Mechanisms for Investigating Changes in Soil Ecology due to GMO Releases

Final Report

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ATKINS

Woodcote Grove, Ashley Road, Epsom, Surrey KT18 5BW
Tel: (01372) 726140 Fax: (01372) 740055

Preface

This study which reviews the impacts of genetically modified crops on the soil ecosystem and the methods available for investigating such effects, has been produced as part of the Department for Environment, Food and Rural Affairs (Defra) *Genetically Modified Organisms (GMO) Research Programme.*

The study was conducted by Atkins Environment in association with staff from the Centre for Ecology and Hydrology (CEH), Oxford.

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EXECUTIVE SUMMARY

This report was commissioned by Defra to review impacts of genetically modified (GM) crops on the soil ecosystem and the various methods available to assay for such impacts.

The soil environment is a complex and highly heterogeneous system with its chemical, physical and biological characteristics varying significantly with location and time. Plants are a key driver of soil processes, and influence soil systems through a range of interactions in which the activities of the plants (rhizodeposition and plant litter, water exchange, and gas and nutrient exchange) modify the physical, compositional and biological characteristics of the soil.

The primary role of plants in the soil ecosystem means that changing plants that are cultivated in a soil, or the manner in which they are cultivated, may be expected to have some effect(s) on the ecosystem of that soil. The significance of the impact(s) caused will depend on what has been affected and whether the characteristics of the soil remain altered when the plant (or the causative agent of the change) is removed. Because changes as limited as using a different cultivar of a crop have been found to have an effect on a soil ecosystem, then the cultivation of a GM crop may be expected to have some effect(s) on the soil ecosystem, either as a direct result of the characteristics of the crop, or because of the agronomic practice required to grow it. Some GM crops, such as those designed to target particular phytopathogens in the soil, may be expected to have a greater effect on the soil ecosystem than others.

The issue of 'significance' and what constitutes an effect on a soil ecosystem is a key component of this report. Soil, as discussed, is a very biodiverse environment containing several trophic levels and numerous varieties of flora and fauna. Changes to any one of these species may be described as an effect on the soil ecosystem. However, because of the degree of fluctuation inherent within diverse systems such as soil, such changes are likely to occur naturally. In order to determine whether such changes are significant it is important to understand (i) the spatial and temporal fluctuations inherent in soils, (ii) the effects that GM plants and associated agronomic practices can have on these fluctuations, and (iii) the availability of methods to determine whether such fluctuations have occurred.

The report is divided into three parts: (i) a review of soil systems as providers of ecosystem services, (ii) a review of soil ecology and the key role of plants as drivers of ecosystem processes, and (iii) a review of the potential impact(s) of GM plants on the soil ecosystem and approaches to their assessment and monitoring.

Whilst all of the studies that have investigated the potential impact(s) of GM plants have focused on effects on the biological component of the soil, the abiotic characteristics of soils are also important in ensuring the correct functioning of the soil ecosystem. Such functions include the production of biomass, the regulation of water quality and quantity, the recycling of nutrients, carbon sequestration, and the provision of mechanical support for plants. The capacity of soils to provide such functions is defined as 'soil quality' with soils that are able to provide such services described as being of good or high quality.

GM plants have the capacity to affect soil quality through altered root architecture, altered decay of plant residues (modified lignin content for example), or altered root exudation for example. Although such effects have not been monitored for (and therefore not reported) it is important that such effects are considered when reviewing possible impacts of GM plants on soil ecosystems.

Understanding the role of plants within the soil ecosystem provides the criteria against which the significance of any effects of GM plants to soil organisms and soil biological processes may be assessed. In any such assessment the importance of the biodiversity of the system and the level of functional redundancy within the soil system needs to be addressed. Biodiversity refers to the number and variety of organisms present, and whilst viewed as an important characteristic of the soil, may undergo changes without affecting the function of the soil. This is particularly true for soils with a high functional redundancy, where the loss of some organisms (and even whole groups of organisms) may occur without a concomitant effect on soil function.

Therefore, before any assessment of the impact of a GM crop is made it is important to determine at what level any effect is to be assessed. Changes in biodiversity can be monitored through a range of genetic (DNA assays), phenotypic (selective plate counts) or functional assays (substrate utilisation tests) but may not measure any differences in soil function. Measurements of biodiversity may provide 'soil function' information if the assays focus on 'keystone' species whose loss is likely to have a significant effect on soil ecosystem function. Keystone organisms are of ecological significance, are susceptible to change, pertinent, have low redundancy within the soil system, and can be identified through practical and verifiable tests. In this report the mycorrhizal fungi, the plant growth promoting rhizobacteria (PGPR), the wood lignin decomposing fungi, and the nitrogen fixing and nitrifying bacteria are identified as the keystone groups of organisms that should be monitored for as part of any assessment of the impact of a GM plant on the soil ecosystem. Methods for the monitoring of each of these groups are discussed.

Studies investigating the effects of GM plants on soil ecosystems have been conducted with plants modified for:

- the release of organic acids into the rhizosphere;
- the exudation of opines which are known bacterial growth substrates;
- improved pest tolerance, for enhanced resistance to phytopathogenic bacteria, invertebrate pests and lepidopteran pests;

- improved herbicide tolerance; and
- the altered decomposition of plant residues.

In the majority of the studies conducted differences were found between the GM and non-GM control for the characteristics studied. In most cases the study was based on assaying for changes in population sizes of particular groups of organisms. Whilst many of these reported changes occurred in the presence of the GM plant, these changes were similar in magnitude to variation observed between different plant cultivars and did not persist when the plant was removed. Such changes are therefore assessed as not significant in terms of their long term effect on the soil ecosystem. This is further reinforced by comments from some of the studies that the effects observed were less than those caused by growing season, different crop species, or the application of herbicide for example.

Given the broad range of potential targets for the genetic manipulation of plants and the existence of variable factors which are specific to soils, their history and use, it is important to generate an assessment and monitoring strategy which integrates both general and specific assays of the soil ecosystem. The report identifies a three part approach to the assessment of the impact of GM plants on soil ecology:

i) the identification of keystone and important groups and processes,

ii) the choice of broad measures of the soil system, diversity and activity, for broader assays of impacts on the soil microbial and faunal communities to improve sensitivity and detection of unforeseen effects, and

iii) the application of a 'soil quality' approach which seeks to more directly assay soil ecosystem health and the ecosystem services provided by soil.

In developing specific lines of assessment consideration is given to the transgene product, its activity, site of expression and persistence. Progress is needed in the development of specific targets for monitoring which have intrinsic and clear definitions of damage. It is recognised here that while diversity *per se* is often no longer regarded as a key determinant of soil function, there is still an inadequate understanding of how community diversity is assembled, the role of factors such as dispersion, the relationship between biodiversity and soil resistance and resilience, and the significance of diversity loss for suppressive soils. It is anticipated that improvements in the conceptual and methodological tools of soil ecology, especially in soil food web interactions and soil spatial heterogeneity, will make important contributions to our understanding of soil ecology and our assessment and monitoring of GM plants. The soil quality approach is identified as particularly beneficial in long term monitoring or post commercialisation monitoring and in focusing on agricultural practices in general.

Research priorities are considered and recommendations made, including the need for additional keystones groups, improving the standardisation of assay procedures and the use of long term assessment of soil system outcomes.

Finally the report stresses (i) the considerable expertise and skill that resides within the communities of soil scientists, soil ecologists, agronomists, and (ii) that the multidisciplinary approach advocated should be addressed to the wider, and more significant, impacts of agronomic and land management practices.

1. INTRODUCTION

1.1 The soil environment is a complex and highly heterogeneous system with its chemical, physical and biological characteristics varying significantly with location and time. In agricultural soils some of these spatial and temporal fluctuations are a result of agronomic practice whilst others are a consequence of broader non-anthropogenic processes such as carbon and nitrogen cycling, weathering/erosion and climatic effects.

1.2 Because of the inherent linkages between the biotic and abiotic components of the soil ecosystem[1] then variations (spatial or temporal) in some parameters within the soil ecosystem have the potential to cause knock-on effects to other components of the system. The likelihood of effects being realised will of course depend on the characteristic involved and the degree of change occurring, as well as the ability of the ecosystem as a whole to absorb the change or recover from a perturbation (termed the 'resilience' of the system)[2] [1, 2].

1.3 The potential impact of a genetically modified organism (GMO) on the soil ecosystem is an important component of the risk assessment process that is conducted by Defra and ACRE[3] for each GMO intended for deliberate release into the environment. Assessment of the impact includes the evaluation of the characteristics of the GMO, and in the case of GMOs released in an agricultural environment, the characteristics of the specific agronomic practice associated with the release or cultivation of the GMO. It is possible that the agronomic practice itself may have a greater effect on the soil ecosystem than the GMO itself.

1.4 The assessment of the potential effects to soil ecosystems can therefore only be made by comparing the potential effects (caused by the GMO and the associated agronomic practice) against the spatial and temporal fluctuations that are inherent to the soil environment. Where the changes assessed to occur are smaller than those inherent to the soil ecosystem, then the risks posed by that particular GMO to the soil ecosystem may be assessed to be low. However, if the effects caused by the GMO

[1] defined as the ecological community present in the soil, functioning as a unit with the soil environment (ACRE Soil Ecology subgroup report, 2003).
[2] the ability of the ecosystem to withstand the immediate effects of a perturbation is described as the 'resistance' of the system (Griffiths *et al.* (2001), *Applied Soil Ecology* 16(1), p.49-61).
[3] Advisory Committee on Releases to the Environment.

are higher than those occurring in the absence of the GMO (or associated agronomic practice), then the risks posed by a release of that GMO to the soil ecosystem may be assessed to be (relatively) higher.

1.5 The comparison of such changes therefore requires an understanding of:

- the spatial and temporal fluctuations inherent to soils;
- the effect(s) that GMOs and associated agronomic practices can have on these fluctuations; and importantly,
- the availability of methods to determine whether such fluctuations have occurred.

1.6 In 2002, the ACRE Subgroup on Soil Ecology was established to consider the potential generic effects that genetically modified (GM) plants and the agronomic practices associated with them, might have on soil ecosystems and how these might be measured (ACRE Soil Ecology subgroup report, 2003). The report recently published by the Subgroup identified a number of key issues for which further information is required. These included the identification of appropriate soil indicators and the methods to monitor them, as well as a greater understanding of the significance of the different fluctuations. The outputs from this report have been structured so as to feed into further discussions by the ACRE Soil Ecology subgroup.

1.7 As a footnote to this study, it is recognised by the project team that the use of GMOs in the UK and some parts of the EU has been the subject of intense public and political interest. Much of this interest has resulted from both a lack of information and a misunderstanding of the information available on the uses of GM crops and the potential implications to the environment and human health. Whilst this proposed study will focus entirely on the scientific issues involved, and is not intended as a forum for the review of the advantages and disadvantages of both GMOs and specific agronomic practices, it is recognised that the public can only benefit from access to current, objective and comprehensive reviews of the information available in this area.

AIMS AND OBJECTIVES

1.8 The aims of the project are:

- to provide a review of current knowledge of the soil ecosystem;

- to compile a list of techniques that have been used to measure changes in soil ecosystems and identify potential indicators for monitoring change in soil ecosystems;

- to collate information from studies that have looked at the direct and indirect impacts of agriculture (involving GM and non-GM crops) on soil ecosystems; and

- to produce a database of relevant literature and to collate hard copies of material that cannot be accessed electronically.

STRUCTURE OF THE REPORT

1.9 The report is structured as follows:

- Chapter 1 – *Introduction*, including the reasons for the production of the report, and the context into which the outputs of the report are intended to fit;

- Chapter 2 – *Review of soil systems as providers of ecosystem services*. The purpose of this chapter and Chapter 3 is to provide the background against which any impact of GM crops can be evaluated. Whilst there are a number of publications that have reported possible impacts of GM crops on soil ecosystems, it is viewed as important for this report to also present some form of framework against which such impacts may be assessed. A key component of the framework is the identification of the fluctuations that are inherent to soil ecosystems.

  The focus on Chapter 2 is the review of the properties that characterise 'soil quality'. Soil quality is defined simply as the "capacity of soil to function" [3] and is determined predominantly by abiotic parameters such as organic matter content or bulk density.

- Chapter 3 – *Review of soil ecology and the influence of plants.* This chapter reviews the existing level of understanding of the function and important (predominantly biotic) components of soil ecosystems, and the roles played by the principal groups of organisms (microorganisms, protozoa, invertebrates and plants). The chapter focuses on the specific influences plants have on the soil ecosystem, and therefore identifies why particular changes to plants may affect the soil ecosystem. This chapter also addresses the issues of diversity within the soil and the importance of redundancy with the ecosystem with respect to the provision of various soil functions.

Both Chapters 2 and 3 also review the various methods available for the monitoring or measurement of the various parameters that define 'soil quality' or characterise the soil ecosystem.

- Chapter 4 – *Review of the potential impact of GM crops on the soil ecosystem and approaches to their assessment and monitoring.* This review is made against the framework established in Chapters 2 and 3. The GM crop (and/or agronomic practice) is assessed to cause a potential impact if the changes caused are outside the fluctuations inherent to the soil and persist in the absence of the GM plant; and

- Chapters 5 and 6 – *Summary and assessment of the issues which need to be addressed by future research*.

2. REVIEW OF SOIL SYSTEMS AS PROVIDERS OF ECOSYSTEM SERVICES

2.1 Soil is a complex mixture of minerals, organic compounds and living organisms whose activities and interactions constitute a living system that is vital to life on earth [4, 5]. Whilst soil was thought of historically as primarily a medium to support the growth of crops, it is now viewed as having a broader role in maintaining environmental quality and health, as well as productivity [4, 6].

2.2 The purpose of this chapter is to review the functions that are performed by soil and that affect the health, functioning and total productivity of terrestrial ecosystems. The review is based on the concept that the quality of a soil must be sustained in order for it to continue to function as required. Anthropogenic activities can and have had an adverse effect on soils. The GLASOD (Global Assessment of Soil Degradation) study by the United Nations Environment Programme (UNEP) reported that as much as 38 percent of agricultural land worldwide has been degraded by anthropogenic processes [7], with degradation defined as the reduction in the current or future capacity of soils to produce goods or services.

2.3 The outputs of this chapter are (in conjunction with Chapter 3) intended to provide the background review of the soil ecosystem (and the properties and functions that support it), against which the assessment of the impact of GM crops (and associated agronomic practices) can be made.

2.4 Ecosystem services are the functions performed by a soil that are necessary for the continuing sustainability of the soil ecosystem and the use(s) to which that soil is employed. The relative importance of each service varies to some degree according to the intended use of the soil, with forestry for example requiring a greater emphasis on biomass production than the phytoremediation of contaminated land.

2.5 However, with all soils the maintenance of the various functions is necessary to provide the infrastructure for a soil ecosystem to operate. Whilst the soil system (comprising of both the abiotic and biotic components) is fluid enough to allow for some changes in the various functions without consequent effects on the ecosystem, significant changes in soil functions (beyond these naturally occurring perturbations) have the potential to cause irreversible changes in the soil system and the uses to

which the land can be employed. It is the purpose of this report therefore to identify whether the growth or cultivation of any GM crops is capable of causing such significant changes in soil functions.

2.6 Although different ecosystems (and land uses) have different requirements in terms of emphasis or importance of the various soil functions, the following characteristics have been identified as key functions required from all soils:

- biomass production;
- regulation of water quality and quantity;
- regulation of the recycling of nutrients and other elements, both within the soil itself and the Earth's biosphere as a whole [4];
- provision of mechanical support for living organisms and their structures. This is viewed by some studies as including the support of man-made structures [4];
- carbon sequestration and regulation of carbon balance; and
- bioremediation of waste (the filtration, buffering, degradation, immobilisation and detoxification of organic and inorganic substances [4, 8]).

2.7 In addition to these key functions, a number of other functions have been proposed by various studies. The relevance of these are likely to be determined more by societal/political pressures than biological or ecological demands. They are therefore outside the scope of this project but have been included here for completeness:

- role of soil as a biological habitat and a gene reserve [9]. This function is viewed in this chapter as a societally determined function as the level of biodiversity within a soil may not have an effect on the capability of that soil to provide the six key functions listed above. In this case, a high biodiversity is something that society may wish to exist within soils, but may not actually be required for that soil to support a healthy and productive ecosystem (the significance of biodiversity to soil function is considered in Chapter 3, with the soil in this example exhibiting some species redundancy);
- role of soil as a source of raw materials such as clay or sand [10]; and

- as a repository of archaeological and palaeontological evidence [10](Blum, 1993; cited by [11]).

2.8 The capacity of a soil to provide and sustain these functions, which are of course fundamental to the correct functioning of the soil ecosystem, is defined as 'soil quality' [3], with the soils that are able to provide the services being described as being of good or high quality.

2.9 At its simplest level, soil quality is therefore the "capacity (of the soil) to function" [3]. The assessment of soil quality provides the means of unifying the multifunctional role of soil as a single concept [9].

2.10 In Karlen's definition [3] the 'function' of the soil is not defined. Other definitions however are broader, describing soil quality as the "capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Soil Science Society of America (SSSA); cited by [12]). The scope of the definition to include functions other than just the production of biomass reflects the current recognition of soil as an environmental buffer protecting watersheds and groundwater from agricultural chemicals and industrial and municipal wastes, and sequestering carbon that would otherwise contribute to global climate change [7].

2.11 The terms 'soil quality' and 'soil health', whilst used interchangeably in some reports [13], are viewed in this report as distinct characteristics. The term 'soil health' is used primarily on the basis of qualitative and descriptive properties using direct value judgements (healthy versus unhealthy) [3], whereas the term 'soil quality' has a more quantitative derivation based on measurements of soil function [3, 14]. The term 'soil quality' (and not 'soil health') has therefore been used in this report to define and characterise soil as a provider of ecosystem services.

2.12 The rationale behind assessing soil quality is that it provides the means to determine whether the function(s) required from a particular soil, such as biomass production or the bioremediation of waste, are being maintained in a sustainable manner. Soil quality also provides a framework against which any impacts on the soil can be assessed as having a positive or negative effect on soil function. With respect to this report, the impacts that are assessed are those caused by the growth or cultivation of a GM crop.

EVALUATION OF SOIL QUALITY

2.13 As discussed, soil quality is the "capacity of the soil to function" [3]. Soil quality cannot be measured *per se* [15], and therefore in order to determine the quality of a

soil, a number of indicators or properties are used with which its function can be evaluated [3, 12, 16].

2.14 Most of the publications produced to date that have reviewed soil quality and the assessment of particular impacts or processes [3, 14, 17] have adopted a similar approach. This is outlined below:

- identification of soil functions, *such as the regulation of water quality*;
- identification of the soil attributes that influence or determine soil function. *Examples of soil attributes that maintain or regulate water quality are the ability of the soil to accept, hold and supply water* [17];
- selection of a minimum data set (MDS) of indicators that may be used as measurable surrogates of the soil attributes [18]. *Soil indicators suitable for measuring water holding capacity include texture and electrical conductivity* [16]; and
- quantification of change in the soil quality indicators. Changes in soil quality indicators are assessed by comparison against agreed threshold values. Assessment of changes can be made at a specific (single indicator) level, or at a more overarching level in which changes (both positive and negative) to all the indicators are incorporated into the assessment. Evaluation of all the indicators requires the use of some form of weighted additive model [17, 19].

Identification of Soil Functions

2.15 Soil functions (ecosystem services) are effectively the requirements that are desired from the soil ("what we want the soils to do" [17]). GM crops have potential applications for use in three areas, namely agriculture, silviculture (forestry) and phytoremediation[4]. Therefore, whilst some functions such as biomass production are common to all three areas, others such as the removal of specific pollutants are more specific and may have a greater relevance to one use area than others.

