan numbers increased in field soil under *Bt* maize relative to conventional maize, whilst microbial community structure and activity were unaffected. Field results for the abundance of nematodes and protozoa showed some negative effects of *Bt* maize, thus contradicting the glasshouse results. However, these negative results were specific to particular field sites and sampling times and therefore were transient. Taking the overall variation found in maize ecosystems at different sites into account, any negative effects of *Bt* maize at field scale were judged to be indirect and no greater than the impacts of crop type, tillage and pesticide use. Although the ECOGEN results were not predictive between the three experimental scales, we propose that they have value when used with feedback loops between the scales. This holistic approach can used to address questions raised by results from any level of experimentation and also for putting GM crop risk:benefit into context with current agricultural practices in regionally differing agro-ecosystems.

Introduction

The soil ecosystem is extremely complex, containing many thousands of different species of bacteria, protozoa, fungi, micro- and macro-fauna. This spatially and temporally variable soil community provides many key 'ecological services' to both agriculture and the wider environment. Soil fertility is primarily dependent on such 'ecological services'. Soil also provides a complex media for many positive and negative biotic interactions with plant root systems in the agro-ecosystem, affecting the productivity and sustainability of the cropping system above- and below-ground (Birch and Wheatley, 2005).

Because of the complexity of soil-based food webs and high degree of spatial and temporal variation as one moves from small to large experimental scales, predictions of the ecological impacts of genetically modified (GM) crop on soil-based ecosystems are particularly difficult. Soil ecosystems involve many more taxa and functional groups than above-ground ecosystems and our understanding of the links between different soil functional groups is still rudimentary (Liu et al., 2005). Since soil community structure and activity are affected by most of the common variables in agricultural practice, including change of crop species, water stress, fertilization, soil tillage, pesticide regimes, soil type and depth, it is to be expected that GM crops will have some effect on soil ecosystems. Generally it is difficult to interpret the ecological significance of results from single studies on GM crop–soil interactions and even more difficult to extrapolate from small (laboratory and mesocosm) to large scale (field based) studies.

GM crops and their associate management systems can potentially influence soil ecosystems positively, negatively or neutrally. The key role of plants as the primary drivers in soil ecosystem functioning has raised some concerns about GM crop associated changes in crops and management practices. Potential impacts of GM crops on soil ecosystems can be: (a) 'direct' (e.g., toxicity of an expressed GM protein on key non-target species or broader functional groups), (b) 'indirect' (e.g., via trophic interactions at multiple levels), (c) caused by unintended changes in the metabolism of the GM plant, such as root exudation, or (d) 'knock on' effects, caused by the regional management regime used with the GM crop, rather than by the GM crop directly (Birch and Wheatley, 2005). Recent reviews on this topic conclude that risk assessment of GM crops on soil ecology and soil quality require both specific and broad assessments using key species and key ecological functions (Lilley et al., 2006; Mendoca Hagler et al., 2006), preferably set within the context of the size and duration of ecological impacts caused by current agricultural practices.

One of the objectives of a European Commission funded project on 'soil ecological and economic evaluation of GM crops' (ECOGEN, www.ecogen.dk) was to integrate information from single species laboratory tests, multiple species mesocosm studies and field experimentation at multiple sites. Combined with data mining, rule based modelling and an economic analysis, this integrative approach was intended to provide a more holistic overview of the risks and benefits of GM crops. The aim was to assess the risks and benefits of proposed GM cropping systems relative to currently accepted crop management practices. Because the project was evaluating GM crops (herbicide tolerant, HT, maize and maize expressing the insecticidal *Bacillus thuringiensis* protein, *Bt*) which had already

undergone extensive environmental risk assessments (ERAs) in several countries, we sought to provide feedback on the predictive usefulness and links between testing methods at the three main scales (laboratory, glasshouse/mesocosm, field) rather than to perform a full pre-release ERA. Methodologies to perform an ERA for assessing impacts of GM crops on non-target organisms and biological diversity have been discussed elsewhere (Andow and Zwahlen, 2006; Andow et al., 2006; Romeis et al., 2006). In this paper we will focus on *Bt* maize expressing the insecticidal protein Cry1Ab in order to examine the value of testing for soil environmental impacts at the three different scales used within the ECOGEN project.