2.16 The soil functions relevant to all three areas are:

- **biomass production** – all three areas of application require the soil to provide the conditions for the production of biomass;

[4] for a review of the application of GM plants for the phytoremediation of chemicals, see the Defra report 'Use of Genetically Modified Organisms for the Bioremediation of Organic and Inorganic Pollutants' (2004). Available at www.defra.gov.uk/environment/gm/research/reports.htm#Completed

- **regulation of water quality and quantity** – the importance of this function varies across the three areas of application. All three areas will require the soil to provide sufficient water for the production of biomass, and will be able to retain the required water without becoming waterlogged or drying out. Soils used to support forestry or agriculture are likely to have a role in the regulation of water replenishing surface or groundwater systems, and will therefore be able to regulate the movement of water through the soil without significant leaching of nutrients, minerals or pollutants, or the destruction of the soil structure through erosion;

- **regulation of the recycling of nutrients and other elements** – as nutrient recycling is mediated by the soil microorganisms and mesofauna (invertebrates), then the role of soil as a regulator of nutrient recycling depends on its ability to support the various biological process being conducted within the soil matrix. Good recycling of nutrients requires effective regulation of water and gas movements within the soil;

- **provision of mechanical support for living organisms and their structures** – requires the maintenance of the soil structure and good resistance to erosion. Plants require the soil to allow good root penetration and growth, and therefore compacted or waterlogged soils provide a relatively poor medium for growth. Soil meso- and macrofauna (vertebrates) also require a good soil structure that promotes good gaseous exchange and transport of water. Waterlogging is likely to limit the activity of many soil fauna although some organisms (such as nematodes) rely on water films to move in the soil and protozoa may be more active in water logged soil;

- **carbon sequestration and regulation of carbon balance** – this function reflects the global importance of soils in the carbon cycle, and also as a carbon sink. A well-structured soil is able to retain carbon in the form of soil organic matter. Excessive breaking-up of the soil structure, through intensive tilling for example will result in the loss of organic matter and the loss of that soil as a carbon reservoir; and

- **bioremediation of waste** – many of the pollutants deposited in soils (either directly, or indirectly through atmospheric deposition) are susceptible to biodegradation, primarily by microorganisms. Therefore a soil environment that promotes good microbial activity (good nutrient availability, good water regulation and gaseous exchange) should promote good bioremediation of waste compounds.

2.17 The review of the key soil functions highlights the inherent linkages involved. Effective regulation of water through the soil will also promote good recycling of

nutrients and is indicative of an effective soil structure. A good structure promotes root penetration and good mechanical support for plants, as well as providing good aeration and the retention of organic matter.

Identification of Soil Attributes and the Selection of Appropriate Soil Quality Indicators

2.18 Soil attributes are defined as specific characteristics of a soil that are essential for the maintenance of a particular soil function [17]. Soil attributes are themselves ascertained by the measurement of soil quality indicators (the measurable surrogates of soil attributes [17]). The indicators that are employed for any assessment of soil quality determine the attributes (and consequently the functions) of the soil that can be measured. Therefore, soil quality indicators must be selected according to which soil functions are to be assessed.

2.19 Most studies reviewing soil quality have proposed some form of minimum data set (MDS) of indicators that can be used to determine soil quality (Table 2.1) [11, 12, 14, 20]. Although the specific indicators selected will of course be determined in part by the types of soil function that are being assessed [8], the MDS of indicators usually includes a number of key properties whose measurement provides information on the more general soil functions such as biomass production and water retention. Examples of key properties proposed by many studies as soil quality indicators are pH, aggregate stability, organic matter level, soluble phosphorus, mineralisable nitrogen and electrical conductivity [21].

2.20 Whilst many of the publications in this field present some form of MDS of indicators, it is important that the actual indicators used will reflect the soil function(s) being assessed. A reliance on a single (generic) set of soil quality indicators to determine the soil quality of all environments can be misleading [14], and has been described by some researchers as unattainable [22]. However, from the information available, the following indicators have been identified as the key characteristics used to assess soil function, and therefore soil quality.

2.21 Soil quality indicators may be physical, chemical and biological properties of the soil, and vary from relatively simple and easily measurable variables such as soil temperature and rate of nitrogen mineralisation, to more complex properties that are derived from a combination of individual measures. Such combinations of indicators (commonly referred to as pedotransfer functions [24]) are suitable for the measurement of soil attributes such as soil aeration which depend on several measurable properties, including water content, bulk density and pore size distribution [20]. Another pedotransfer function, the soil tilth index incorporates measures of bulk density, strength, aggregate uniformity, soil organic matter and plasticity index [25].

Table 2.1 – Proposed MDS of indicators of soil quality and the soil function(s) they are associated with (adapted from [3, 14, 16])

Soil Indicator	Relationship to soil function Rational for selecting the indicator	Ecologically relevant measurement values	Other soil quality indicators that affect the selected indicator
Physical			
Bulk density	Indicator of the potential for penetration by plant roots, the amount of water- and air-filled pore space and also the level of biological activity. Therefore provides a measure of both soil structure and soil strength (penetration resistance).	g cm^{-3}	Organic matter content, aggregation, topsoil-depth, exchangeable sodium percentage (ESP), biological activity.
Texture	Measure of the size of particles present in the soil. Provides information on the retention and transport of water and chemicals.	%age sand, silt, clay content.	
Infiltration	Indicator of the potential for runoff and leaching, and therefore erosion of the soil.	min/2.5 cm of water.	Organic matter content, electrical conductivity, ESP.
Water holding capacity	Provides a measure of water retention in the soil, which in turn indicates the potential for water transport through the soil and the possibility of erosion. Low water holding capacity will result in more rapid runoff and greater erosion potential.	% (cm^3/cm^3)	Calculated from bulk density, texture and organic matter.
Aggregation	Provides an indication of soil structure, erosion resistance, crop emergence, and infiltration.		Organic matter content, microbial (especially fungal) activity, texture.
Topsoil depth	Gives a measure of rooting volume for crop production, water and nutrient availability. Can also provide an estimate of productivity potential and erosion levels.	cm or m.	
Chemical			
pH	Indicator of nutrient availability, pesticide absorption and mobility.		

Organic matter content	Provides an indication of nutrient cycling, pesticide and water retention, soil structural stability, crop water availability, and erosion control. Measure of organic matter content provides the most significant single indicator of soil quality.	kg C ha^{-1} 30cm depth.	Organic matter content influences aggregation, structural stability of the soil, water holding capacity and nutrient holding capacity.
Forms of nitrogen	Availability to crops, leaching potential, mineralisation and immobilisation rates.		
Conductivity or salinity	Water infiltration, crop growth, soil structure.		
Available nutrients	Capacity to support crop growth, environmental hazard. Provides an indicator of nutrient limitation. Nitrogen is the most limiting element for plant growth.		Organic matter content, pH, topsoil-depth, texture, microbial parameters (mineralisation and immobilisation rates).
Biological			
Microbial biomass	Biological activity, nutrient cycling, capacity to degrade pesticides.		Organic matter content, aggregation, bulk density, pH, texture, ESP. Significant correlation reported for soil pore space and microbial biomass levels, with the highest biomass reported for soil pores of 0.2µm and 3.0µm. Pore spaces within this size range are accessible to microorganisms but not their predators [23].
Potentially mineralisable nitrogen	Soil productivity and nitrogen supplying potential.		
Soil respiration	Measurement of microbial activity, but not necessarily of high biomass. Polluted stressed soils can have an elevated respiration rate.		

2.22 Irrespective of the degree of complexity, the most relevant indicators are those that are sensitive to management induced changes, easily measured, able to demonstrate both positive and negative change, relevant across sites or over time, inexpensive, and adaptable for specific ecosystems [7, 12, 16, 17].

2.23 Biological indicators in particular are often very dynamic and sensitive to changes in soil conditions. Consequently they are often used as markers of short-term changes in soil quality [8]. Biological indicators include populations of micro-, meso- and macroorganisms, respiration rate (as an indication of activity of the microbial population), ergosterol content (as an indicator of fungal populations) and more detailed evaluation of the soil organic matter [8, 23].

2.24 The identification and measurement of biological indicators is addressed in more detail in Chapter 3 and Chapter 4, and is only referred to here because studies often cite soil quality indicators as being physical, chemical or biological in nature [3, 8, 12].

Physical indicators

2.25 Of the key physical indicators, bulk density, along with soil texture and infiltration (or penetration of resistance) provides a measure of the level of compaction of the soil, as well as an indication of the translocation of water and air and the movement of roots. Increased bulk density of the soil is indicative of a low level of porosity and increased compaction. The level of compaction is enhanced through traffic by agricultural machinery or livestock trampling but may be reduced through ploughing (which also promotes aeration and infiltration) [26]. However, excessive ploughing to the same depth can also increase bulk density, through the creation of a ploughpan, cause by the smearing and compacting action of the plough sole. This can be avoided through ploughing at different depths [26].

2.26 Soil texture is a key variable affecting soil organic matter and site productivity [27] as the size of the particles present has a significant effect on the physical properties of the soil, particularly the drainage, water-holding capacity and the ease with which the soil can be cultivated [26].

2.27 Because of their high porosity and high infiltration rates, coarse-textured soils have a low soil moisture storage capacity and excessive internal drainage. In semi-arid and sub-humid areas the low moisture storage capacity of such soils has a negative effect on crop growth and yields, because excess moisture from previous rains cannot be stored sufficiently long in the soil profile [26]. Whilst semi-arid and sub-humid conditions are not usually associated with the UK, low rainfall (<600 mm annually) in areas such as East Anglia means that these areas may be described as semi-arid.

2.28 Infiltration provides a measure of the compaction of the soil and the potential for leaching. However, the usefulness of infiltration data is limited due to wide natural variation that occurs for this property within soils.

2.29 Aggregation is a useful indicator of erosion potential of the soil [26]. Soils that are devoid of vegetation and whose surface layers are non- or poorly-aggregated are susceptible to wind erosion when dry. These soils are also susceptible to erosion when wet, especially where the silt content is high as the silt material will seal when moistened by rain (or irrigation water) forming a surface crust. This causes a reduction in infiltration rate and an increase in runoff [26].

2.30 As topsoil is the part of the soil that is most important for biomass production, soil management and degradation control [26], then topsoil depth provides a measure of the amount of soil available for these functions[5]. The United Nations report [26] defined topsoil as extending to either a depth of 30 cm, or the depth at which root growth becomes inhibited (whichever is shallower).

Chemical indicators

2.31 Of the chemical indicators used to characterise soil quality, pH and soil organic matter content have been identified as key characteristics. pH defines biological and chemical activity thresholds and therefore provides key information on the potential for various processes to occur within the soil [14]. Potentially toxic compounds become more soluble and therefore more mobile with increasing soil acidity [10]. Microbial activity also tends to decrease with increasing soil acidity. Optimal pH range is 5-8 [10].

2.32 With respect to soil organic matter content, whilst no single soil quality indicator has been identified as being suitable for the measurement of soil quality on its own [7], some indicators have been described as more significant than others, with soil organic matter (SOM) identified by many studies as the most significant single indicator of soil quality[6] [7, 18].

2.33 SOM is defined as the 'organic fraction of the soil exclusive of undecayed plant and animal residues' (SSSA, 1987; cited by [7]). The maintenance of SOM content is described as paramount in sustaining the quality of the soil [7], with both the amount and location of the organic matter in the soil profile being important [28].

2.34 The importance of SOM is a consequence of its multifunctional role in soil and the linkages to other soil characteristics. As described in Table 2.1, SOM content influences aggregation, cation exchange capacity, water holding capacity and

[5] The terms 'soil management' and 'degradation control' are viewed as encompassing the soil functions of regulation of water quality, nutrient recycling, provision of mechanical support, carbon balance and bioremediation of waste.

[6] Although this should be viewed in the context that it is also the single soil property determined most frequently in research studies 7. Reeves, D.W. (1997). The role of soil organic matter in maintaining soil quality in continuous cropping systems. *Soil & Tillage Research*, 43(1-2): p. 131-167..

nutrient holding capacity of the soil. These in turn affect soil bulk density and infiltration for example as a decline in SOM levels leads to the degradation in soil structure and little resistance to consolidation following compression by farm machinery or livestock [7, 23, 26]. The key role of SOM in influencing these characteristics is its ability to bind soil particles together [26]. If the level of SOM decreases then binding is reduced leading to lower aggregation and reduced structural stability of the soil (and consequently enhances the potential for erosion). SOM is also able to hold up to twenty times more water than an equivalent quantity of mineral particles, and therefore may have a significant effect on water holding capacity of the soil [26].

2.35 Linkages between SOM content and aggregation are two way, with the decay of SOM in soil reported to be modified slightly by the physical protection conferred by the soil aggregates. This is demonstrated by observations that rates of decay of SOM compounds in the absence of soil are an order of magnitude higher than in the presence of soil [28]. Therefore, changes in the physical structure of the soil will alter the decay and ultimately the level of SOM in that soil. This is demonstrated by flushes of SOM released from soils following disruption of their aggregate structure [28].

2.36 SOM has been used as an indicator of soil quality both in terms of its concentration in the soil, and also its location within the soil horizon. The majority of the SOM in soils subject to zero or negligible tillage has been found to be located above the mineral soil as a litter layer with only 20 percent present at a depth >25 cm. In conventionally tilled soil the SOM is spread more homogenously throughout the ploughed layer [28].

2.37 In addition to ploughing, good mixing of the SOM and mineral particles is best achieved in well-saturated soils. In poorly saturated soils the degree of mixing is limited, leading to inadequate aggregation and a poorly developed soil structure [26]. Mixing of the SOM with soil mineral particles has the following effects on the characteristics of the soil [26]:

- enhances aggregation;
- increases structural stability. This is more pronounced on sandy rather than more finely textured soils;
- increases water holding capacity;
- contributes to the nutrient holding capacity;
- buffers against potential acidification;

- binds toxic substances to the soil matrix; and

- provides the soil with N, P and S and other nutrients which were stored in the above ground vegetation.

2.38 SOM content is correlated positively with biological activity in the soil. Because of the important role of SOM in biological activity, the location of the SOM within the soil profile will affect the levels of activity of organisms present. The concentration of SOM in the surface layer of untilled soil means that biological activity is concentrated in that area [28]. The effect of this on other soil properties is varied. The concentration of SOM in the surface layer means that further additions of organic matter to the surface will further heighten the disparity of SOM levels in the soil profile. As falling leaves represent a significant input of organic matter to soils, then seasonal changes have a more pronounced effect on biodegradation rates in no-till soils. In conventionally-tilled soils seasonal variation on biodegradation is much weaker [28].

2.39 Greater mixing of the SOM through the soil by conventional tillage may enhance biodegradation where the SOM comes into contact with soil solutes such as nitrate [28]. This may result in an improved rate of nitrogen mineralisation, thereby reducing the need for the addition of nitrogen fertiliser in soils of high organic matter [29]. However, where the SOM contacts the clay matrix, biodegradation is reduced [28].

Biological indicators

2.40 The level of fungal biomass in the soil is usually measured (and reported) as the concentration of ergosterol per gram soil. Ergosterol is an important cell wall component of most fungi and is produced almost exclusively by fungi [23]. The consensus view of the publications reviewed for this report is that ergosterol is a useful indicator to determine fungal biomass in soils.

Linkages between soil quality indicators

2.41 The soil quality indicators described are not independent determinants [7], with various linkages and correlations existing between many of the properties. Linkages between indicators such as bulk density, strength and aggregate uniformity are demonstrated by the replacement of these individual indicators with pedotransfer functions such as the soil tilth index [25].

Quantification of Changes in the Soil Quality Indicators

2.42 Whilst there appears to be a basic consensus of the key indicators that should be used to assess soil quality, and therefore the means to make **qualitative** assessments of various impacts on soil quality, there is little information on how changes in those indicators should be interpreted in order to make a **quantitative** evaluation of changes to soil quality [16]. A similar problem applies to assessments of the biological indicators of soil quality (see Chapter 3).

2.43 In theory, the quantification of the changes in the soil quality indicators, and the comparison of those changes against agreed threshold values (or critical limits[7]) [16], provides the means by which a particular impact (such as a GM crop or associated agronomic practice) on the soil can be assessed and compared with other impacts. A crop or management practice that affects a soil quality indicator beyond the (upper or lower) threshold value is assessed to have a significant effect on that aspect of soil quality.

2.44 However, whilst this approach provides the mechanism to assess changes in soil quality quantitatively, applying the approach is dependent on the selection of critical limits for each indicator and understanding the linkages between the individual indicators. Although a range of models have been developed to study changes in soil quality, they can only identify impacts that have significant changes to soil quality if critical limits for the various indicators have been identified. Without the information on critical limits it is difficult to determine whether the effects caused by the impact are outside (higher or lower) of those that occur as natural perturbations.

Modelling changes in soil quality indicators

2.45 As a consequence of the linkages between soil quality indicators, it is more appropriate to assess soil quality on the basis of changes of all of the indicators, rather than changes in specific indicators (and the attributes that they represent).

2.46 The values for all the indicators may be combined as a pedotransfer function [19], or by using a weighted additive model [17, 21] to provide a single soil quality index. This then allows an overall assessment of the impact (on all soil quality indicators) to be made, and is equivalent to assessing the impact on overall soil function.

2.47 In Figure 2.1, the values of the individual soil quality indicators are represented by the radii of the individual segments, with overall soil quality equivalent to the total area occupied by all the segments. The angle of each segment illustrates the

relative importance of that indicator as a measurement of overall soil quality. The thick line represents the baseline soil quality. In Model A each of the soil quality indicators are equivalent to their baseline values and the overall soil quality is therefore also equivalent to the baseline. In Model B however, three of the indicators are above (i.e. greater than) the baseline value (pale highlighting), four are below the baseline (medium highlighting) and one is unchanged (dark highlighting). The overall effect of the impact on soil quality is therefore negative. Such a conclusion would not have been drawn if the assessment was based on changes to indicator 'A' for example.

Threshold values for soil quality indicators

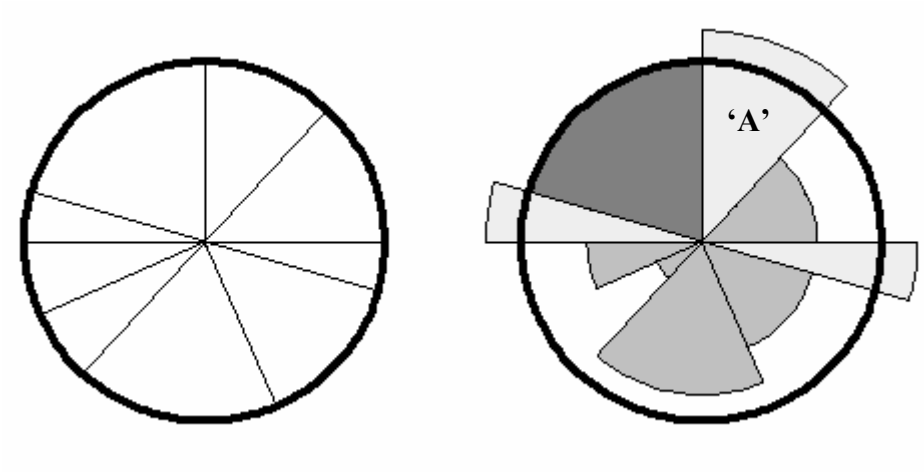
2.48 Whilst models to determine soil quality have been proposed (for example Figure 2.1), they can only be applied to a field situation if a baseline or threshold value exists that can be used as the foundation for the model.

2.49 Threshold values are defined as the range of values for a selected soil indicator that must be maintained for normal functioning of the soil ecosystem [16]. However, whilst threshold values for soil quality indicators have been described as a key component of soil quality assessments, there is little information currently available (2004) on actual values that can be used [10, 11, 16]. This is due in part to the significant variability both within and between different soils, which results in threshold values determined for one soil not being applicable to other soils. There is also variation between different crops due to the varying conditions tolerated by each plant. Blueberries for example can tolerate acidic soils at pH 4 without any effect on yield, whereas alfalfa yield is reduced if the pH drops below pH 6.5 [16].

2.50 The linkages between soil quality indicators mean that a critical limit for a particular indicator can be ameliorated or reduced by changes to other indicators [16]. In selecting threshold values it is therefore appropriate to select a particular environment, with the values of the various soil quality indicators of this 'baseline' environment forming the critical limits for any assessment.

[7] defined as the desirable range of values for a selected soil indicator that must be maintained for normal functioning of the soil ecosystem (Arshad and Martin (2002). Identifying critical limits for soil quality indicators in agro-ecosystems. *Agriculture Ecosystems and Environment,* 88(2) p153-160).

Figure 2.1 – Representation of overall view required when addressing the potential effects of changes in soil quality. (Adapted from Arshad, 2002 [16])



Selection of the baseline environment

2.51 The baseline environment selected must be appropriate for the impacts being investigated. It does not need to represent an 'ideal' environment against which only negative effects can be realised, and should ideally be well characterised with a good database on information on the soil quality indicators and their natural fluctuations. Burger and Kelting (1999) [17] stated that threshold may best be set at levels equivalent to those that occur naturally for the particular region or site.

2.52 The purpose of this report is to assess the impact of GM crops (in agricultural, silvicultural or phytoremediative applications). Because of the differences in the function of soils used for agriculture, forestry or phytoremediation it is appropriate that they are assessed separately, with different baseline data sets and different threshold values. It may though be more appropriate to focus on the identification of biological baseline components such as keystone species, rather than abiotic characteristics as these exhibit high variability within soils. The use and suitability of different biological indicators is discussed in the following chapter.

3. REVIEW OF SOIL ECOLOGY AND THE INFLUENCE OF PLANTS

3.1 The purpose of this chapter is to review the biological component of the soil ecosystem, and in particular to review the role of plants within the soil ecosystem and the types of effects that they can confer on both soil organisms and soil processes. Also discussed in this chapter are the issues of diversity and redundancy within the soil ecosystem, and whether changes to the diversity of organisms present within the soil are important if the soil continues to function as required.

3.2 As with the previous chapter, the purpose of this Chapter 3 is to provide the background against which an assessment of how plants (and specifically how do GM plants) effect soil ecosystems.

3.3 It is recognised that to date (2004) a significant body of research has been conducted into soil ecosystems, the various organisms involved and their particular functions and interactions. The purpose of this report is therefore not to review all of this information but to provide an overview of the soil ecosystem, against which any impact of GM crops can be assessed. (A more detailed review of soil ecology and plant soil interactions, including a comprehensive review of the physical, chemical and biological properties of soil, is provided by Killham (1994) [30], and this text is recommended if further information is sought in this area).

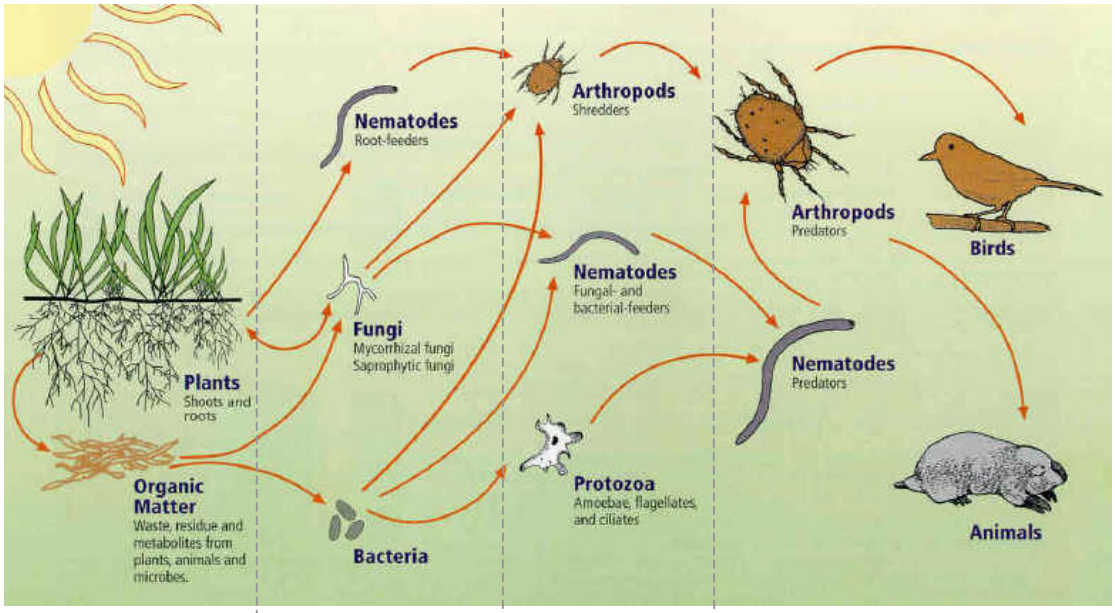
3.4 With respect to the impact of GM plants on the soil ecosystem, the key consideration is whether the effect(s) actually matter(s) in terms of the overall functioning of the ecosystem. Changes as small as growing a different cultivar of the crop have been found to affect components of the soil ecosystem, irrespective of whether the plant is genetically modified or not [31, 32]. Studies investigating the effects of traditional breeding showed that rhizosphere microflora characteristics can be changed both qualitatively and quantitatively by substituting $^{1}/_{21}$ of the genetic information from one variety to another (Neal *et al.* 1970; cited by [33]). Therefore the issue in determining the potential impact of GM crops is not whether any changes in the soil ecosystem can be measured, but the significance of these changes with respect to the functions and ultimately the quality of the soil.

3.5 Therefore, the introduction of a GM crop will have some impact on the soil ecosystem, purely because of a different plant being grown. Changes to just one or two genes may though mean that non-specific/indirect effects on the soil ecosystem are lower than those caused by changing the species of crop. However, the important consideration is whether the impact is significant, i.e. is greater than the fluctuations in soil ecosystem parameters observed during the cultivation of the non-GM equivalent.

3.6 The interaction between plants and the soil ecosystem is a two way process, with plants both influencing and being influenced by the soil in which they are grown. Whilst the objective of this report is to assess the effect of GM plants on soil

ecosystems, and therefore the effect(s) that plants have on soils, it is also recognised that if a crop has an impact on the soil ecosystem then cultivation of that soil in subsequent years may be affected.

Figure 3.1 – Soil food web illustrating basic interactions between the various groups of organisms present.
(Adapted from diagram produced by United States Department for Agriculture)



1st trophic level: Plants as the primary producers and drivers of the soil ecosystem.	2nd trophic level: Decomposers, mutualists, pathogens, parasites and root-feeders.	3rd trophic level: Shredders, predators and grazers	4th and higher trophic levels: Higher level predators

Glossary of technical ecology terms used

3.7 A number of technical ecology terms are used in this chapter to describe the role of plants and how they influence the soil ecosystem. It is viewed as appropriate to define these terms here:

- *autochthonous* – describes an organism with more stable, specialised growth characteristics, i.e. slower growth rate, only able to use a limited number of growth substrates and requiring specific stable environmental conditions.
- *biodiversity* – the biological heterogeneity of a system [34].
- *dominance* – where one organism or group of organisms accounts for the majority of the organisms present in that environment.
- *ecosystem* – an ecological community together with its environment, functioning as a unit.

- *evenness* – the degree of similarity of characteristics exhibited by different species.
- *(soil) function* – what the soil does.
- *functional diversity* – refers to the range of functions present.
- *functional redundancy* – refers to where a particular function is provided by multiple sources within a system. The loss of a source of that function will therefore not cause that function being lost from the system. The source may be a gene, a plasmid or a species. The term 'species redundancy' may sometimes be used instead of functional redundancy where different species fulfil the same function or role in a single ecosystem. The loss of one or more of those species from the ecosystem will cause no concomitant loss of ecosystem function.
- *K-selective conditions* – promotes growth of autochthonous organisms.
- *nitrogen fixation* – conversion of nitrogen (N_2) to ammonia (NH_3) by nitrifying bacteria.
- *oligotrophic* – nutrient poor environment.
- *OTU* – operational taxonomic unit.
- *resilience* – the ability of the soil to return to the state prior to the disturbance [35].
- *rhizodeposition* – the deposition of organic carbon from living root systems to soils, including compounds lost through root exudation, sloughing of dead cells during root growth, and fine root turnover [36].
- *rhizosphere effect* – collective term for the intimate influences of plant roots on soil organisms and biological processes [30].
- *r-selective conditions* – promotes growth of zymogenous organisms.
- *species richness* – the number of different species present in a community.
- *zymogenous* – describes an organism with opportunistic growth characteristics, i.e. rapid growth rate, ability to utilise a range of growth substrates and tolerable of a range of environmental conditions. Such organisms are characteristic of changing 'unstable' environments.

HOW PLANTS INFLUENCE SOIL SYSTEMS

3.8 Any impact of a GM plant will ultimately have been caused by some influence of the plant on the soil ecosystem. Plants influence soil systems through a range of interactions in which the activities of the plant(s) modify soil systems which in turn modify the conditions for plant growth. The key interactions are rhizodeposition (the deposition of organic carbon from living root systems to soils, including compounds lost through root exudation, sloughing of dead cells during root growth, and fine root turnover [36]), the deposition of plant litter, water exchange, and gas and nutrient exchanges. These interactions have an impact on the physical structure, composition and biological activity of soils. The intimate influences of plant roots on soil organisms and biological processes are known collectively as the 'rhizosphere

effect', and have an important role in the formation and activity of soil systems in the proximity of root structures [30, 37, 38].

3.9 A key effect of plants on soil systems is the supply of carbon into the soil through rhizodeposition and plant litter. This effect means that plants have a major influence on the microbial community (the primary decomposers in the soil) which are themselves fundamental to many soil system functions such as nitrogen cycling, decomposition of wastes and mobilisation of nutrients.

Plants are drivers – plant root exudates and plant litter

3.10 The supply of organic material from plants is crucial to soil microbial communities whose growth is carbon limited. The type and amount of nutrients released will affect both the numbers of microorganisms and their diversity. This primary carbon supply to the soil system arrives through plant litter and more directly from roots (through a variety of processes collectively described as rhizodeposition). These include the release of plant exudates, many of which appear to be simply lost by leakage from the root.

3.11 Plant exudates contain carbohydrates, amino acids, organic acids, lipids, hormones, vitamins and enzymes. Some polysaccharides are actively secreted by the plant through systems involving energy expenditure. Lysates are also released by the autolysis or bacterial colonisation of older root cells (often sloughed). Mucilages are released from root cap cells, some epidermal cells such as root hairs and by degradation of the walls of dead cells. These mucilages together with bacteria, their metabolic products, colloidal minerals and other organic matter combine to produce the mucigel, a gelatinous layer on the root surface.

3.12 The mucigel may fade into or have an abrupt boundary with the soil. The rhizosphere influence from fine roots will reach beyond the mucigel, and may extend between one and two millimetres into the soil. With larger roots however the rhizosphere influence may extend several centimetres or be continuous in densely rooted soils.

3.13 Bacterial densities decline with increasing distance from the rhizoplane (root surface) as the exudates are exhausted. Bacteria often occupy only four to ten percent of the root surface, with aggregations of bacteria forming at sites where there are sloughed or dead cells or at some cell junctions which are probably sites of exudate leakage. Cells of the roots cortex and vascular tissue are also colonised by non-pathogenic bacteria [39-41]. Bacteria have been identified in intercellular spaces, inside dead cortex cells and in the cytoplasm and tonoplasts of healthy cortex cells. These colonising bacteria are taxonomically diverse and have been observed to be common soil-rhizosphere types. Further information on root exudates, mucilages and the

rhizosphere is available in the reviews of Walker *et al*., (2003) [37], Killham (1994) [30], van Elsa *et al*., (1997) [42], Whipps, (1990) [43] and Curl and Truelove (1986) [38].

3.14 The quantification of root exudates, and therefore an assessment of whether levels vary between plants, has proven a technically difficult task, especially differentiating between plant root and bacterial activity. Comparison of results from different studies is also difficult due to bias of the methods chosen [44]. Estimations of the carbon fixed in photosynthesis that is then released as exudates vary with crop and conditions. Levels are typically five percent of photosynthate but can be considerably higher (30 percent) [43]. The rapid incorporation of ^{13}C-labelled photosynthate in soil DNA and RNA revealed higher rates of microbial RNA turnover (~20 percent day^{-1}) and ^{13}C residence time (15-20 days) in the microbial RNA fraction, verifying the rapid passage of C fixed in photosynthesis from the plant to soil microorganisms and on to the atmosphere as respired CO_2.

The rhizosphere effect

3.15 The rhizosphere effect is the most immediate influence of plants on soil systems. It has been observed in studies comparing the conditions and communities of root free soil and adjacent soil associated with (and influenced by) roots, and has three main components:

(i) Higher densities of root soil-associated microbial populations. Microbial numbers are elevated considerably in the rhizosphere [30, 38, 45] and this is expressed as the count ratio (R/S) of rhizosphere soil to root-free soil [46]. Bacterial R/S values may be as high as 100 although values of 3 to 24 are typically reported for a range of crop plants [47, 48];

(ii) Increased metabolic activity of rhizosphere bacteria. These have been shown to have higher growth rates and respiration rates [49] and be more metabolically active (as determined by reduction of INT[8] [50], and exhibit larger cell size (up to 5.4 mm for example in studies with barley roots) [51].

(iii) A qualitative change in bacterial diversity. Bacteria isolated from root-associated bacterial communities tend to have higher proportions of Gram-negative, nonsporing, and rod-shaped bacteria; whilst bacterial communities in root-free soil are characterised by Gram-positive, nonsporing rods and cocci, pleomorphic rods, and aerobic spore-forming bacteria [38].

[8] 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride

3.16 Qualitative changes in bacterial diversity are a consequence of the different conditions encountered in the rhizosphere relative to the wider soil environment. Generally, soil is a nutrient-poor (oligotrophic) environment where spatial and temporal discontinuities in the distribution of energy substrates, including the rhizosphere, provide opportunities for opportunistic (zymogenous) bacteria (rather than stable or specialised (autochthonous) organisms). Information from culture-based studies shows that bacterial communities in close association with roots have increased proportions of *Pseudomonas*, *Flavobacterium, Bacillus* and *Streptomyces,* while in bulk soil *Arthrobacter* have been reported to dominate [52-54]. During development of the plant's root structure from seedling to mature root the dominance of the zymogenous groups of bacteria over the autochthonous types has been found to decline [38, 55, 56]. This reduction probably reflects a transition from so called 'r-selective' conditions (food supply is improved and niche opportunities expand with plant growth) to K-selective conditions (population density approaches the carrying capacity of the environment) [57].

3.17 The rhizosphere effect has been found to be strongest generally amongst unicellular bacteria followed by actinomycetes, then fungi, then protozoa and algae and then the microfauna [47, 52]. This means therefore that changes in the rhizosphere effect are likely to have a greater and more immediate effect on bacteria rather than on protozoa or algae.

Affects of plants on the decomposer microflora biomass

3.18 The linkages between plants and soil microflora mean that increases in plant activity are associated generally with improvements in both microbial biomass and activity. Studies with plant microcosms have shown that in addition to reducing the growth of the plant, shading of the plant seedlings also reduced below-ground carbon allocation and the microbial biomass [58].

3.19 Despite the influence of the plant and rhizosphere effects there is evidence that bacterial levels in soil are frequently observed to be "top-down" regulated by predation (i.e. bacterial numbers are controlled by their predators). Wardle *et al*., (1995) [59] reported a field study of changes in the decomposer food-web over a three year period when the system was supplemented with sawdust as an additional utilisable carbon source. The carbon supplement resulted in a prolonged increase in soil fauna without an increase in bacterial biomass indicating predative regulation of this bacterial community. The top down regulation of bacterial populations can limit the utility of measuring bacterial numbers and biomass as a means to detect the impact of the genetic modification of a plant.

3.20 Fungal populations, by contrast, are more typically regulated by fungal competition for substrates and antagonistic chemical conflicts [60, 61], although grazing of fungal hyphae by microfauna may also regulate fungal populations.

3.21 Regulation of the fungal biomass through substrate competition and chemical conflicts is more likely to reflect the plant activity, with increases in plant activity resulting in increased fungal biomass for example. In upland grasslands Bardgett *et al*., (1999) [62] found that microbial biomass and activity were more affected by changes in plant species composition and dominance than by the addition of nitrogen (N). The affects of N supplements (and the consequent increase in plant productivity) on microbial biomass were found to be inconsistent, indicating the lack of a simple relationship between plant productivity and microbial responses. The study concluded that 'the functional characteristics of dominant plant species are important determinants of soil biological properties, and hence ecosystem functioning in temperate upland grasslands'. Plant productivity therefore has an impact on soil processes but the effect(s) caused are not straightforward to predict. The influence of plant productivity on soil processes is an important issue which is considered further below.

Affects of plants on the soil fauna biomass

3.22 Whilst the relationship between increases in plant productivity and microbial biomass have been found to not be straightforward, increased plant productivity is assessed to have some effect and increase the microbial biomass and/or turnover. Consequently, soil fauna populations should also increase with increasing plant productivity (as the soil fauna occupy the trophic levels above the microorganisms).

3.23 However, in reviewing the relationship between soil fauna and plant productivity, Wardle (2002) [61] reported mixed responses of soil fauna to plant productivity. "Bottom-up" regulation (availability of primary nutrients) of the soil fauna has been observed where the experimental addition of glucose to soils resulted in increases in a variety of soil fauna. This method was used in a limestone soil by Scheu and Schaefer (1998) [63] to manipulate microbial biomass and study the response of the fauna. Increases in both microbial biomass and in the biomass of earthworms were observed. Another study of the bottom-up influence of plants on soil fauna followed changes in microbial activity and populations of protozoa and nematodes in decomposing barley root material, and in soil fractions with increasing distance from the root material [51]. These results demonstrated distinct successional patterns in the microbial food web with a sequence of population developments from microorganisms to protozoa and nematodes. Invertebrate detritivores can be expected to be even more directly influenced by the availability of plant material in soil.

3.24 The mechanism of faunal population regulation can though be complex. Chen and Wise (1999) [64] have demonstrated substantial bottom-up limitation of a detritus-based food web through the increased populations of predaceous arthropods in response to increases in prey arthropods. The increase in prey arthropods occurred in response to experimentally supplementing their resource base with mushroom, potato, and fruit fly medium. Nonetheless, the soil fauna seem to be often regulated by predation [61, 65, 66].

3.25 The relationship between plant activity, microbial biomass and soil fauna is multifaceted and often does not follow straightforward patterns. Soil moisture levels, for example, can change patterns of selection and predation with drying conditions progressively reducing the activity of types of predators. As with the microflora, the soil fauna population levels and activity will be influenced by bottom-up (plant and prey activity), top down (predation) and abiotic factors (such as soil moisture).

Conclusions on the affects of plants on the decomposer microflora and the soil fauna biomass

3.26 The influence of plant productivity on soil processes is an important issue [61, 67, 68]. Plants play a key role in soil systems providing the major source of organic carbon. It is to be expected therefore that changes in the plant community or in plant activity (especially plant productivity) will affect changes to the soil system. The carbon flow from plants provides the major substrates for detritivores and decomposers present in the soil.