Scales of experimental testing used in the ECOGEN project

The ECOGEN project tested for environmental impacts of *Bt* and more recently HT crops at three increasing scales. Each scale has its own benefits and limitations in terms of predicting potential direct and indirect impacts of GM crops on soil ecosystems and the environment.

In laboratory tests, single 'indicator species' were selected and fed purified GM protein products (e.g., *Bt* protein) or processed GM plant material (e.g., leaf powder) as part of a controlled diet under set environmental conditions. This enabled standardized dose–response characteristics to be defined, typically for high dose (relative to the receiving environment) and short exposure scenarios (days to a few weeks). This approach has been equated to an estimate of a 'worst case scenario' of GM crop impacts on non-target organisms by some authors (e.g., Dutton et al., 2003), particularly focusing on direct effects of the GM protein on a restricted range of pre-selected indicator species. The test species are generally selected because they are relatively easy to culture under laboratory conditions and have a history of use in testing of pesticides, but those species are restricted to a few taxonomic groups selected from the vast array of soil organisms in the ecosystem. Some authors (e.g., Lovei and Arpaia, 2005) argue that single species laboratory tests are not ecologically realistic because the non-target species selected may not represent the most sensitive examples from the functional group in the regional food web. The necessarily simple laboratory designs have several limitations. They usually provide no food choice, exclude important trophic interactions and minimize environmental stress factors which can modify responses when novel GM proteins are consumed by non-target organisms in a complex mixture of plant produced nutrients, plant toxins and anti-metabolites. Exposure routes are often simplified, particularly if the GM crop is replaced by a purified GM protein in an artificial diet. Furthermore, test conditions are, usually highly controlled and standardized resulting in a much higher statistical power than is possible under variable conditions in the glasshouse or field. In laboratory-scale feeding experiments great care needs to be taken to ensure GM proteins purified from non-plant sources (e.g., extracted from GM yeasts or bacteria) are identical in structure and biological activity to the GM protein produced in the test GM plant (Prescott et al., 2005). Impacts of longer term exposure to GM proteins and derived metabolites are also not easily addressed in laboratory tests. In certain soil conditions where GM proteins can persist for several weeks or months (Tapp and Stotzky, 1998; Stotzky, 2004) tests designed to assess acute toxicity over short term exposure may not predict effects of chronic exposure (e.g., sublethal direct or indirect effects on non-target species over multiple generations, or indirect effects on trophic interactions and soil functions) which are better assessed at other scales.

The glasshouse or mesocosm experiments were designed to increase the ecological realism by using actively growing GM plants in field soils and studying multiple species representing different functional groups from a typical food web for the GM crop in its receiving environment. In this way, simplified (compared to field scale) trophic interactions could be studied, allowing detection of some indirect effects (e.g., resulting from trophic interactions or unintended effects on the GM plant's physiology and metabolism) which would not be detected in laboratory tests using purified *Bt* toxin rather than *Bt*-expressing plants. The plant's growing environment (i.e., temperature, light, soil water) is partially or fully controlled, but at least some aspects of genotype $\times$ environment interactions affecting trophic levels in soil food webs can be included (Lilley et al., 2006). Mesocosm and glasshouse experiments can be run for the full growing season of the GM plant (Griffiths et al., 2006) so that longer term exposures to the plant produced GM product and any effects of unintended changes in plant metabolites can be studied (Latham et al., 2006). If field soils are used, as in ECOGEN, rather than using sterilized composts with added 'indicator species', responses of functional groups within broader taxa (e.g., nematodes, functionally divided into fungal feeders, plant

feeders, bacterial feeders and omnivores) can be compared.