3.27 However, plant productivity does not play a straightforward role in regulating the soil biota and it is necessary to integrate both bottom-up and top-down processes to any assessment made. The question of whether soil trophic levels are more regulated by resource limitation (including competition) or by predation is a complex one and has been reviewed by Wardle [61]. This review reports a range of responses by both primary consumers (bacteria and fungi) and secondary consumers (those feeding on bacteria and fungi) to net primary production (NPP). Increased plant productivity (and therefore greater organic carbon flow to the soil system) is not observed to result in a consistent response in the primary consumer biomass or the secondary consumer biomass, as these have been observed to rise, fall and be neutral in a variety of different contexts. In considering this Wardle observed that in the complexity of soil systems it is not possible to vary NPP experimentally while holding all other factors constant and that these and the context may have significant interaction affects. These interactions, such as plant-microflora competition for resources, may counter some of the benefits expected to microflora and fauna from increased NPP.

3.28 Nutrient cycling by microbes may also be more specifically affected by plants through the release of organic substrates in response to conditions including browsing by herbivores. These substrates may include signals affecting bacterial gene expression, stimulating the release of exoenzymes and promoting the release of nutrients via protozoan predation [67].

3.29 This complexity in interactions is important to this review of the impact of GM plants on soil ecology. Clearly, assessing impact just by tracing causal links between changes and effects, or by relating observed changes to mechanisms, may be prohibitively complicated. Furthermore, extrapolations of potential impacts from observed causes, effects and impacts may be especially difficult.

3.30 There is no doubt from the studies conducted to date (2004) that plants are the major drivers in soil ecosystems, and that the relationship between plant production and microbial biomass and activity is complex. Key to these systems are the mineralisation and cycling of nutrients. Therefore, understanding the effect different plants and plant activity have on microbial mineralisation of N and other nutrients is also important in determining the effect(s) of plants on soil ecosystems.

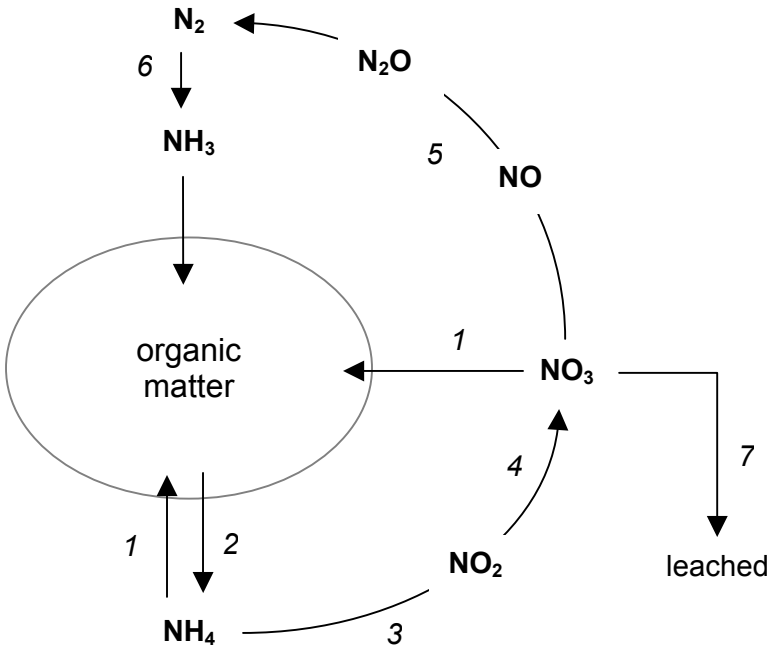
3.31 Plant influences on nutrient cycling are mediated by a myriad of interactions in which the health (success) of the system is often judged by the resulting conditions for plant growth (fertility). The issues raised with respect to the relationship between the N-cycle (and also the phosphorus and sulphur cycles) and plant activity extend to a variety of nutrients which are recycled via mineralisation in soil systems where both generalist and specialist microbial populations play key roles in resolving the availability of these essential nutrients.

ROLE OF PLANTS IN SOIL SYSTEMS

Plants and the nitrogen cycle

3.32 Nitrogen is a key requisite of life and is used in agriculture as a common fertiliser supplement necessary to maintain high levels of soil fertility. In addition to animal waste, plant residues and othe organic inputs, nitrogen enters the soil ecosystem when atmospheric nitrogen (N_2) is fixed as ammonia (NH_3) by specific bacterial populations. Nitrogen fixation is a reductive energy intensive process and nitrogen-fixing bacteria are often associated with plants that can provide energy and fixed carbon. Further, ammonia may be oxidised by nitrifying bacteria to nitrite (NO_2^-) and on to nitrate (NO_3^-). This nitrification of ammonia means that the soluble nitrate is more readily available to plants and microbes but is also more likely to be lost from soil by leaching, thus decreasing the available fixed nitrogen.

Figure 3.2 – Nitrogen cycle in soil or aquatic habitats. (Adapted from notes from the University of Edinburgh, Institute of Cell and Molecular Biology)



(*1*) Uptake of NH_4 or NO_3 by organisms; (*2*) Release of NH_4 by decomposition; (*3*)(*4*) Microbial oxidation of NH_4 (yields energy in aerobic conditions); (*5*) Denitrification (NO_3 respiration) by microbes in anaerobic conditions (NO_3 is used instead of O_2 as the terminal electron acceptor during decomposition of organic matter); (*6*) Nitrogen fixation from the atmosphere; (*7*) Nitrate leaching from soil.

3.33 Although agricultural supplements and the fixation of new nitrogen are important, a key aspect of soil fertility is the cycling back of fixed nitrogen from wastes (plant and animal) to forms which are available to plants. In this mineralisation process nitrogen in organic wastes is liberated as ammonium by a diverse community of microbes and animals.

3.34 In soil, ammonia and nitrate may be taken up and utilised by plants or microbes. Bacterial assimilation of NH_4^+ or NO_3^- is called immobilisation. Alternatively, denitrifying bacteria may use the nitrate as an electron acceptor in anaerobic respiration. This involves the reduction of nitrate to nitric oxide (NO), to nitrous oxide (N_2O) and on to N_2. The release of these gases depletes the fixed nitrogen of the soil system. This denitrification happens when soils or parts of soil systems become anoxic (low oxygen availability).

3.35 The carbon to nitrogen (C:N) ratio of plant rhizodeposition is an influential factor in N cycling as low ratio values result in N-limited microbial growth and increased microbial immobilisation of N. Plants, through rhizodeposition and other plant-soil exchanges, also have a significant effect on the diversity of the microbial community

and its behaviour. Paterson (2003) [67] reviewed the coupling of plant productivity and nutrient cycling, and identified a number of mechanisms by which plants can influence nutrient mineralisation by soil microbes, and by which microbes can influence plant productivity.

3.36 Changes in plant species, and potentially in the introduction of GM varieties of the same crop, will therefore result in altered plant-soil interactions which affect the mineralisation of N and other nutrients by soil microbes. A key issue raised by Paterson (2003) [67] is to understand how plant driven changes in the microbial community influence soil-system function. This is considered later in this chapter.

Plants and the phosphorus cycle.

3.37 As with nitrogen, the soil system recycles phosphorus (P). This compound is essential for life and taken up by plants (and microbes) from soil as phosphate (PO_4^{3-}). Although mineral phosphorus enters the soil system as phosphates from geological sources, the recycling of phosphorous by decomposition of plant material, animals and wastes is important for soil fertility. Even with the addition of phosphate-based fertilisers, the majority of phosphorus used by a crop (70-90 percent) will be derived from soil reserves.

3.38 The availability of phosphates may be limited or stimulated by a variety of factors including microbial immobilisation, plant uptake, immobilisation on plant residues, release from organic wastes, temperature and weathering of mineral sources. The bioavailability of P is greatest when soil pH is just below neutral and declines with increasing or decreasing pH. Plant access to phosphates may be limited by their poor solubility. Mycorrhizal plants benefit from a symbiotic relationship with a root infecting fungus which can scavenge phosphates that are too far from the roots and otherwise unavailable. This activity is especially important in drought conditions where low water availability limits the movement of phosphates through the soil. Differences between plants, including GM plants, will affect the phosphorus cycle by stimulating different levels of microbial activity, by promoting specific mycorrhizal associations and by root-specific scavenging of phosphorus.

Plants and the sulphur cycle

3.39 Sulphur like nitrogen and phosphorus is required for life. It is available in soil to plants and microbes mostly as sulphates (SO_4^{2-}). Sulphates arrive in soil through soil minerals, atmospheric deposition, fertilisers and pesticides. As pollution has declined, sulphur inputs too have declined, and sulphur deficiency in British soils has emerged as an issue.

3.40 In soil the various reduced forms of inorganic sulphur are oxidised to sulphate by a variety of microbial populations including heterotrophic bacteria, fungi, actinomycetes and chemoautotrophs (notably the thiobacilli). This variety probably accounts for the soil to soil variability in factors affecting the availability of sulphate [30]. Whilst inorganic sulphur is readily oxidised, the mineralisation of organic sulphur from plant and other wastes is more typically limiting. Many microbial populations participate in the mineralisation of organic sulphur. The rate of mineralisation is therefore particularly dependent on the conditions for their growth, which, as has been discussed will be influenced strongly by plant type and activity.

3.41 Sulphate may be lost from the system through anaerobic respiration and reduction to hydrogen sulphide. This is limited to anaerobic and therefore to wet soils. The bacterial populations involved are heterotrophs (notably the genera *Desulfobacteriaceae*, *Desulfovibrionaceae* and *Desulfovibrionaceae*). Plants, through rhizodeposition, will directly influence the activity of these Gram-negative sulphate reducers which are dependant on the supply of organic carbon for energy.

Plants and soil pH

3.42 Soil pH is an important property of a soil as it influences many activities. The pH of a soil affects the solubility and therefore availability of many nutrients needed for the growth of plants and microbes. Neutral to slightly alkaline soils are favourable for dissolving many nutrients while a lower pH (acid conditions) can create an environment that is toxic to plants by mobilising and increasing the bioavailability of aluminium, copper and manganese. The pH of a soil has a major influence on the microbial decomposer populations and plant types that will flourish. Acid conditions reduce primary decomposer activity and select for acid tolerant plants such as conifers.

3.43 Acidification of soils can arise from field management practices through the conversion of ammonium from fertilisers to nitrate (nitrification process) generating H^+ ions. When fertilisers are applied to excess, the H^+ ion load can no longer be naturally neutralised in the soil and begins to accumulate resulting in the formation of more acid conditions.

3.44 Soils are usually characterised by a simple pH. However, the soil particles, water, substrates and microbes are heterogeneously distributed through this complex matrix in which pH can be seen as a 'local' phenomenon [69]. The microsite pH is affected by the distribution of microbial activity, clay minerals and organic materials which buffer hydrogen ions, and sites of root activity. The take-up of nutrients, metals, nitrates and ammonia involves the root in ionic exchanges with the soil. The uptake of ammonium (NH_4^+) for example involves root proton pumps releasing H^+ ions and acidification of the local immediate rhizosphere. Changes in plant variety will affect

the microscale pH processes and plant associated changes in field management (fertilisers) can affect changes in soil pH with a variety of consequences for soil fertility and soil system function.

BIODIVERSITY IN SOIL SYSTEMS

3.45 Diversity, which includes both the species present and the community structure, is both a key concept and focus for ecologists, and is an important issue that must be understood and addressed when assessing the impact of plants on the soil ecosystem. Because "diversity" *per se* is regarded by many as a value in its own right and important to protect and preserve, it is important to understand what is meant by diversity and to be able to separate the values of "diversity" and "function". Changes in diversity of organisms in a soil do not necessarily change the function of that soil.

3.46 Many human activities cause a reduction in soil diversity and it is necessary to evaluate these reductions and assess their significance. The issues of the nature and significance of diversity are addressed in the following sub-sections of this report (although it should be noted that much of the following review is based on research which of necessity is limited to methods which are better at detecting change than they are at characterising the communities studied).

3.47 The measurement of soil diversity, especially microbial diversity, has many problems and short comings. These include:

(i) difficulties in making comprehensive inventories;

(ii) difficulties in evaluating relative abundances; and

(iii) a shortage of soils for which comparable investigations have been made.

The high diversity of the soil ecosystem

3.48 The diversity present within soil ecosytems is greater than in any other habitat [61]. The sheer size of this diversity raises question such as 'how it is achieved without species-species competition excluding more species', or 'how can they all have distinct niches'? No doubt, a part of the answer lies in the spatial and temporal heterogeneity of the soil habitat. The tremendous spatial heterogeneity resides in the way that even small (µm) distances in a soil crumb or pore can traverse gradients in, for example, oxygen, water and carbon flow, which favour substantially different life styles and therefore different organisms.

3.49 Temporal heterogeneity driven by season and plant development cycles is enhanced by the capacity of much of the soil biota to remain passive when conditions are unfavourable. The diverse nutrient sources available in soil, combined with the spatial and temporal heterogeneities facilitate substantial resource and niche specialisation. Despite considerable progress in understanding this subject area, the diversity of soil systems is still widely regarded by researchers as an important and exciting challenge.

Diversity is influenced by plants and soil management

3.50 As discussed, plants are able (and do) modify the conditions for life in soil. This influence may be direct as with nutrient exchanges with the soil system, or less direct as with cultivar associated changes to field management practices. The literature contains numerous examples confirming that the diversity (species present and community structure) of microbes in soil communities is influenced strongly by plant species and a wide variety of soil variables. Factors observed to result in detectable differences in the composition and structure of soil communities include plant species [70, 71], root zone [72], water stress [73], fertilisation [74], field management [75], tillage [76], fungal disease [77], plants and field management [78], grassland improvement [79], nitrification [80] and soil depth [81].

3.51 The diversity of soil fauna are also influenced by soil and plant factors. The protozoa for example, are known to be sensitive to a variety of factors including acid rain, fertilisers, biocides and heavy metals [82]. Soil invertebrate diversity and counts have been shown to be reduced in pesticide free cornfields compared with conventionally farmed soils. The diversities of soil microarthropod and soil nematode communities are sufficiently sensitive to environmental factors to encourage their use as bioindicators [83-85]. In these communities changes in diversity may be assessed at the taxonomic level or by characterising the relative abundances of functional types.

3.52 The density and diversity of soil invertebrates have been shown to be reduced by the intensification of crop management with changes in the relative abundances of springtail saprophages, mites, predators and crop feeding invertebrates [86]. As with the microbial community the soil fauna are engaged in complex interactions with the biota of the soil and rhizosphere. Understanding the relationships observed between roots and soil microbial communities involves recognition of the influence of the soil fauna on rhizosphere microbial populations and plant growth [87].

3.53 It should be noted that the effects of plants on soil microbes are frequently evaluated in the rhizosphere where plant soil interactions are most intimate. Individual plant species or cultivars planted in a common soil do develop rhizosphere communities with differing diversities. While this habitat is most sensitive to plant influences there

is a shortage of research evaluating the effects of plants or cropping regimes on the total soil diversity and its spatial distribution in the local system. A key factor here is evaluating the contribution of dispersal to local soil microbial diversity, which is largely unknown [88]. Whilst it is not clear at what point a plant community or crop will change the diversity pool, there is some evidence of soil communities recovering common diversities when a variety of perturbations are removed. These studies are reviewed in Chapter 4 (Review of the Impact of GM Crops).

3.54 Transgenic (GM) crops will often require associated changes in field management, such as different pesticide or fertiliser treatments and variations in tillage requirements. These changes are likely to affect the diversity of the soil biota. For example, studies by Webster *et al.* (2002) investigated the effects of N fertilisers on populations of bacterial ammonia oxidiser populations in grassland soils [74]. The studies compared unimproved and semi-improved grassland pastures and found that the diversity of ammonia oxidiser populations was greatest in the unimproved soils which were dominated by bacteria from two *Nitrosospira* clusters and one *Nitrosomonas* cluster, compared with improved soils which were dominated by bacteria from one *Nitrosospira* cluster.

The role of diversity in ecosystems is controversial

3.55 Biodiversity has become a more prominent issue through the increased awareness of the effects that human activity, especially changing land use have on it [89]. In soil and other systems, the questions have been posed as to whether (or when) ecosystems will be undermined by declines in the diversity of organisms present within them, or whether the function of the soil, as determined by the activities of all the organisms as a whole, is more important. The dichotomy of these two issues means that the role of biodiversity in soil ecosystem function is controversial [90, 91]. Some studies for example have indicated a strong role for diversity in ecosystem function [92], whereas others have reported that soil function can be maintained with a relatively low diversity of species present [91].

3.56 Following the experimental manipulation of diversity, Naeem *et al.* (1994) [93] found evidence that a reduction in biodiversity would alter ecosystem performance. In a study at eight European field sites, Hector *et al*., (1999) [94] attempted to assess the role of plant diversity on the soil ecosystem by comparing grassland community plots each containing different numbers of plant species. The study found:

(i) an overall log-linear reduction of above ground biomass with loss of species; and

(ii) that for a given species richness (number of species), productivity declined as the number of functional groups in the plant community was reduced.

3.57 In long-term grassland experiments Tilman *et al*. (2001) [92] reported maximal productivity and carbon stores in high diversity plant communities. However, it has generally proven difficult to assess whether the association of diversity and ecosystem function may be related to:

(i) species-diverse communities exploiting more opportunities and cooperating in ways unavailable to monocultures, or

(ii) whether a relatively small number of species constitute the requisite functional diversity [91].

3.58 Communities with high diversity and species redundancy are expected to be more efficient in maintaining systems when challenged with change [95], with the species redundancy acting as a form of insurance against unfavourable or changing conditions [96]. Species redundancy is observed in many ecosystems and occurs when a number of species may fulfil the same ecosystem roles such that some species may be lost without detrimental affects on the system as a whole. The species redundancy described here is a form of functional redundancy where the ability to conduct function 'X' is held by several different species within the ecosystem.

3.59 There is consensus 'that at least some minimum number of species is essential for ecosystem functioning under constant conditions and that a larger number of species is probably essential for maintaining the stability of the ecosystem process in changing environments' [91].

The role of diversity in soil systems

Poor support for diversity-function relationship

3.60 Diversity is often considered to be a positive attribute and many studies have been conducted to investigate the relationship between diversity in the soil biota and the various functions of the soil system. There is though a lack of reports supporting a diversity-function causal link among the soil communities and it has been proposed that this is because there is no causal mechanism linking these two characteristics [97]. The role of biotic diversity in soil systems has been reviewed by Wardle [61], Bardgett [68] and Nannipieri *et al*. [98].

3.61 Generally, reviewers have concluded that there is 'no predictable relationship between diversity and function in soils' and that species richness is not an important factor in the overall function of the soil [68, 99]. It has been observed that the extinction of many nematode species in highly disturbed soils for example does not affect the decomposition process in the soil (in which nematodes are known to play a

key role) [100]. This suggests that a high diversity of nematode species is not required to maintain the decomposition function of that soil. Other observations have shown that reduced functional diversity is not consistently associated with reductions in activity such as decomposition, which also indicates the lack of a causal link between diversity and function [60]. Although plants are identified as the primary drivers in soil systems, there is no evidence to suggest that a relationship between diversity and plant productivity exists [61], and whilst agriculture is generally associated with causing declines in the diversity of soil fauna this is not consistent in a number of studies such as those with worms [101].

3.62 Diversity however continues to be a major focus of soil ecology and it has been proposed that the identification of the connections between genetic diversity, community structure and soil system function remain a key issue [70]. Despite the lack of a relationship between diversity and soil function, and the ease with which differences in microbial diversity can arise, the possibility remains that there are underlying common trends in the establishment of microbial communities in soil systems. This has been investigated by assaying microbial heterotrophic evenness (carbon substrate respiration rates) at different stages in the development of five different soil and plant systems [102]. No general trends were identified with three patterns of change in heterotrophic evenness being observed among the five systems. However, within three of the soil systems the study found significant linear correlations between heterotrophic evenness and basal respiration ($r = 0.52$ to $r = 0.88$) and in one other a near significant correlation ($r = 0.69$. $P < 0.06$) [102].

Ecosystem functions are maintained by communities

3.63 Soil ecosystem services include many important functions performed by bacterial communities. These functions and services are necessary for the continuing sustainability of the soil ecosystems and the uses which are made of soils. While it is possible to identify populations both carrying and performing (*in situ*) key functions, their presence and activity will be dependent on the context of the wider community. Further, the growth of microbial communities in biofilms, on surfaces and in semi-solid habitats frequently exhibit stages of community accumulation marked by distinct succession and interaction. There is increasing evidence that these interactions generate community structures which are optimised for ecosystem function [103]. These interactions, including cross feeding, context dependent-gene expression and cell-cell signals, can be expected to affect community structure and stability. Further, soil microbial communities are dynamic affairs responding to changes in their habitat and continually adapting to new conditions. These changes to community structure and community physiology represent the interplay of:

(i) the plasticity of bacterial expression,

(ii) the fortunes of individual populations,

(iii) inward colonisation from a variety of levels of dispersal [104], and

(iv) community responses (interactions).

3.64 Given the very high bacterial diversity and complexity of interactions performing numerous functions in soil ecosystems, it is often not appropriate to measure or assess impact on a single process or numbers of a single organism. Bacterial diversity is a pertinent component in assays of soil ecosystem function because:

- changes in community structure are a key component in ecosystem function with the potential to indicate perturbations and a wide variety of complex effects.
- bacterial diversity is considered a value in itself, *per se*.

3.65 However these are two important general difficulties which must be considered before reviewing the methods available for:

- the identification of the appropriate units of bacterial diversity;
- coping with very high diversity; and
- relating diversity and function.

♦ *Identifying the appropriate units of bacterial diversity*

3.66 In their review of the bacterial species concept Rossello-Mora and Amann [105] note that generally there is 'agreement that the species concept currently in use is useful, pragmatic and universally applicable within the prokaryotic world'. They suggest that species could be described as `a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics, and is diagnosable by a discriminative phenotypic property'. However the identification of diversity to the species level in soil habitats is a highly 'non trivial' task. This problem arises from the low proportions of bacteria cultured, the very high diversity and the practical difficulties of assigning species classifications to bacteria from a wide taxonomic range.

3.67 The use of ribosomal sequences for establishing phylogeny has removed much of the uncertainty from the characterisation of environmental isolates and facilitated the

extension of sampling to include taxa which are not cultured. The application of threshold levels to 16S rRNA sequence differences is used to define taxa, though it has insufficient resolution at the species level. However, environmental surveys of diversity are greatly facilitated as stable and reproducible operational taxonomic units (OTUs) may be identified.

3.68 An OTU may be defined by specific isolates or at various taxonomic levels including species, genus and family. Species diversity is used throughout this report as a term of convenience when referring to bacterial diversity. In practice however most considerations in this report with regard to bacterial diversity could and should be applied equally to a variety of taxonomic levels defined by OTUs. OTUs defined by specific levels of difference in 16S ribosomal subunit sequence (or RFLP) have become the favoured unit of bacterial diversity for many environmental and ecological studies. This is because the use of 16S sequences for assigning OTUs permits:

- the relatively reliable and reproducible typing of isolates;
- the generation of libraries or community fingerprints independent of culturability; and
- the investigation of community structure.

3.69 When new types or species are encountered this approach has no difficulty in placing them in a scheme permitting some phylogenetic and sometimes phenotypic assumptions. Whether or not 16S is actually the most appropriate level at which to resolve OTUs must depend on the questions to be asked. A difficulty with the 16S OTU is the very high diversity to be sampled and that therefore a large number of bands can be generated from universal primers and may be too many for useful resolution. Any attempt at an assay of general diversity using an even lower taxonomic unit would have to cope with the subsequent analysis of very 'noisy' data. Therefore the use of species or sub-species level assays are more appropriate for targeting specific populations or functions such as that of Phillips *et al*., (2000) [106] who used a competitive PCR method to amplify 16S rRNA genes specific for the beta-subgroup of proteobacterial ammonia oxidising bacteria.

3.70 OTUs based on higher taxonomic levels are appropriate in a number of circumstances, especially the use of *in situ* probes where the physical location or frequency of a specific group within a sample is assayed. Changes in diversity at the genus level or higher can be used to detect and investigate more substantial habitat changes. The question as to whether OTUs should be set at higher levels such as genus or family is discussed below.

- *Coping with very high diversity*

3.71 The problems of characterising and assaying bacterial communities are amplified by the very high diversity observed in soil habitats. DNA based culture independent methods have shown that soil communities, even at small spatial scales such as one gram, contain very high bacterial diversity. This was estimated in one study at 6400-38000 taxa with one tonne of soil therefore containing 4×10^6 taxa [107] Even reasonably complete species (or other taxa) lists for soil samples are still unrealistic and will probably remain so for some time as tools for use in field studies.

3.72 The classical approach to this problem is to assay and compare community structures using diversity indices, species or OTU richness (adjusted for comparable sampling efforts), or distributions as in collectors curves, rank abundance curves and rarefaction curves. These structural assays of diversity are suitable for bacterial communities and some measures such as diversity indices may be applied to a variety of assay methods including CLPP, identification of isolates and DNA based methods [108, 109].

3.73 Two important questions are:

(i) how well does a method assay/inventory the diversity present; and

(ii) how representative are the samples

3.74 All sampling methods whether DNA based (cloning, annealing and PCR), culture based, or CLPP based will introduce their own sampling biases limiting the capacity of these methods to make absolute statements about community diversity. The biases and limitations associated with a number of these methods are considered below. Further, there may be concern that changes in bacterial diversity are sensitive indicators of perturbation and are misleading by looking at the predominant groups but ignoring the underlying population. That all these measures address only a portion of the community may not be quite the problem it first appears. This is because the aim of such assays is to detect changes of ecological significance and these methods will include biases toward populations which are more numerous or more active and thus biasing samples toward populations making a disproportionately elevated contribution to ecosystem function.

3.75 The selectivity of such methods is illustrated in a study [110] which has shown that soil communities inoculated into sterile soils at dilutions from 10^{-2} to 10^{-8} generated communities between which the number of 16S rDNA DGGE bands did not differ significantly (an overall mean of 44 ± s.e. 1.6 bands). Therefore, the removal of a large number of less common types did not affect the amount of diversity that could

be distinguished. However, the biases arising from the different methods should be offset by the inclusion of various and contrasting assays of diversity.

3.76 The high diversity of bacterial communities therefore limits *in situ* studies to comparisons of relative diversity and structure. In complex diverse habitats such as soil it has not been feasible to evaluate the correspondence between diversity indices, estimations of diversity, the underlying models of community structure and the actual structures. When plotted, samples with very high diversity will generate steep near-linear species accumulation curves which are often considered to carry insufficient signal to estimate (extrapolate) the true diversity (though see Lunn *et al*. (2004) [111] for their analysis of samples from hyperdiverse communities (many singletons) where they estimate the probability that a sample could have come from a community with a particular diversity). Samples large enough to generate concave downward curves facilitate the estimation of asymptotes and thus may be used to produce estimates of true diversity. The point has been made that if accumulation curves are found to extrapolate well (cross only infrequently) then bootstrapping to generate rarefaction curves 'may be a valuable way to compare the relative diversity of communities' [109]. Interestingly, while diversity indices are popular in GM impact studies, estimating richness has not yet entered this field.

♦ *Observations:*

- Comparing the structure of communities using relative rather than absolute measure alleviates many of the difficulties associated with species definitions and the inevitable biases of sampling and focus on the dominant rather than the full complexity.

- Different methods (CLPP, PLFA, DGGE, changing substrate or primer targets etc) should be combined to reduce the bias toward limited community components and improve the case for statements made regarding community changes.

- More studies of the structure and composition of microbial communities are needed to develop and test the 'relative comparison' methods which are so convenient.

♦ *Relating diversity and function*

3.77 As we have seen there is a lack of a clear relationship between bacterial diversity and soil system function. There is also scant assessment of the relationship between diversity measures and functional measures such as CLPP. In practice this is difficult to evaluate because suitable (controlled) manipulations of bacterial diversity

are not practical and because high levels of functional redundancy result in weak associations between bacterial diversity and the distribution and activity of functional traits. Further because the various methods (e.g. DGGE, CLLP, PLFA, T-RFLP) are used to generate data analyses of different types (parametric/non-parametric, pattern analysis, principle component analysis) many of the studies using these methods do not test their correlations. Frequently, as we have seen in Section 4 (Review of the Impacts – Specific Effects Caused by Transgenic Plants), these methods show marked differences in sensitivity and appear to address distinct parts of the community or its activity. Where these methods are combined, relationships between them are often weak. A survey of grasslands with different management intensities [79] has compared %G + C content, CLPP and PLFA. These diversity measure were highly correlated with various factors (PLFA with calcium, phosphorus, Sodium, nitrogen and organic matter content and pH; CLPP with sodium and organic matter content and pH; and %G + C content with pH). However, among the three measures a small but significant correlation was only observed between the CLPP and PLFA data. Indicating some detection of similar components of the microbial community.

The role of redundancy

3.78 The poor relationship between diversity and function in soils (as discussed in the previous section) is often attributed to functional redundancy. This is defined as the existence within the soil of a number of species that can each fulfil the same soil function, and that therefore some species may be lost without the loss of that function from the soil [112].

3.79 There is evidence of substantial functional redundancy in the soil biota, both as a whole and also within specific groups of organisms including, bacteria [35], nematodes [100], the beta-proteobacterial ammonia-oxidising bacteria [113] and in microarthropod communities. In the latter group diversity has been observed to be only weakly related to 'recovery from a disturbance' and which were functionally redundant with respect to plant growth [96].

3.80 A number of studies have investigated the hypothesis that soil biota does have a high functional redundancy, and have found indications that substantial thresholds of diversity loss (loss of species) must be achieved in order to impair the function or resilience[9] of the soil [35, 114]. Griffiths *et al*. (2000) [35] used chloroform fumigation to reduce the diversity in the soil studied by up to 40 percent, but found no consistent negative impact on soil system functions of decomposition, respiration, growth, denitrification, nitrification and methane oxidation. Using fumigation and fumigation-reinoculation to manipulate soil biodiversity, Degens (1998) [114] also found that

[9] resilience refers to the ability of the soil to return to the state prior to the disturbance.

reductions in soil microbial diversity did not consistently result in reductions in soil function.

3.81 There is a shortage of experiments manipulating faunal diversity to test the significance of faunal diversity to function. In grassland microcosms Cragg *et al*. (2001) [115] manipulated the diversity and composition of soil fauna within a trophic group (the microbial-feeders) and tested for effects on litter decomposition and nutrient flux. Impacts on these ecosystem processes were found to arise from differences in the composition of the community, rather than the number of species present. These findings supported the notion that changes in the diversity of microbivorous fauna may not have a predictable effect on decomposition process rates, and that the functioning of the microbivorous trophic group is influenced mainly by the physiological attributes of the dominant animal species present.

3.82 In arctic soils manipulations of the soil food web resulted in changes in the composition of the nematode fauna. Estimates of functional properties at the sites (e.g. microbial biomass, plant growth) indicated the existence of considerable redundancy among the nematode fauna [116]. In grassland microcosms Bradford *et al*. (2002) [117] manipulated the soil fauna community composition using body size and found that major ecological measures were significantly affected (plant community composition, microbial and root biomass, decomposition rate, and mycorrhizal colonisation), while others (aboveground net primary productivity and net ecosystem productivity) surprisingly were not. Bradford *et al.* (2002) [117] suggested that positive and negative faunal effects in soil communities may have cancelled each other out, resulting in a lack of ecosystem effects.

3.83 However, whilst the soil ecosystem has been shown to have sufficient functional redundancy to absorb relatively high reductions in diversity, and that therefore losses of soil species may not matter in terms of the function of the soil, soil systems with high redundancy (and therefore high diversity) may be considered, by definition, more resilient when challenged. Such challenges may include physical perturbations (ploughing for example), addition of pollutants (pesticides) or nutrients (fertilisers) or increased predation. Therefore, it is the resilience of the system, rather than its diversity or function that some have proposed is a valuable characteristic of the soil ecosystem and should be conserved.

Diversity, functional redundancy and resilience

3.84 The role of diversity in soil function has been investigated with experiments to assess the impact of reductions in biodiversity on soil function. As discussed above, Griffiths *et al*. (2000) [35] found that considerable reductions in diversity produced no consistent trends in a range of soil functions (respiration, decomposition, denitrification, growth and methane oxidation). Notably, however, the reductions in

diversity did reduce the resilience of the soil when challenged with added copper or heated to 40 °C. In a further study Griffiths *et al*. (2001) [2] added communities from a non-sterile soil into samples of the same soil that had been sterilised. The inoculant samples were serially diluted to reduce diversity. As with the other studies, considerable reductions in biodiversity (15%, 40% and 60%) produced no correlative changes in soil function or resilience, demonstrating no consistent relationship between biodiversity and soil functions, including resilience.

3.85 An investigation of the effects of cropping-associated reduced catabolic diversity found that such reductions and land management associated changes in soil properties could reduce the resistance of microbial communities to challenge [118]. The effects of the soil challenges caused by reducing pH, increasing salinity, heavy metal contamination and wet-dry or freeze-thaw cycles, were assayed against changes in the catabolic evenness. Catabolic evenness was calculated from the soil respiration rates for a range of simple carbon substrates. Interestingly this report regarded the greater declines in catabolic evenness that were observed in the challenged crop soil as evidence of reduced resistance to stress or disturbance. In practice, changes in structure may also reflect the exploitation of opportunities by specific populations and do not anyway confirm the loss of the relevant populations from future responses by a soil system.

3.86 This view of functional redundancy does not exclude the key role of specific or small groups of populations in specific functions. An example of this was reported by Laasko (1999) [65], who in forty week experiments manipulated the soil fauna in forest soil birch seedling microcosms, and like others, found that plant productivity was not affected by reductions in species or trophic group diversity. However, loss of the microbe and detritus feeding *Cognettia sphagnetorum*, reduced plant N uptake and accumulation of plant biomass.

3.87 While the points discussed in these preceding paragraphs suggest that diversity *per se* is not suitable as a simple divining rod of soil quality, understanding the causes of diversity and changes in diversity remain important targets. It is increasingly thought that soil systems are regulated more by dominant species, their traits and the complex species interactions that arise, rather than the diversity of organisms present [61, 68]. Bengtsson (2002) [97] suggested that it was probably more relevant to investigate the linkages between key species or functional groups and ecosystem function, rather than relationships between total soil diversity and ecosystem function. That is stability may be a trait of communities arising from their structure such as food webs and interactions.

3.88 In another study, noted above Cragg *et al*. (2001) [115] manipulated the microbivorous soil fauna and resolved that changes in their diversity may not have predictable effects on decomposition process rates and that the functioning of this

group was mainly affected by the physiological attributes of the dominant species present. In their review Bardgett and Cook (1998) [119] observed that the effect of agricultural activity and increases in its intensity reduced the diversity of the soil fauna. Bardgett and Cook (1998) [119] noted that previous studies that have investigated the role of food-web complexity in soil system stability and nutrient cycling have paid insufficient attention to taxonomic composition and species redundancy within functional groups. In their opinion, 'the central question to be addressed is how soil biodiversity influences the stability of soil ecosystems, both in terms of their structure and their function' [119].

CONCLUSIONS

- Soil diversity is primarily a result of the structure and conditions prevailing in a soil.

- There is no consistent relationship between diversity and ecosystem function in soils.

- The dominant species and food web structures, with their complex interactions, are probably the key biotic factors in the function of soil systems.

- Changes in diversity are sensitive indicators of perturbation.

- Diversity *per se* may be valuable as a source for resilience (more research needed).

4. REVIEW OF THE POTENTIAL IMPACT OF GM CROPS ON THE SOIL ECOSYSTEM AND APPROACHES TO THEIR ASSESSMENT AND MONITORING

4.1 The purpose of this chapter is to review reported and potential impacts of GM crops and associated agronomic practices on the soil ecosystem. The chapter is divided into two sections with the first reviewing impacts of GM crops, and the second reviewing how such impacts might be measured and assessed. As discussed in the previous chapters, the complexity of the soil ecosystem in terms of both species present and function, and interactions with plants means that it is not appropriate to measure or assess impact on a single process or numbers of a single organism. As addressed in Chapter 3, the objective of the impact assessment is important. There are significant differences between impacts as defined by:

- the numbers of organisms present;
- the function of the soil as a whole being maintained; and
- the resilience of the soil (ability to recover).

4.2 An impact of a GM crop may be measured and assessed at each of the levels. However, the consensus from the scientific literature is that function and resilience of the soil ecosystem are the key characteristics that should be assessed. Changes to either of these that result in an irreparable loss in soil function (a function is lost permanently) are viewed as significant.

REVIEW OF THE IMPACTS OF TRANSGENIC PLANTS ON SOIL SYSTEMS

4.3 Studies of the impacts of transgenic plants have developed in close association with studies of the ecological interactions between the soil biota and plants. This association has resulted from the use of conceptual and methodological tools from soil ecology as well as the exploitation of manipulations made available by genetic modification to investigate how plant soil systems function. The findings from various studies conducted to assess the effect of GM plants on soils are reviewed below. The issues raised by the effects with respect to future risk assessment and monitoring are also considered.

4.4 For the purposes of this report the review of the impact(s) is presented in two parts. Both types of impact are assessed as having the potential to affect the soil ecosystem:

(i) impact(s) caused by changes in agronomic practice that occur as a consequence of the cultivation of the GM crop. In general, agricultural practices cause huge perturbations in soil systems, with changes in management practices having serious and long-lasting effects on soil microbial communities [75, 120]; and

(ii) impact(s) caused specifically by the GM crop[10]. In many of the examples described the plants have been modified for the specific purpose of altering the composition of the soil ecosystem, through the enhanced secretion of various compounds into the rhizosphere or the suppression of specific components of the soil flora or fauna. Studies investigating changes caused by GM crops with commercially-relevant traits such as pesticide or herbicide tolerance are more limited although some investigations have been conducted [31, 32, 121-123].

Whilst effects on soil microorganisms have been reported, a study by Kowalchuk *et al.* (2003) [124] concluded of the studies investigating the impact of GM plants on the soil ecosystem, limited significant non-target effects were detected. Many of the studies reported only minor non-target effects [31, 32, 121, 125-135] or no detectable non-target effects [122, 136-143].

4.5 The effects that may be caused simply through changing the type or cultivar of crop being grown are not addressed. As has been discussed previously in this report, such effects on the soil ecosystem may occur irrespective of whether the new crop is GM. However, as discussed, the issue is not whether the introduction of the GM crop causes an effect, but what that effect is, and how significant it is in terms of altering the function and resilience of the soil system. Changes to the biological diversity within the soil, with no concomitant effect(s) on the resilience are not assessed to be significant effects. As discussed in Chapter 3 'The Role of Redundancy', it is the resilience of the soil system, rather than its diversity or function that is the key characteristic of the soil ecosystem that should be conserved.

4.6 This does of course assume that the characteristics of the soil prior to the disturbance are desirable and should be conserved. With respect to the use of GM

[10] This section is complete with respect to the published literature (May 2004).

plants for the bioremediation of contaminated soils[11] it is the characteristics of the soil prior to the pollution that are desirable. The successful phytoremediation of polluted soil should result in irreversible changes in the compounds within the soil, and therefore in the soil ecosystem present.