Indirect and direct effects of GM crops on non-target organisms can be complex in nature so experiments need to take into account both the specific GM plant and the receiving environments (i.e., biotic and abiotic factors affecting agroecosystems and the regional cultivation practices) in which it will be grown (Birch and Wheatley, 2005; Andow and Zwahlen, 2006; Andow et al., 2006; Romeis et al., 2006). Well-designed and replicated field trials can provide unique data which are lacking from smaller scale studies, particularly on complex food web interactions. Field trials, however, are costly to perform and sometimes difficult to interpret. At the field scale, effects of the GM trait on key ecological functions in the local receiving environment can be studied within a more ecologically realistic context of multiple biotic and abiotic stressors, acting over single or multi-season time scales. Results from laboratory and glasshouse experiments can provide important information on the eco-toxicological activity of the GM protein and metabolites, sites of plant expression (ecological exposure routes for non-target organisms) and persistence in representative field soils. Thus, smaller scale test results should help to focus more complex field scale studies, by prioritizing any key direct or indirect effects already noted (Hilbeck et al., 2006). Because species diversity in soil ecosystems is vast and functional redundancy is common (key soil functions can be maintained by relatively small number of key species) there is often a lack of a predictable relationship between species richness and function (Lilley et al., 2006). This problem is not easy to resolve, but in the ECOGEN project we focused on several keystone indicator species within ecologically important taxonomic groups (e.g., nematodes, protozoa, micro-arthropods, earthworms) and studied them in detail at more than one experimental scale where possible, to check for any consistent patterns of effects.

In the ECOGEN project, we attempted, wherever possible, to put the size and duration of any detected field scale effects of the GM crop into context with a range of factors involved in building ecological baseline data. These included crop variety-based effects (i.e., isogenic non-GM lines or commercially grown conventional cultivars), multi-seasonal variation, effects caused by current agricultural practices (e.g., use of pesticides, soil tillage treatments), differences caused by different crop types (e.g., grass, maize) and differences in the receiving environment itself (e.g., soil type, climate). This type of context setting for environ-mental impacts of new technologies, which is not possible at the two smaller experimental scales, sets an important precedent for risk:benefit analyses (Hails, 2002; ACRE, 2006. Consultation report) and is in contrast to currently accepted practices. It also allows any detected environmental effects (positive or negative) to be ranked so that any GM crop effect can be assessed within the range of acceptable risks and benefits in current agriculture.

Assessment of standardized representatives of non-target groups (e.g., soil microbial activity and community structure, protozoa, nematodes, micro-arthropods, enchytraeids, earthworms) across two to three testing scales (laboratory to field) enabled us to assess how useful smaller scale tests are in forming testable hypotheses for predicting field scale impacts (e.g., on key ecological functions affecting soil health and resilience to stressors and on persistence of any effects over one or more growing seasons) and in identifying gaps in current knowledge affecting the level of predictability (Mendoca Hagler et al., 2006). Because GM crops are generally developed with specific crop management packages (e.g., minimum tillage with HT crops, reduced pesticides against target pests in *Bt* crops), we also included field scale assessment of any indirect effects of the associated GM crop management systems, particularly altered tillage and pesticide regimes (see Andersen et al., 2007).

Outcomes from experiments at different scales

The results presented in Table 1 summarize existing ECOGEN project data, either published or in preparation. The table is intended as an overview across scales, not a detailed examination of each scale. Detailed information is available in the ECOGEN publications cited. This summary table was used to examine the presence or absence of predictive patterns of *Bt* maize impacts across ECOGEN's testing scales, and focused on nematodes, protozoa, micro-arthropods and earthworms as representative indicator groups.

Single species laboratory tests

Taxonomic groups represented in ECOGEN included nematodes (*Caenorhabditis elegans*; Scott-Fordsmann et al., personal communication), ciliate protozoans (*Tetrahymena pyriformis* and *Colpoda steirnii*; Scott-Fordsmann et al., personal communication), micro-arthropods (Heckmann et al.,

Table 1. Comparative results of the effects of *Bt*-maize on non-target soil organisms across experimental scales within the ECOGEN project