REVIEW OF THE IMPACTS – EFFECTS CAUSED BY ALTERED AGRONOMIC PRACTICE

4.7 The purpose of this section is to review potential impacts on soil ecology caused by changes to agronomic practices that might occur with the introduction of particular GM crops. Unlike the effects addressed in the following section, the impacts caused are not specific to the GM crop but may occur due to changes in agronomic practice required by the GM crop. Effects caused may though be significant and are likely to be greater than those caused by the genetic modification. The practices identified are:

- changes in the level of mechanisation. This includes altering the tillage requirements, levels of pesticide or herbicide needs and harvest frequency. Reduced mechanisation may occur for example with GM crops that are herbicide or pest tolerant and require potentially fewer chemical applications, or plants that are more drought tolerant and therefore require less irrigation; and

- cultivation of GM plants with modified growth characteristics, particularly altered salt, drought or temperature tolerance.

Changes in the level of mechanisation

4.8 Changes in the levels of mechanisation of the soil may occur through the adoption of a zero-tillage or low-tillage strategy, the application of fewer doses of herbicides or pesticides, and in the case of forestry the use of longer harvest rotations. Any reduction in mechanisation will mean less traffic across the soil and therefore less soil compaction, and no adverse effect on soil bulk density or soil pore water retention [23, 144]. Changes to tillage, rotation or post-harvest treatments of crop residues all affect the amount and quality of the organic matter that returns to the soil [76, 145]. This in turn is likely to affect the characteristics of the soil ecosystem, including pathogen viability and distribution. The addition of organic amendments is reported to reduce soil-borne diseases [145]. A reduction in the level of mechanisation is also likely to involve a decrease in the degree of intensification of the farming practices. This has been found to affect density and diversity of soil invertebrate populations [86] (see Chapter 3 for more details).

[11] See Defra report 'Genetically Modified Organisms for the Bioremediation of Organic and Inorganic

4.9 Altering the tillage strategy used (conventional, reduced or zero tillage) can change both the abundance (by 2-9 times) as well as the composition (diversity) of earthworm populations [101]. Increasing the level of tillage is reported to reduce the number of deep burrowing (anecic) earthworm species, whilst numbers of the more shallower dwelling (endogeic) species may increase if sufficient food is available [101]. The roles of the various groups are not clear, although earthworms have a more significant role to play in soils with a reduced tillage strategy because of their role in nutrient cycling and their abilities to modify the physical characteristics of the soil [101]. With respect to effects of tillage on microbial communities, comparisons of zero tillage and conventional tillage reported 60-140 percent higher microbial biomass carbon content in zero tillage soils [76]. Differences in PLFA (phospholipids linked fatty acid) profile of the soil microbial community were also reported between the two soils, although the differences were most pronounced during the first half of the growing season [76].

Cultivation of plants with modified growth characteristics

4.10 The application of GM techniques to modify a plant's tolerance to drought, salt or temperature has been reviewed in Defra's report 'Effect of compositional traits on the survivability and persistence of GM crops' (2004) [146]. Whilst such modifications are not assessed as having any direct effect on the soil ecosystem, the ability of the modification to allow the cultivation of a crop in an environment which under conventional practices would be unable to support that crop, will inevitably have an effect on the ecosystem of that soil. Arid soils are normally only able to support slow growing xerophytic or xerotolerant plants. The introduction of a non-xerotolerant plant that has been genetically modified to tolerate arid conditions is likely to result in different types and quantities of compounds being released to the soil from the plant's roots. Whilst information describing these effects has not been identified, such effects may need to be considered in any assessment of GM crops with modified growth characteristics.

REVIEW OF THE IMPACTS – SPECIFIC EFFECTS CAUSED BY TRANSGENIC PLANTS

4.11 The purpose of this section is to review those reports that have investigated whether the presence or growth of a GM plant has an effect on the composition or function of the soil ecosystem. Effects have the potential to occur through the horizontal transfer of the novel genes from the GM plants to soil microorganisms and more direct effects posed by the release of novel compounds from the GM plants into the soil (either during the lifetime of the plant or during decomposition of plant matter) and the subsequent effects they may have on soil biota [33].

Pollutants' (2002).

Horizontal gene transfer (HGT) from GM plants to soil microbes

4.12 Horizontal gene transfer (HGT) is often cited as an issue of concern with respect to the release of GM plants in the environment [124, 147, 148]. The issue and potential effects caused by HGT are less specific than many of the other examples addressed in this section as the possible nature of the impact will depend on the nature of the gene(s) transferred from the GM plant to the soil microbial community. The consensus from the scientific literature is that horizontal gene transfer is likely to be very rare, with no successful examples of the transformation of DNA from plants to soil microorganisms reported under field conditions [33]. The potential for HGT between plants and microorganisms is greatest where the DNA added to the plant shares some degree of sequence homology with microbial genomes [124]. Minimisation of the use of bacterial vector DNA in gene constructs added to plants should therefore reduce the potential for HGT to occur [124].

4.13 HGT from plants to bacteria has been demonstrated under laboratory conditions [149], with the availability of competent bacteria in the vicinity of transformable DNA suggested as the rate-limiting factor [33]. However, even where the experiments were designed to maximise gene transfer, through the use of highly recipient strains such as *Acinetobacter* sp. BD413 [148-152] and *Pseudomonas stutzeri* [153] and specially designed DNA cassettes with shared transgenes and strong bacterial homologies, transfer rates were still low.

4.14 From these studies it is recognised that the horizontal transfer of transgenic DNA from plants to bacteria in the soil community will be a very rare event. Further, if the transgenes and vector(s) are not of bacterial origin, then the rate of transfer will be exceedingly low.

4.15 Given the low rates of expected horizontal transfer, then a huge number of plant roots or quantity of soil would need to be sampled to detect a transfer event. However, even if such events were to occur it is expected that the recipient microorganisms would not persist within the soil ecosystem in significant numbers. However, if through some fitness advantage or hitch-hiking effect a transgene was to persist in the soil bacterial community it is unlikely to be of serious ecological significance [124]. This is partly because the potential for acquisition of a transgene and ecological effects is not different to the same real potential for acquisition of plant or animal genes by soil bacteria.

4.16 Laboratory assays transforming bacteria with transgenic DNA can usefully confirm that the transgene design avoids homologies which would otherwise facilitate horizontal gene transfer (albeit at a low rate of transfer). Many transgenes would not express a selectable phenotype in bacteria which lowers the sensitivity of environmental monitoring by several orders, making detection of *in situ* transfer

events very unlikely. More information on the conditions for transfer and persistence is expected from the growth of genomics and the future experiments are expected to provide further information.

Effects caused through the release of novel compounds

4.17 In most cases the effects on the soil ecosystem have been monitored because the GM plant was expected (and in some cases designed) to have an effect on soil flora and fauna. The plants described in these examples often have little commercial relevance as they would not be grown in an agronomic context. However, some studies have sought to assess the effects of commercially relevant GM plants (such as those designed for altered pest resistance or herbicide tolerance) on the soil ecosystem.

4.18 For the purposes of this report the various studies have been presented according to the purpose of the modification, rather than by crop species or specific gene. A summary of the studies conducted is presented in Table 4.1. The areas reviewed are:

- modification of the expression and release of organic acids;
- controlled exudation of opines;
- modified pest tolerance (including expression of Bt toxin and T4 lysozyme);
- modified herbicide tolerance; and
- altered decomposition of plant residues.

4.19 In addition, some studies reported as investigating the impact of GM plants on the soil ecosystem have in fact been designed to test the efficacy of various analytical methods [129, 130, 140]. Such studies are of less interest but have been included in this report for completeness.

4.20 Several studies have compared the effects of GM alfalfa expressing either bacterial (*Bacillus lichenformis*) genes for α-amylase or fungal (*Phanerochaete chrysosporium*) genes for Mn-dependent lignin peroxidase, on the soil microflora [129, 130]. Di Giovanni *et al* (1998) [129] assessed effects using the Biolog GN microtitre plates with DNA fingerprinting (enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR)) of bacterial communities present in the wells. Analysis of the results found differences in the rhizosphere populations from the soil of the different plants, with the lignin-peroxidase$^+$ plants having the most distinctive rhizosphere

populations (although this may have been a consequence of the stunted nature of the transgenic plants themselves) [129]. Donegan *et al.* (1999) [130] also used metabolic fingerprinting (Biolog plates) and DNA fingerprinting to assess effects caused. The study reported distinct metabolic fingerprints of the soil bacterial communities in the soil, and higher numbers of culturable bacteria in the soil of the lignin peroxidase$^+$ plant. No effect on protozoa, nematodes and microarthropods and DNA fingerprints of indigenous soil bacteria and rates of substrate induced respiration were reported [130].

Modification of the expression and release of organic acids

4.21 Transgenic plants with elevated organic acid exudation are of interest agronomically for their potential to improve the uptake of phosphorus and other nutrients and to improve tolerance of plants to aluminium [154, 155]. The effects of altered organic acid exudation into the rhizosphere (both amount and composition) from transgenic lines of alfalfa on the diversity of rhizobacteria and soil nutrient availability has been reported by Tesfaye *et al*. (2003) [156].

4.22 The study involved a transgenic alfalfa cultivar which over-expressed a nodule-enhanced malate dehydrogenase cDNA. This modification was known to change the pattern of expressed organic acids and was reported to increase organic acid production seven-fold in the rhizosphere [156].

4.23 The study which was field-based, with rhizosphere soils from the transgenic plants and untransformed alfalfa compared after 53 weeks, investigated both effects on bacterial diversity and activity.

4.24 Effects of the transgenic plant on the diversity of rhizobacteria were tested by analysis of 240 16S rDNA clone sequences. Eleven bacterial phyla and their major subdivisions were detected in the two alfalfa rhizosphere samples. There were qualitative changes in the abundance of some bacterial phylogenetic groups between rhizosphere soils of transgenic and untransformed rhizospheres. Notably, significantly more *Flexibacter* (9.2% versus 4.1%) and unclassified (10.9% versus 5.8%) sequences were cloned from the untransformed alfalfa rhizosphere while significantly more *Nitrospira* (1.7% versus 6.6%) and *Fibrobacter/Acidobacterium* (4.2% versus 9.1%) were cloned from the transgenic alfalfa rhizosphere.

4.25 The approach used was however relatively conservative, with differences between soils only detected at the group level, and at only a weak level of significance ($P < 0.1$). Of the seventeen bacterial groups identified, fourteen were found in the rhizosphere of both the transgenic and untransformed plants. The other three groups were present in such low proportions that considerably larger samples would have been needed to confirm a significant decline in their numbers.

4.26 The catabolic activity of the bacterial community was profiled using Biolog GN microtitre plates. The Biolog substrate utilisation patterns showed a clear separation of the untransformed and transgenic rhizospheres. The rhizosphere bacteria of the transgenic alfalfa utilised significantly more substrates and had a greater functional diversity than bacteria from the untransformed control soil.

4.27 Initially the transgenic alfalfa plants were slower growing but no significant differences in yields were observed overall and no significant differences were observed in colony forming units of bacteria counted on R2A (a low nutrient agar). The concentrations of nitric acid extractable P, K, Mn, Zn and Cu increased significantly in the transgenic alfalfa rhizosphere and this was probably directly associated with the increased exudation of organic acids.

4.28 The study observed that the elevated release of organic acids by the alfalfa roots influenced rhizosphere microbial diversity and the availability of macro- and micro-nutrients. Given the nature of the modification, these results are certainly of a type and scale that might be expected. It should also be noted that the changes (diversity of bacteria and functions) noted are probably the product of changes in relative numbers and or activity rather than the loss or acquisition of new populations by the rhizosphere.

Controlled exudation of opines

4.29 Opines (small amino acid and sugar conjugates) are derivatives of arginine and known bacterial growth substrates. There is interest in manipulating the opine production of crops to promote populations of rhizobacteria of benefit (e.g. antagonist of plant pathogens) for specific crop cultivars [128, 134, 157]. The promotion of specific populations of microorganisms in this way may offer an alternative to the use of chemical fertilisers [157]. It is also reported to be more successful than just adding cultures of microorganisms direct to the soil as it provides a selective pressure to maintain the population of the target microorganisms [157].

4.30 Oger *et al*., (2000) [134] tested the effects of transgenic opine production by *Lotus corniculatus* (bird's-foot trefoil) on soil bacterial populations including opine-utilisers. Transgenic effects were tested for in continuous cultivation, and with removal of plants and replanting with non-transgenic trefoil or wheat or left fallow. Greenhouse experiments were in non-sterile soil and were sampled up to 42 weeks. Counts on selective agars were made to enumerate total (culturable) counts, fluorescent pseudomonads, agrobacteria, heat-resistant spore-forming bacteria, 42°C thermotolerant bacteria, mannopine utilisers, and nopaline utilisers.

4.31 The study found that in soil and on root tips of the transgenic plants there were no significantly different densities of total viable bacterial populations, thermotolerant or

heat-resistant spore-forming bacteria. The densities of mannopine and nopaline utilisers were elevated (by 23 fold in the soil and 475 fold on root tips). The fluorescent pseudomonads were elevated on the root tips of transgenic plants and the agrobacteria were elevated in the soil from transgenic plants.

4.32 When wild type and transgenic plants were removed, the fallow bacterial populations studied were very similar regardless of the prior planting. A similar result was obtained where the soil was replanted with wheat. In soils previously planted with transgenic plants, the mannopine utilisers, however, declined more slowly and were still apparent as elevated counts after 4 months. The authors observed that the catabolic bias generated by transgenic opine-producing plants did remain in the soil for a period of time following the removal of the GM crop, albeit at a lower level than when the GM plants were present. The study concluded that bacterial populations can be manipulated temporarily to favour target bacterial populations by plant rotations with GM (*Lotus corniculatus* and *Solanum nigrum* (black nightshade)) and wild type plants [134, 157].

4.33 Although the authors observed that some alterations to the rhizosphere microbial community (populations of mannopine utilisers) induced by the cultivation of transgenic plants may sometimes be persistent, for most of the measures made the report illustrated a more general phenomenon of recovery seen in studies of impacts in soil microbial communities. This means that perturbations in communities are readily affected but following either the withdrawal of the challenge from a treatment or its dissipation with time, the system will return to that present in the control (i.e. the soil system recovers/returns to its original state) [134].

4.34 The observed resilience of the soil system when exposed to opine-producing GM plants means that the effects caused by the plants on the soil ecosystem are concluded as not significant.

Modified pest tolerance

4.35 The development of transgenic crops modified for enhanced pest tolerance may affect soil biota through the direct and indirect effects caused by the genetic modification. Studies reported to date that have investigated such effects have focused on the following different modifications:

- expression of T4-lysozyme;
- expression of lectins;
- expression of the lytic peptide cecropin B; and

- expression of the Bt insecticidal toxin.

Effect of plants modified to express T4-lysozyme

4.36 The expression of the bacteriophage T4-lysozyme has been proposed as a strategy for the inhibition of phytopathogenic bacteria [137, 158]. T4-lysozyme has activity against both Gram-positive and Gram-negative bacteria either by its muramidase activity against the bacterial cell wall component murein or by a nonenzymatic mechanism which may involve disruption of membranes [142].

4.37 A study by Ahrenholtz *et al.* (2000) [158] with T4-lysozyme modified potatoes found that expression of the T4-lysozyme resulted in increased killing of *Bacillus subtilis* on root hairs relative to the non-GM control. The level of killing was quantified by fluorescence microscopy with a known quantity of bacillus cells added to the roots at the start of the (greenhouse-based) experiment.

4.38 Interestingly the non-GM potato lines (Désirée) also exhibited a degree of bactericidal activity on the root surface, although this was 1.5-3.5 fold less than the GM lines. The bactericidal activity of the non-GM potato plants was probably due to secretion of other bactericidal compounds such as benzofurans, terpenoids, butyrolactones, and other phytoalexins (Mansfield, 1983; cited by [158]).

4.39 Whilst the study by Ahrenholtz *et al.* (2000) [158] demonstrated the bactericidal effects of T4-lysozyme the design of the study meant that the occurrence of any effect of such GM plants on soil bacteria in the field could not be determined [158].

4.40 A field-based study by Heuer *et al.* (2002) [142] used potato plants (*Solanum tuberosum*) modified to express T4-lysozyme and secrete it into intercellular spaces. Such plants have been reported to be less susceptible to infection by the plant pathogen *Erwinia carotovora* [159]. The secretion of the compound into intercellular spaces means that some loss of the T4-lysozyme into the rhizosphere will occur, thereby causing bactericidal effects on the root surface [142].

4.41 Studies by Heuer *et al.* (1999, 2002) investigated the effects of T4-lysozyme expression in potatoes on both the phyllosphere [137] and rhizosphere [142] microbial communities. Whilst some differences in numbers of culturable bacteria were observed in the phyllosphere studies, the assessment of the rhizosphere microbial community found no effect of T4-lysozyme expression.

4.42 Effects of T4-lysozyme expression were investigated using both culturable and non-culturable techniques. Culture-based methods included heterotrophic plate counts, determination of species composition and diversity based on fatty acid analysis of

isolates, and community level catabolic profiling. Non culture-based analyses were based on denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments amplified from rhizosphere DNA using primers specific for bacteria, Actinomycetales, or α- or ß-Proteobacteria. Several bands of the DGGE patterns were further characterised by sequence analysis. None of these methods found a significant effect on the rhizosphere community that was attributable to the expression of T4-lysozyme, although effects caused by environmental factors of season, field site (two field sites used) or year (three year study) were identified [142].

4.43 The field release of GM potato plants modified to express T4-lysozyme conducted by Lottmann *et al.* (1999) [138] measured changes in the:

- numbers of plant-associated bacterial populations (selective isolation plate counts);
- functions of potentially beneficial bacteria (measured by assaying for the production of indole-3-acetic acid (IAA)); and
- diversity of antagonistic bacterial species (particularly antagonists to *Erwinia carotovora* and the phytopathogenic fungus *Verticillium dahliae*).

4.44 Crops from two transgenic lines, the transgenic control and a non-transgenic control were compared at two field sites through two years.

4.45 The study found that surprisingly the expression of the T4-lysozyme did not result in significant differences in viable counts between transgenic and non-transgenic plants. No transgenic plant effects were detected on potentially plant beneficial bacteria. The diversity of those bacteria with antagonisms to phytopathogens were compared between the plant lines. Of twenty-eight antagonists detected, seven were found only on control plants but this was minor compared with the natural variability observed [138].

4.46 In a further experiment T4-lysozyme expressing potatoes, transgenic control potatoes and non-transgenic parental potatoes were inoculated with two bacterial strains: i) *Pseudomonas putida* QC14-3-8, an antagonist of *Erwinia carotovora* ssp. *atroseptica* isolated from the tuber surface (the geocaulosphere), and ii) *Serratia grimesii* L16-3-3, antagonistic toward the plant pathogenic fungus *Verticillium dahliae*, isolated from the potato rhizosphere [139]. These were, respectively T4-lysozyme tolerant and sensitive. In a field crop both bacteria colonised the rhizosphere and geocaulosphere in a similar way in both the transgenic and non-transgenic potato lines except during flowering. At this stage T4-lysozyme levels

were at their highest, and significantly higher counts of T4-lysozyme tolerant *P. putida* were observed on the two transgenic T4-lysozyme producing lines.

4.47 The impacts of the treatments on the bacterial community were assayed by inspection of the patterns obtained from DGGE separation of PCR-amplified whole bacterial community 16S rRNA gene fragments. This method detected differences associated with season and between rhizosphere and geocaulosphere communities. No differences were detected associated with the bacterial inoculants with plant lines.

4.48 The study has tested for negative affects of plant beneficial bacteria using both general indicators (IAA production) and assays with specific bacterial and fungal pests. The finding of no negative effects is strengthened by the use of two field sites and in some cases two years.

Expression of lectins

4.49 Griffiths *et al*. (2000) [132] evaluated non-target effects of transgenic potato lines expressing lectins (concanavalin A (Con A) and *Galanthus nivalis* agglutinin (GNA)) to discourage feeding by invertebrate pests. The study was field-based and observed transient effects, within season, on soil protozoa and microbial activity (soil dehydrogenase) and transient effects on CLPP. None of the effects reported persisted into following seasons.

Effect of plants modified to express cecropin B (lytic peptide)

4.50 The genetic modification of potatoes to express the lytic peptides has been reported as a potentially effective strategy for the suppression of bacterial pathogens. Traditional breeding has had little impact in developing resistance in crops such as potatoes and chemical control of bacterial pathogens is not feasible [160]. The potential advantages of lytic peptide expression are that such compounds exhibit strong antimicrobial activity *in vitro* and significant antibacterial activity in potato and tobacco plants *in vivo* [160].

4.51 Cecropins are a group of small, highly basic lytic peptides first isolated from the pupae of the giant silk moth *Hyalophora cecropia.* All cecropins exhibit lytic and antibacterial activity against several Gram-negative and Gram-positive bacteria *in vitro*. Cecropin B has particular activity against a number of phytopathogenic bacteria including *Ralstonia solanacearum* and *Erwinia* spp. (Nordeen *et al.* 1992; cited by [160]).

4.52 Bacterial samples were obtained through selective isolation on ATCC medium 552 (designed for the isolation of *Bacillus* spp.) with subsequent analysis of strains conducted using PCR-RFLP analysis of the 16S rRNA gene and the 16S-23S rDNA

intergenic spacer. Representative isolates were analysed further by partial 16S rDNA sequence analysis [160].

4.53 Differences in the diversity and community structure of culturable *Bacillus* spp. in the rhizosphere of the GM potatoes compared to the non-GM control were reported, with the biggest changes observed during the flowering stage of the potato crop. Differences at the tuber production stage were smaller [160]. *Bacillus megaterium* for example showed significantly higher abundance in the rhizospheres on the cecopin-producing plants at both flowering and tuber producing stage [160]. Further studies investigating the effects of the modification on other microbial groups, particularly the plant growth promoting bacteria were proposed [160].

4.54 Observed effects on spore-forming strains of *Bacillus* spp. suggest that the cecropin peptide was released into the rhizosphere. The transgene construct was designed to either lack an N-terminal signal peptide or contain plant-specific hordothionin N-terminal signal peptide, and should therefore have remained in the plant cell [160]. The release of the peptide into the rhizosphere probably occurred following the death of root cells and highlights the fact that effects to the rhizosphere as a whole should be addressed for such modifications. The extra-*planta* effects of the release of the T4-lysozyme described by Ahrenholtz *et al.* (2000) [158] was conferred by the presence of an α-amylase leader peptide in the transgene construct which results in the enzyme being secreted into the intercellular space (and consequently to the rhizosphere).

Effect of plants modified to express Bt toxin

4.55 Many crops are now protected against lepidopteron pests by genetic modification to express insecticidal toxins from the soil bacterium *Bacillus thuringiensis*[12]. These so-called Bt toxins are known to bind to active sites on the surface of soil particles where they may persist for months retaining their insecticidal activity through cycles of wetting drying and freezing [161, 162]. The targeted effect of the Bt toxin to a component of the soil fauna means that the cultivation of Bt-expressing GM crops may have a direct effect on the soil ecosystem through adverse effects to specific organisms, as well as indirect effects on other organisms elsewhere in the soil foodchain. The accumulation of the Bt toxin in the soil could also have a potential detrimental effect to non-target organisms. The impact of the release of Bt toxin from transgenic plants to soils, particularly during the decomposition of the Bt plant material, has been evaluated by a number of studies [131, 141, 163].

4.56 An investigation of the effects of Bt transgenic rice straw on basic biochemical characteristics of a waterlogged soil [163] found some differences in protease,

neutral phosphatase and cellulase activities between soil amended with Bt-transgenic rice straw and non-transgenic rice straw at the early stage of incubation, although none of the differences were persistent. Measurements of dehydrogenase activity, methanogenesis, hydrogen production and anaerobic respiration were however found to be greater in the GM straw amended soil compared to the control, with the differences persisting over the course of the 80 day study. The changes detected were reported to indicate a shift in microbial populations or a change in the metabolic abilities of the microbial community as a result of substrate availability (including the presence of the Bt toxin) in the soil [163].

4.57 A transgenic corn cultivar expressing *B. thuringiensis* insecticidal toxin cry1Ab gene was evaluated for its impact on the soil biota [141]. The transgenic corn (Bt corn) had been shown to release the toxin into the soil as an exudate and in corn litter/biomass [161].

4.58 In the study natural sieved soils in pots were planted with Bt or non-Bt corn or were amended with biomass from these plants. Pots were placed in growth chambers and sampled at 40-45 days.

4.59 The study found that in the soil and guts and casts of earthworms, the Bt toxin was found to persist and retain its activity. The toxin was undetected in earthworm guts within a few days of transfer to fresh soil. The Bt toxin did however not affect the earthworms, nematodes, protozoa, bacteria, and fungi [141]. Impact was assayed by biomass and mortality (earthworms) and counts. A further study [162] confirmed the release and persistence for up to 180 days of bioactive toxin from twelve transgenic corn hybrids from three transformations confirming that appearance of Bt toxin in soil was likely to be a common phenomenon.

4.60 The authors cautioned that before definitive conclusions were made, that these results should be extended to more worm species and a more detailed evaluation of potential changes in the composition and diversity of bacteria, fungi, protozoa, and nematodes be conducted. Given the large area planted to Bt corn, Saxena (2001) [141] proposed that Bt crops should probably be evaluated for potential ecological effects. The study advocated the application of methods such as DGGE, Biolog, and evaluation of nutritional groups of protozoa and nematodes. These methods were proposed as having the potential to confirm the absence of the effect of the insecticidal toxin on biodiversity in soil.

4.61 Another study involving Bt corn assessed the impact of the GM crop on the earthworm *Lumbricus terrestris* [164]. The experiment was a 200-day study

[12] Bt-based insecticides are also employed in organic agriculture, although the Bt toxin is added onto the cultivated area, rather than being produced by the crop.

conducted to investigate the impact of transgenic Bt corn on adult and immature *Lumbricus terrestris* in the field and in the laboratory. The laboratory experiment was imitated with adult earthworms and the field experiment with immature earthworms. Earthworms were fed with Bt+ or Bt- corn litter.

4.62 The study reported that mortality in both laboratory and field experiments was low and was not significantly different for Bt+ corn and Bt- corn fed earthworms. In the field experiment with immature worms no significant differences in relative weights of Bt+ and Bt- corn fed worms were found. In the laboratory experiment with adult worms, their mean relative weight was no different for Bt+ and Bt- corn feeding up to 160 days. However at 200 days significant differences were reported with Bt+ corn fed worms having a mean weight loss of 18 percent and Bt- corn fed worms having a mean weight gain of 4 percent.

4.63 Zwahlen *et al*. (2003) [164] used the same earthworm species as Saxena and Stotzky (2001) [141] and their detection of differences in earthworm weights may result from their longer test period. As noted by the authors, more studies are needed to resolve whether the weight differences persist and if so whether they are caused by the Bt toxin itself or whether other causes such as changes nutrient quality of the transgenic plant litter may account for it. The authors recommended that life history traits should be included in long-term impact assays including longevity, development to sexual maturity, cocoon production, and fertility. It should also be noted that 200 day old microcosms will lack a certain realism and it would be sensible, given the widespread growth of these crops to make these long term studies in the field.

4.64 In a separate study [121], three Bt expressing transgenic cotton lines were evaluated against a parental control for affects on the numbers and species of indigenous soil bacteria and fungi [121]. The assays included biochemical tests to identify individual cultures, community substrate utilisation patterns and DNA fingerprinting. In experiments lasting up to 56 days, only two of the transgenic cotton lines displayed any effects on the microbial community. All of the effects observed were temporary but included significant elevations in bacterial and fungal counts, as well as changes in bacterial community composition, community substrate utilisation and DNA fingerprinting. The lack of similar effects when Bt toxin was added directly and a lack of similar effects from the third transgenic lines indicated that the different effects of the two transgenic lines were probably not caused by release of the Bt toxin but more probably arose from differences between the plants generated during the genetic manipulation or culturing of the plants.

4.65 The accumulation of Bt toxin in soil following the decomposition of Bt maize and its effects on non-target organisms was investigated by Escher *et al.* (2000) [131]. The effects of foliage from Bt maize on the decomposer organism *Porcellio scaber*

(woodlouse) and leaf-litter-colonising microorganisms were assessed. Changes in weight, reproduction, growth and survival of the woodlice were recorded throughout the feeding study, with woodlice on the transgenic diet exhibiting faster weight gain and lower offspring mortality compared to the non-GM control. Changes in the microbial populations (bacterial and fungal) were determined by counts on selective isolation media. Microbial numbers were found to be the same on both the GM and non-GM foliage [131].

4.66 Analysis of the chemical composition of the maize leaves (Bt and non-Bt) showed that initial contents of fructose and soluble carbohydrates were significantly higher in the non-Bt maize, whereas lignin decomposed more quickly in the Bt plants (although lignin content in both plant groups were similar at the start of the study[13]). Levels of starch, cellulose, hemicellulose and ash did not differ between the two samples [131].

4.67 The differences reported for the woodlice populations were proposed to be due to a higher nutritional quality of the Bt (GM) maize over the non-Bt maize. Whilst consumption of the maize foliage by the two groups of woodlice was the same, the slightly lower C:N ratio, lower lignin content and higher content of soluble carbohydrates conferred a greater nutritional advantage for the Bt fed group [131].

Herbicide tolerant transgenic plants

4.68 Modified herbicide tolerance, along with altered pest resistance, represents a significant percentage of the traits present in the GM crops grown commercially worldwide [165, 166]. Whilst the plant-orientated targeting of the modification means that direct effects on the soil ecosystem are unlikely, changes in weed content and cover in the soil may have some indirect effects on the soil ecosystem. Given the large area planted to herbicide tolerant crops worldwide, the occurrence of any effects of herbicide tolerant GM plants on soil ecology may be significant.

4.69 Studies at the University of Saskatchewan have investigated the rhizosphere and root-endophytic (within the root) bacterial communities of transgenic and non-transgenic herbicide tolerant cultivars of oilseed rape [31, 32, 167-169], maize [143] and wheat [170].

4.70 An interesting feature of this work was that it isolated thousands of bacteria on standard media and then identified and typed them using analysis of fatty acid methyl esters (FAMEs). This limits the detection to the culturable fraction but probably increases the sensitivity to reflecting changes in physiological condition as well as changes in numbers present.

4.71 These studies have shown notably different structure and diversity in bacterial communities colonising the rhizosphere of some non-transgenic and transgenic oilseed rape cultivars. In one study the transgenic oilseed rape (modified for improved tolerance to glyphosate) contained reduced numbers of *Bacillus*, *Micrococcus* and *Variovorax* isolates, but higher numbers of *Flavobacterium* and *Pseudomonas* isolates in the root interior. The study also reported reduced numbers of arthrobacters and bacilli isolated from the rhizosphere [31]. In another field study, with three transgenic and three non-transgenic oilseed rape cultivars, 35 percent of the bacteria (n=2257) isolated and identified were *Pseudomonas* species [168]. This study focused on the pseudomonads as important rhizosphere bacteria and many of the pseudomonad strains identified were isolated from all six cultivars. Metabolic characterisation (Biolog Gram-negative GN2 plates) and enzyme profiling of the two most common species, *Pseudomonas putida* and *Pseudomonas chlororaphis*, showed different cultivar associated carbon utilisation profiles. However, no transgenic plant specific affect was observed on the rhizosphere pseudomonad populations.

4.72 The differences observed are compatible with the scale of differences observed in the same fields between the bacterial communities colonising the rhizosphere of conventional wheat and oilseed rape crops [170]. This study involving non-GM plants found that the rhizoplane communities of oilseed rape and wheat grown at the same site differed significantly. Here the wheat rhizosphere had strongly elevated levels of *Bacillus* sp. while the oilseed rape rhizosphere had a more uniform distribution of bacterial species.

4.73 This study with the non-GM wheat and oilseed rape plants [170] illustrates well a real dilemma facing soil ecology impact assessment in GM plants. Changes in community structure are complex, and result from many interactions and subtle changes in the plant-rhizosphere interaction. These non-GM studies have shown that the composition of the root-endophytic bacterial community of oilseed rape will differ between cultivars, and raise the questions:

- does the reduced diversity observed in the transgenic oilseed rape root-endophytic bacterial community matter? and
- is it enough to observe that the GM effects are within the scale of the differences associated with cultivar-cultivar or crop-crop differences?

4.74 An important approach from the Saskatchewan research, to answering this question was reported by Dunfield and Germida (2003) [169]. Having shown that some

[13] The similarity in lignin content between the Bt and non-Bt maize is at odds with the findings of Saxena and Stotzky (2001) who reported that Bt-transgenic maize has a higher lignin content than

transgenic cultivars affect the structure and metabolic function of the rhizosphere bacterial community [32], a further study has addressed the impact of transgenic plants on non-target soil bacteria [169].

4.75 The rhizosphere microbial communities of transgenic and non-transgenic cultivars of oilseed rape were compared in a two year study at two field sites. Importantly, this study investigated whether the changes observed remained/persisted in the absence of the plants, or were only observed whilst the plants were present. Bacterial community structures were assayed using:

- community-level physiological profiles (Biolog GN2 plates);
- fatty acid methyl ester profiles; and
- terminal amplified ribosomal DNA restriction analysis profiles.

4.76 Comparisons were made between transgenic, non-transgenic and fallow field plots. All three measures of community structure detected differences in the microbial community associated with cultivar and with year. However, with respect to long-term changes in the microbial community, the study found that when the microbial communities were assessed after winter, there were no differences between microbial communities from field plots that had contained transgenic oilseed rape plants and microbial communities from field plots that had not contained plants during the field season. Therefore the study concluded that whilst the GM plants caused changes in the microbial community structure, the changes were temporary and did not persist into the next field season [169].

4.77 In a separate study [143], the effects of transgenic maize (conferring glufosinate herbicide resistance) on bacterial community structure were evaluated in a field-based comparison with an isogenic non-transgenic cultivar [143]. Total rhizosphere bacterial DNA was extracted and partial sequences from conserved 16S rRNA gene regions were PCR amplified. The amplified fragments were converted to single-strand conformation and single-strand conformation polymorphism (SSCP) patterns were visualised by separation in polyacrylamide gels. With genetic profiles of 40-60 bands depending on the conserved region (the study evaluated three), low variability was observed between independent replicates.

4.78 Schmalenberger (2002) [143] observed no effect of either the transgene or the herbicide on the SSCP profiles. However, clear differences were observed between dates (35 and 70 days after sowing) and between the maize rhizosphere SSCP profiles and those from sugar beet (grown as a control crop). The SSCP profiles

non-Bt maize. (*American Journal of Botany*, 88 p.1704-1706).

from the rhizosphere of transgenic herbicide-resistant sugar beet and a non-transgenic control have also been evaluated [171]. Whilst no effect from the genetic modification or application of herbicide was detected, the analysis did detect differences between two seasons and different patterns reflecting the location of the plant in the field.

4.79 An investigation with transgenic herbicide-tolerant (glufosinate) oilseed rape evaluated greenhouse (field soil) trials against the wild-type cultivar with either herbicide application or weeding [123]. Rhizosphere pseudomonad and eubacteria communities were characterised from DGGE patterns of PCR 16S rRNA gene fragments and by sequence analysis of dominant pseudomonads. The eubacterial and pseudomonad communities were observed to be altered but these differences were minor when compared with the differences observed through the plant season and with the transient changes caused by herbicide applications.

Altered decomposition of plant residues

4.80 For the soil system, an important interaction between plants and the soil microflora is the mineralisation of nutrients by biodegradation of plant residues. There is the potential that some transgenic plant tissues will have an adverse affect on the community of degraders and thus decompose more slowly (or reduce decomposition of other plant litter). This was a consideration when the ecological impact of transgenic tobacco expressing an insecticidal proteinase inhibitor was assessed by Donegan *et al*. (1997) [122]. In this study the authors tested the transgene effects by burying transgenic, and non-transgenic leaves in litterbags at a field site. The study reported counts of the transgenic plant litter associated collembola populations were reduced while nematodes were increased and had an altered community structure with elevated proportions of fungal feeding over bacterial feeding nematodes.

4.81 Decomposition of transgenic tobacco stems was investigated by placing stems in four soils over 77 days [135]. This revealed that the transgenic stems decomposed faster than the wild type stems and was probably due to altered resistance to microbial degradation resulting from the manipulated composition and conformation of the transgenic lignin.

Observations from the studies reported

- Considerations of the transgene product, its activity, site of expression and persistence are important guides to developing, on a case by case basis, lines of investigation into likely or important potential effects.

- The majority of the studies reported found some effect of the transgenic plants on the soil ecosystem relative to the non-GM control. However, these

effects were often minor compared with changes associated with cultivar-cultivar comparisons or the influence of weather and season. The more valuable studies were those that:

- assayed for changes caused by the GM plant as well as changes in the natural variation in the system (including variation arising from differences in field management); or
- investigated whether the effects were transient and not maintained in the absence of the GM crop. As discussed earlier in this report, transient effects are viewed as less significant as they demonstrate no fundamental or irreversible effects on the soil ecosystem.

- Only one potentially long-term effect of a GM plant on the soil ecosystem was reported [134]. Such effects are defined as those that persist in the absence of the GM plant. The single study investigated the effect of opine-producing birds foot trefoil on soil microorganisms. Whilst the GM plants caused an increase in both nopaline and mannopine-utilising microorganisms, following the removal of the GM plants, the mannopine-utilising population did not return to the level found with the non-GM control. Numbers of mannopine-utilising strains decreased to a level between the non-GM control and the GM plant [134]. Such changes were described by Oger *et al.* (2000) as 'persistent' although the changes were only measured over a four month period.

- Differences between non-transgenic treatments and controls can provide a baseline for expected or accepted variation in the soil system. However, the response of the system when the transgenic challenge is removed provides an important measure of impact. In practice, in these studies and many others in soil ecology the return of many soil parameters to match those in control soils is frequently observed and provides a useful threshold criterion when evaluating the impacts of GM plants.

- The majority of the studies investigated effects on the soil microbial community, rather than impacts on invertebrates or protozoa. Of the microbial studies, many of these focused on effects to the culturable component of the soil microbial community. The findings from such studies may therefore not be applicable to the soil microbial community as a whole.

- When monitoring observes the absence of microbial taxa in transgenic or control conditions it is unlikely that they are actually lost from the soil system. That is many of these changes are probably in the structure (relative numbers) rather than the composition of the community. Soils are very high diversity habitats in which a fall in relative numbers would then require

excessive efforts to confirm either the persistence or loss of a population. The large pool of diversity held in soil the system is further enhanced by the dispersal and colonisation of populations. It is not clear how significant dispersal is to the diversity observed in soil.

- Community structures are dynamic, changing through the season and plant development. These changes confer an added level of complexity as transgenic-non-transgenic differences are not fixed (context dependent) and, in many studies, not systematic in character through the season. This underlines the importance of regular sampling and longer term studies, at least two years for some studies and certainly longer for others.

- Arising from the case by case approach, specific targets for monitoring are established which have intrinsic and clear definitions of damage. However, there is a lack of monitoring activity which is linked to a concept of damage to the system. Many researchers hope that the more general assays of diversity will provide more general monitors of soil quality, and consider that it would be useful to have a better understanding of the relationship between soil factors (diversity, activity, etc) and soil function.