Indicator group	Single species	Mescosm/glasshouse		Field (2002–2005)		
	Effect	Effect	Where	Effect	Where	When
Bacterial functional profile	N/a	No effect[a]		No effect[b]		
Microbial community structure	N/a	No effect[a,g]		No effect[b]		
Amoebae	N/m	Non *Bt* < *Bt*	Foulum[a]	*Bt* < non *Bt*	Varois[b]	October-02
Flagellates	N/m	No effect[a]		*Bt* < non *Bt*	Varois[h]	May-05
Ciliates	No effect	No effect[a]		*Bt* < non*Bt*	Varois[b]	October-02
				Bt < non*Bt*	Narbons[b]	October-03
Nematode abundance	No effect[c]	No effect[a]		*Bt* < non*Bt*	Foulum[h]	October-05
		Non*Bt* < *Bt*[a,g]	Pooled	*Bt* < non*Bt*	Pooled[b]	October-03
Nematode community	N/a	*Bt* < non*Bt*[a]	OM; Pooled	*Bt* > non*Bt*	FF, PF; Varois[h]	October 04/05
Micro-arthropods	No effect[d]	No effect[a]		Non*Bt* < *Bt*	Foulum[i]	Pooled
				Bt < non*Bt*	OR, Varois[i]	October-02
				Bt < non*Bt*	Narbons[i]	October-03
Earthworms	No effect[e]	No effect[j]		No effect[j]		
Snail	No effect[f]	*Bt* < non*Bt*[f]	Infected snails only	N/m		
Decomposition	N/a	N/m		No effect[k]		

The 'effect' refers to a comparison of populations under *Bt* vs non-*Bt* maize, while 'where' is stated only if the effect is specific to: soil type; nematode trophic group; data pooled across soil type but not for individual soil types; or snail health, and 'when' refers to sampling date.

N/a = not appropriate (for testing at that level).
N/m = not measured.
FF = fungal feeding nematodes.
PF = plant feeding nematodes.
BF = bacterial feeding nematodes.
OM = omnivorous nematodes.
OR = oribatid mites.
[a]Griffiths et al. (2006).
[b]Griffiths et al. (2005).
[c]Caul S., personal communication.
[d]Heckmann et al. (2006).
[e]Vercesi et al. (2005).
[f]Kramarz et al., (2007).
[g]Griffiths et al. (2007).
[h]Griffiths et al. (2007).
[i]Cortet et al. (2007).
[j]Krogh et al. (2007).
[k]Cortet et al. (2006).

2006), earthworms *Aporectodea caligosa*; Vercesi et al., 2005) and snails (*Helix aspersa*; Kramarz et al., 2007). For short duration tests on protozoan and nematode species, purified *Bt* Cry1Ab protein was tested in solution (100 µg µl^{-1}). This maximum test concentration exceeded ecological levels of *Bt* quantified in ECOGEN soils, plant residues or exudates measured under glasshouse and field conditions (Griffiths et al., 2006). Tests on earthworms and snails used *Bt* leaf residue (ground *Bt* leaf powder) rather than purified *Bt* protein, to simulate a more realistic release rate for longer duration bioassays which were run for several weeks.

From Table 1 it is evident that direct effects of Cry1Ab protein were not detected using the selected soil indicator species. Finely ground *Bt* maize leaves added at an ecological realistic range of concentrations to soil from the ECOGEN field site at Foulum had no negative effects on earthworm fitness parameters including mortality, growth rate and cocoon production. However, *Bt* leaf material incorporated into field soil (Vercesi et al., 2005) at the highest concentration tested (representing a worst case scenario) reduced earthworm cocoon hatch rates by 20% relative to the control (non-*Bt* maize leaf powder). The snail *H. aspersa* was only adversely affected by *Bt* maize leaf powder when it

was pre-infected with the parasitic nematode *Phasmarabditis hermaphora,* indicating the potential for interactions between *Bt* maize and biotic stressors in the ecosystem, such as snail parasites (Kramarz et al., 2007).

Mesocosm and glasshouse experiments

Field soil from the ECOGEN sites at the Foulum and Varois sites was used to grow two maize lines (*Bt* and non-*Bt*) to maturity (83 days). Neither microbial community structure nor soil micro-arthropods were significantly affected by the *Bt* trait, in contrast to the large effects attributable to soil type and plant growth stage (Griffiths et al., 2006). However, a small but significant increase in nematode numbers and a small shift in nematode community structure (fewer omnivorous nematodes) were also detected under *Bt* maize compared with the non-*Bt* cultivar Monumental (Griffiths et al., 2005). Amoebae responded positively, in terms of numbers, to soil from *Bt* maize, but only in field collected soil from one site (Foulum) and only at one plant growth stage (5 leaf). Amoebal biomass increased from 234 (non-*Bt* maize) to 531 $\mu g \, kg^{-1}$ (*Bt* maize). Other protozoan groups (ciliates, flagellates) were numerically unaffected by soil from *Bt* maize. The growth rates of the earthworm species *Allobophora caliginosa* (Vercesi et al., 2005) and the snail *H. aspersa* (de Vaufleury et al., 2007) were not affected by the presence of actively growing *Bt* maize plants, thus supporting the results obtained from parasite-free snails in the laboratory tests.

Field trials

At the field scale, soil microbial biomass, as indicated by the total amount of phosphorus in the fatty acid fraction (PFLA-P) was not significantly affected by *Bt* maize grown in Denmark, Eastern France and South West France. This microbial index generally increased during the cropping season and to a greater extent under grass than maize, demonstrating a large 'crop type' effect. Community level physiological profiles (CLPP) were also determined (Griffiths et al., 2005, 2007), as a more general estimate of microbial activity. CLPP tests showed differences between all three field sites and between sample dates at any one site, but generally showed no difference in microbial profiles between *Bt* and non-*Bt* maize when data was pooled across sites and sampling dates. Any effects of *Bt* maize cultivation on microbial community structure and activity were considered no greater

than effects of insecticide application on non-*Bt* maize and were smaller than site and sampling time-related effects.

The biomass of amoeboid, ciliate and flagellate protozoa were all reduced under *Bt* maize compared with conventional maize, but these effects were site and sample time specific (i.e., transient) and did not persist over the growing season. For example, total protozoa (pooled data for protozoan subgroups) were reduced at the Varois and Narbons sites under *Bt* maize compared with conventional maize, but only on the October harvest date. When data was pooled across all three ECOGEN sites and all sampling times over the 2 year study, any positive or negative numerical effects of *Bt* maize on protozoa fell within the overall normal variability of maize agro-ecosystems (Griffiths et al., 2005).

Small decreases in nematode numbers sampled from *Bt* maize field plots were detected, in contrast to the mesocosm results. This small reduction in nematodes was a generic effect, occurring at all three field sites after pooling differing soil type data (Griffiths et al., 2005). This decrease was not confined to specific nematode taxa or nematode feeding types (plant, bacterial, fungal feeders, omnivores), indicating that no specific functional group was adversely affected. Nematode community structure differed between the three field sites, demonstrating the need for multiple sites to take account of natural variation in agro-ecosystems. The small size and transient nature of the observed *Bt* effect on nematodes was found to be well within the range of variation observed due to field site, sample date, crop type (grass, maize) and maize cultivar in these agricultural systems.

Discussion

Generally we found a lack of predictability between the experiments at the different scales. Bruinsma et al. (2003) also commented that smaller scale tests are not predictive of field scale impacts on soil ecosystems. For the *Bt* trait, the lack of direct effects of the Cry1Ab protein or plant tissue residues containing the protein on our selected species in laboratory tests is in general agreement with other studies on soil organisms (Yu et al., 1997; Saxena and Stotsky, 2001; Zwahlen et al., 2003; Clark et al., 2005). Although tests using purified *Bt* protein from non-plant sources may help to provide insight into potential direct effects and possible mechanisms, their ecological relevance is

probably low (Clark et al., 2005). A lack of direct effects of *Bt* Cry1Ab protein on non-target soil organisms does not rule out possible direct or indirect effects of *Bt* crops, which can sometimes be detected at the larger scale. Effects of purified *Bt* protein and *Bt* crops on non-target natural enemies from small scale experiments have been reviewed recently (Lovei and Arpaia, 2005). This review showed that there is potential for both positive and negative impacts of *Bt* crops on non-target organisms at different trophic levels, often via indirect effects. Although the mechanisms causing indirect effects on non-target organisms are often not fully understood, exposure routes via trophic interactions have been demonstrated under field conditions. For example, uptake of *Bt* Cry1Ab protein by higher order predators in maize agro-ecosystems has indicated the potential for longer term exposure routes via trophic interactions (Harwood et al., 2005). Indirect effects can also be caused by unintended changes in the GM plant's metabolism and physiology, which only become evident in specific environmental conditions. For example, although the mechanism causing nematode reductions with *Bt* maize under some field conditions is not known it is likely to be an indirect effect of *Bt* maize cultivation, possibly due to the lower soil moisture under the *Bt* maize event than under the conventional maize lines tested (Griffiths et al., 2005). In a two year (2004–2005) follow-up study incorporating soil tillage as an additional treatment comparison (Griffiths et al., 2007), nematode numbers were transiently reduced under *Bt* maize compared to non-*Bt* maize, but only at one of the three sites tested (Foulum, Denmark). The effect of *Bt* maize cultivation in reducing nematode numbers was found to be significantly smaller than seasonal, soil tillage, soil type, crop type and cultivar effects. This type of 'ecological context setting' is important when judging the risk of any negative impact caused by a GM crop, vs. impacts caused by currently accepted agricultural practices.

Specific differences were detected between the micro-arthropod populations (transient and site-specific reductions in the biomass of oribatid mites and total micro-arthropods) under *Bt* and non-*Bt* maize. However, the differences between populations under contrasting non-*Bt* maize varieties was often of the same magnitude as that between *Bt* and non-*Bt* maize, leading to the conclusion that the effects seen in the field were varietal effects and not specifically due to the *Bt* trait (Cortet et al., 2007). The value of including several conventional (non-GM) varieties in GM trials is that they enable the size of any observed effects to be put into context of the variation due to existing agricultural practices. In ECOGEN field trials we included four such conventional varieties at the Foulum site in Denmark, and at the Narbons and Varois sites in France.

The mechanism behind the small reduction in earthworm cocoon hatchability due to ingestion of *Bt* maize leaf material observed in the laboratory is unknown, but is likely to be indirect, since the earthworm's gut pH (6–7) is well below the optimal pH (>10) required in insect guts to activate the *Bt* toxin. Our findings in the field confirm the results of other studies for other earthworm species (Saxena and Stotsky, 2001), which indicate that cultivation of *Bt* maize poses low risk to earthworms as far as growth and reproduction are concerned. Although sublethal effects on earthworms resulting from *Bt* maize cultivation have been reported under field conditions (Zwahlen et al., 2003), *Bt* crops are likely to have a smaller impact on earthworm populations relative to other agricultural practices such as soil tillage. Interactions of GM crops with factors such as seasonal variation in climate, soil tillage and pesticide regimes cannot be realistically tested in mesocosm or glasshouse studies, and therefore, necessitate field scale experimentation. The integrative approach adopted by ECOGEN enabled us to put any small scale effects into a broader context in the field and to be compared against typical variation in maize agro-ecosystems caused by different growing conditions (e.g., climate, soil type) and crop management regimes (e.g., varieties grown, pesticides applied, soil tillage treatments).

Although field experiments are more costly to conduct than smaller scale tests and need to be repeated over several sites and growing seasons, they do verify whether effects seen in the laboratory and glasshouse are detectable in a range of receiving environments and if they persist or are transient in nature. Our studies demonstrate that any impacts (positive or negative) of *Bt* maize observed in single species or in mixed species mesocosm tests, were either not detectable at the field scale or fell within the normal variation found in maize agro-ecosystems. Field tests also provide valuable information on the risk/benefit ratio of new technologies versus existing technologies and crop management systems (ACRE, 2006).

Caution needs to be exercised in interpreting ecological impacts based on numerical changes in the non-target taxonomic groupings being monitored. Increases or decreases in numbers or biomass do not necessarily translate into a deleterious or beneficial change in the agro-ecosystem due to complex interactions in time and space,

compensatory responses in the plant and to the common occurrence of functional redundancy between species within functional groups (Bruinsma et al., 2003; Birch and Wheatley, 2005; Andow et al., 2006; Lilley et al., 2006; Mendoca Hagler et al., 2006). In addition, a broad taxonomic grouping such as "nematode" contains a large range of functional groups, some of which are harmful to the crop and some of which are beneficial. In our studies, although nematodes were sometimes numerically reduced when *Bt* maize was cultivated this could not be attributed to a specific functional group, such as plant parasitic nematodes or bacterial feeding nematodes (Griffiths et al., 2007). Therefore it seems that this was a general response of all nematodes under certain environmental conditions and the balance between nematodes beneficial or detrimental to the crop was not adversely affected by *Bt* maize. Before fully appreciating any potential impact of a new crop management system it is necessary to understand the ecological function of each taxonomic group affected and how it interacts in the local agro-ecosystem. We therefore advocate the use of a holistic approach with feedback loops (Fig. 1) rather than a sequential, linear approach used in several tiered eco-toxicological approaches for testing effects pesticides or GM crops on the environment. Within the ECOGEN project we have developed multi-attribute modelling approaches to understand how multiple changes in the soil

ecosystem could affect soil quality and ecosystem resilience (Bohanec et al., 2007). From an agronomic point of view it is also important to know how GM crops and their associated management systems affect crop yield and quality. In our studies we have found that the yield and quality (as assessed by nitrogen content in crop dry matter) were similar in *Bt* and conventional maize. At field sites where target pest populations occurred (*Sesamia* and *Ostrinia* corn-borer larvae) the growing of *Bt* maize increased yield and/or allowed decreases in insecticide application compared with conventional maize (Andersen et al., 2007).

Given the apparent lack of predictability when up-scaling experimental results for soil organisms and functions, an approach that incorporates levels of increasing complexity is beneficial. Using this approach, with feedback links between the levels (Fig. 1), information on direct or indirect effects of GM crops on soil ecosystems can be gathered, checked for consistency between the levels and put into a broader agricultural context at the field scale. If necessary, results from one level can be re-examined to address specific knowledge gaps, by designing additional experiments at a higher or lower level. Techniques for prioritizing soil processes for particular GM crop/receiving environment combinations are now being developed, using selection matrices to score and rank each process against agreed criteria. These methods should help target which key soil function parameters should be
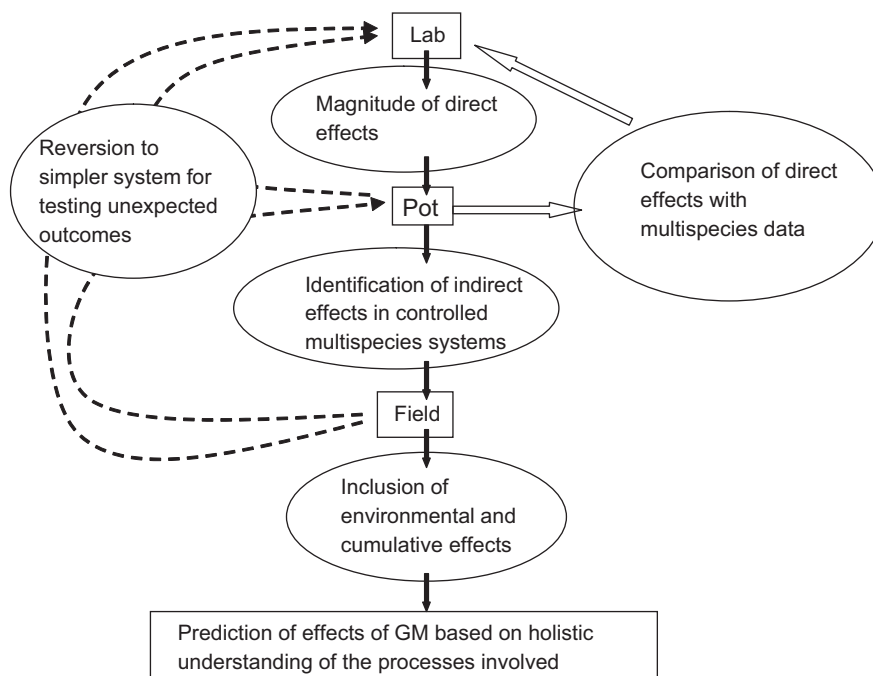
Figure 1. Schematic diagram of the inter-relationships between eco-toxicological and ecological testing methods at the three experimental scales (laboratory, glasshouse and field) discussed in this paper.

assessed where time and financial resources are limited (Mendoca Hagler et al., 2006).

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