Table 4.1 – Summary of studies investigating the effect of GM plants on soil ecology

Novel trait	Plant and modification	Method(s) used	Observed effect on soil ecology	Ref.
α-amylase or lignin peroxidase production	Alfalfa modified to investigate changes in soil bacterial ecology	GN Biolog plates and ERIC-PCR	Yes relative to the control.	[129]
α-amylase or lignin peroxidase production	Alfalfa modified to investigate changes in soil bacterial ecology	Biolog plates and DNA fingerprinting	Yes relative to the control	[130]
Organic acid expression	Alfalfa modified to overexpress a nodule-enhanced malate dehydrogenase cDNA	16S rDNA sequence analysis and metabolic fingerprinting with Biolog GN plates	Little change in bacterial diversity but some change in bacterial activity.	[156]
Opine production	Bird's-foot trefoil modified for enhanced opine production Oger P, 1997 #405} [134] Black nightshade modified for enhanced opine production [157]	Selective plate counts (enumeration of total counts, fluorescent pseudomonads, agrobacteria, heat-resistant spore-forming bacteria, 42°C thermotolerant bacteria, mannopine utilisers, and nopaline utilisers)	Increased numbers of opine utilisers in the presence of the GM plants. When the GM plants were removed levels of nopaline utilisers reduced to a level equivalent to the non-GM plants, whilst levels of mannopine utilisers decreased to levels intermediate between transgenic and normal plants.	[128, 134, 157]
T4-lysozyme expression	Potato modified to express the anti-pathogenic compound T4-lysozyme	Fluorescence microscopy	Enhanced bactericidal effect against *Bacillus subtilis* added artificially to the plant root hairs.	[158]
T4-lysozyme expression	Potato modified to express the anti-pathogenic compound T4-lysozyme	Heterotrophic plate counts, determination of species composition and diversity based on fatty acid analysis of isolates, and community level catabolic profiling. Also DGGE of 16S rRNA gene fragments amplified from rhizosphere DNA using	No effects found	[142]

Novel trait	Plant and modification	Method(s) used	Observed effect on soil ecology	Ref.
		primers specific for bacteria, Actinomycetales, or α- or ß-Proteobacteria.		
Expression of T4-lysozyme	Potato modified to express T4-lysozyme	Selective isolation plates, production of IAA and antagonistic activity to the bacterium *E. carotovora* and the fungus *V. dahliae*.	No significant differences between the GM and non-GM control	[138]
Expression of T4-lysozyme	Potato modified to express T4-lysozyme	Selective isolation plates, and analysis of PCR-amplified fragments of the 16S rRNA genes of the whole bacterial community after separation by DGGE.	No significant differences between the GM and non-GM control	[139]
Expression of T4-lysozyme	Potato modified to express T4-lysozyme	Enumeration of culturable bacteria	No effect on antagonistic bacteria	[140]
Expression of lectins	Potato modified to express lectins to discourage feeding by invertebrate pests.	Enumeration of microbial and protozoan numbers. Microbial activity determined by dehydrogenase assay.	No significant effects apart from transient reductions of ~40% soil protozoa and ~10% microbial activity.	[132]
Expression of cecropin B	Potato modified to expression the antibacterial lytic peptide cecropin B.	Bacteria isolated on bacillus-selective medium. Subsequent analysis of strains conducted using PCR-RFLP analysis of the 16S rRNA gene and the 16S-23S rDNA intergenic spacer. Representative isolates were analysed further by partial 16S rDNA sequence analysis.	Transient effects observed on populations of *Bacillus* sp. No other effects analysed for.	[160]
Expression of Bt toxin	Effect of straw from Bt rice on biochemical characteristics of waterlogged soil.	Measurement of protease, neutral phosphatase, cellulose, and dehydrogenase activities, as well as methanogenesis, hydrogen production and anaerobic respiration levels.	Changes to all characteristics, although only dehydrogenase activity, methanogenesis, hydrogen production and anaerobic respiration persisted over the course of the study.	[163]
Expression of Bt toxin	Maize modified to express Bt toxin	Enumeration and measurement of earthworm population.	No significant differences found between GM and non-GM control.	[141]

Novel trait	Plant and modification	Method(s) used	Observed effect on soil ecology	Ref.
		Selective isolation of bacteria, actinomycetes and fungi. Enumeration of nematodes and protozoa.		
Expression of Bt toxin	Maize modified to express Bt toxin	Enumeration and measurement of earthworm populations.	No significant differences between the Bt and non-Bt fed worms.	[164]
Expression of Bt toxin	Cotton modified to express Bt toxin. Cotton leaves placed in the soil.	Selective isolation counts of total culturable bacteria and fungi. Community substrate utilisation tests and DNA fingerprinting	Higher bacterial and fungal counts reported for two of the three leaf samples tested.	[121]
Expression of Bt toxin	Maize modified to express Bt toxin	Analysis of populations of woodlice, culturable fungi and bacteria on foliage cut from Bt maize. Chemical composition of the maize also investigated.	Faster weight gain and lower juvenile mortality in woodlice fed on Bt maize. No differences in microbial populations. Bt maize proposed to offer higher nutritional quality.	[131]
Insect resistance	Tobacco modified to express Proteinase Inhibitor I	Enumeration of Collembola and nematode populations in soil surrounding plant foliage (GM or non-GM).	Increased number and different species of nematode in the GM study, but lower numbers of Collembola. Carbon content in the GM sample decreased significantly during the time of the experiment.	[122]
Herbicide tolerance (glyphosate)	Oilseed rape modified for improved tolerance to glyphosate	Selective isolation and FAME analysis of isolates.	Reduced numbers of *Bacillus*, *Micrococcus* and *Variovorax* isolates, but higher numbers of *Flavobacterium* and *Pseudomonas* isolates in the root interior. Reduced numbers of arthrobacter	[31]

Novel trait	Plant and modification	Method(s) used	Observed effect on soil ecology	Ref.
			and bacilli in the rhizosphere.	
Herbicide tolerance	Oilseed rape modified for improved herbicide tolerance.	Selective isolation and enumeration of pseudomonads.	No GM plant specific changes	[168]
Herbicide tolerance	Oilseed rape modified for improved herbicide tolerance.	Substrate utilisation (Biolog), FAME analysis and terminal amplified ribosomal DNA restriction analysis.	Changes in substrate utilisation patterns between GM and non-GM rhizosphere. However, the changes did not persist after the growing season.	[169]
Herbicide tolerance, glufosinate tolerance	Maize modified for enhanced tolerance to the herbicide glufosinate ammonium.	Analysis of genetic diversity of the total bacterial community.	No effects caused by the GM plant when compared to a non-GM control in the same field and during the same growing season.	[143]
Herbicide tolerance, glufosinate tolerance	Oilseed rape modified for enhanced tolerance to the herbicide glufosinate ammonium.	Analysis of rhizosphere pseudomonad and eubacteria communities from DGGE patterns of PCR 16S rRNA gene fragments and by sequence analysis of dominant pseudomonads	Minor changes between the GM and non-GM samples. However changes were much less than those caused by plant season and herbicide application.	[123]
Herbicide tolerance	Oilseed rape modified for enhanced herbicide tolerance	Substrate utilisation and FAME analysis	Endophytic and rhizosphere microbial communities differed between the GM and non-GM cultivars grown on the same field site.	[167]
Lignin production	Tobacco modified for altered lignin biosynthesis. Effects on soil decomposer organisms investigated. Material from all plants decomposed more rapidly.	NMR and carbon analysis of plant material and soil to determine rate of lignin decomposition.	No effect on soil ecology investigated.	[135]

APPROACHES FOR THE ASSESSMENT OF THE IMPACT OF GM PLANTS ON SOIL ECOLOGY

4.82 Studies designed to assess the impact of GM crops on the soil ecosystem reviewed for this report have identified negligible long term impact of the crops on the soil ecosystem. Whilst some of the crops have been found to have an effect relative to non-transgenic controls, the effects are (when measured over a long term) found to be temporary and not maintained in the absence of the GM plant.

4.83 However, the regulatory requirement to assess the potential environmental risks posed by the GM plant prior to its release into the environment, and the important role of plant soil interactions as vital ecosystem drivers, means that some strategy to assess the potential impact of GM crops on soil ecology is required (and would be of benefit to assessors).

4.84 As discussed however, soil processes, plant activity and the activities of the soil populations are involved in very complex interactions which may be prohibitively complicated to disentangle. The development of environmental risk assessments for GMPs has been reviewed and discussed by Bruinsma (2002), Kowalchuk (2003), Batie (2003), Poppy (2000), Sutherland (2001) and Dale (2002) [124, 172-176].

4.85 The purpose of this section of the report is to evaluate the strategies proposed to date (2004) and to suggest where further modification of the approach may be required.

4.86 The basis of any assessment approach is that it is not possible to assay all aspects of a soil system receiving GM plants. The numbers of ecosystem components and the complexity of the interactions mean that any assessment must focus the risk assessment and monitoring process on clear priorities. Generally this is a three part process:

(i) assess the proposed release;

(ii) identify aspects of the soil system which may be affected or vulnerable given the nature of the GMP; and

(iii) identify methods which may be used to assay aspects identified in (ii) and assay for unforeseen ecosystem effects.

4.87 This process will typically start by taking account of whether the introduced genes are expressed (as opposed to regulatory functions), the activity of their products and when and in what plant tissues are they expected to be active. As discussed above

the answers to these questions can lead to very different concerns, such as the persistence of the Bt toxin in soil [162] or reductions in plant beneficial bacteria [138]. Whilst risk assessment and monitoring must proceed on a case by case basis there is a need for a systematic approach, rooted in soil ecology, which can guide the making of assessments and relevant research targets.

4.88 In practice, risk assessments and monitoring are tiered, starting in the laboratory and scaling up to the variety and complexity of conditions in full field trials [174]. Field trials are important as observed behaviours do not always 'scale up' from the laboratory and greenhouse to the open environment. Field trials of transgenic lectin producing potatoes detected transient effects on CLPP, microbial activity and nematode populations which were not detected in laboratory and pot assays [35]. Even when naturalistic microcosms are created, the open environment is less constrained with additional sources of variation including dispersal which can dissipate some affects while new affects may become apparent [174]. Long term microcosms suffer the additional problem of most becoming increasingly unrealistic.

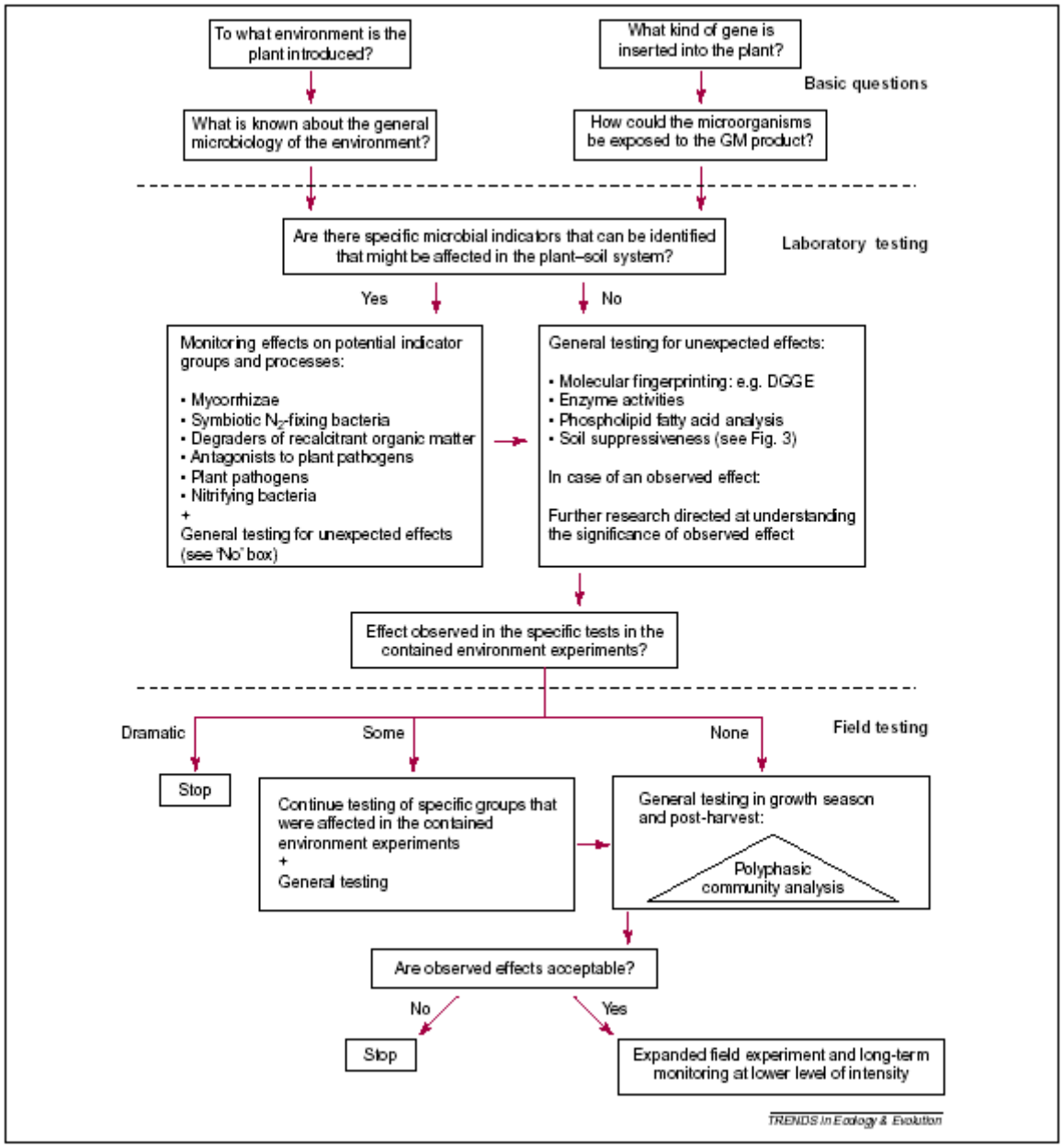
4.89 The most comprehensive reviews and discussions regarding the monitoring of GMP impacts on soil systems have focused on the soil microbial community [124, 172]. This focus has arisen, in part, because on roots and litter the microbial community will be in intimate association with any GMP, making them first sentinels of perturbations and because of their key role in soil food webs and nutrient cycling. Kowalchuk *et al.* (2003) [124] have proposed a two part approach to monitoring GMPs:

(i) Monitoring indicator groups or processes which are considered vulnerable given the soil system or the nature of the genetic modification; and

(ii) Making broad assessments of changes in microbial community structure.

4.90 A schematic of their approach to assaying the impacts of genetically modified plants on soil microbial communities is reproduced in Figure 4.1. This approach identifies and monitors both general and specific measures of perturbation in a tiered methodology scaling up from contained experiments to field trials and longer term studies. At each stage the aim is to assay indicators of GMP impacts and assess the significance of these. In reviewing how GMP impacts might be assessed, this report will also distinguish between approaches based on keystone approaches and approaches based on broader measures of soil function. However, this approach will be extended here to include the soil fauna and soil processes.

Figure 4.1 – Schematic approach outlining a new framework for measuring the potential effect of genetically modified plant on soil-borne microbial communities and functions (taken from Kowalchuk *et al.* (2003) [124]).



4.91 On the basis of the information present in the reviews of Kowalchuk (2003) and Bruinsma (2002) [124, 172], and other studies, the approaches and methods available for assessing and monitoring the risk to the soil ecosystem are presented, under the following three categories:

- GM plant effects (1)[14]: keystone and important groups and processes

[14] The effects of GM plants have been sub-divided into three groups and addressed separately in the report.

- GM plant effects (2): broad measures of the soil system, diversity and activity

- GM plant effects (3): soil quality

4.92 In each category the groups and processes have been reviewed for vulnerability and importance to the soil system, and potential role as sentinels or as keystone indicators.

GM plant effects (1): keystone groups and processes

The use of keystone indicators of soil system perturbation and damage

4.93 Whilst the characteristic of functional redundancy has been described as significant within soils, and that losses in diversity may not have a significant effect on soil function, there are some soil processes and functions that have a low redundancy and are therefore mediated by a very limited number of organisms. Such organisms are referred to as 'keystone' organisms [66] and are proposed as an important component of any risk assessment strategy.

4.94 A recent conference 'Effects of genetically modified plants on soil ecosystems' in the Netherlands [172], and other publications [124] addressed the issue of using keystone indicators for monitoring impacts. The use of keystone indicators is sensible as it is inconceivable to assay all components in a soil system, and allows the assessment strategy to focus on those organisms whose loss is likely to have a significant effect on soil ecosystem function. A number of criteria for choosing these indicators have been proposed:

- *Ecological significance*. When changes observed to indicator species, groups or activities, it should be possible to relate these to potential ecological effects in the soil system.

- *Susceptible to change*. Indicators chosen need to change fast enough and to a sufficient extent to be assayed.

- *Pertinent*. Indicators need to be relevant to the specific soil system and GM-plant type. Many potential indicator populations may be absent from specific soils perhaps through pH or water levels.

- *Low redundancy*. Many important functions are undertaken by a wide variety of populations. An extreme example is carbon mineralisation which is undertaken by a huge diversity of populations. With these high redundancy functions, changes to specific populations may well not influence the soil

function. Low redundancy improves the prospect that changes detected in a population are likely to be associated with changes in that function.

- *Practical*. Impact assessments need to be based on methods which are economic, for which the materials and machines are available, and which are appropriate for the scale of sampling.

- *Validated*. The tests need to be validated, to be reproducible and for suitable controls to be identified

4.95 The Netherlands conference report [172] considered specific microbial groups as potential indicators of perturbations caused by GMPs. The groups selected were the Mycorrhizae, symbiotic N_2-fixing bacteria, antagonists, wood decaying fungi and nitrifying bacteria. These groups were chosen on the basis of their sensitivity to change and that they perform key soil functions with a relatively low level of redundancy. These and other potential target groups are now considered in more detail.

Mycorrhizal fungi

4.96 Mycorrhizal fungi enter plants through their roots and establish mutualistic associations in which they provide nutritional benefits to the plant. These associations play an important role in the health of most vascular plants. In the ectomycorrhizae the fungal hyphae are found between root cells while endomycorrhizal hyphae are also found in living root cells [177, 178].

4.97 A major group of the endomycorrhizal fungi are the arbuscular mycorrihizal (AM) fungi which establish specific structures for nutrient transfer and are found in the roots of many plants. A mycorrhizal fungus will be distributed through a soil and invade many roots. The fungal hyphae extending from the plant root increase the area from which nutrients needed for plant growth may be acquired, thereby benefiting the plant. The plant in return supplies the fungi with nutrients and energy which are distributed through the hyphae [178].

4.98 The presence of mycorrhizal fungi can improve plant growth in low phosphate soils by hyphal transport of phosphate from soils too far from the root for normal uptake. Other nutrients which may be similarly scavenged are potassium, trace metals (magnesium, copper, boron, molybdenum and zinc), nitrogen and sulphur. The effectiveness of AM fungi is influenced by bacteria which can promote root colonisation or play a role in solubilising nutrients transported by the hyphae. AM fungal colonisation can also reduce the susceptibility of plants to pathogens [178].

4.99 The mycorrhizal fungi are important contributors to plant health and soil fertility. They are sensitive to some fungicides, ploughing, fertilisers and flooding. Microscope-based methods are preferred for assessing impacts by assay of the proportion of roots colonised, as the persistence of spores reduces the efficacy of direct counts. Recently, molecular methods have been developed for the assay of diversity. It has been reported that increased land use intensity is correlated with a reduced diversity of AM fungi favouring species that colonise roots slowly but form spores rapidly [179]. The AM fungi are also affected (diversity and activity) by plant community diversity [180-182], and host density [183].

4.100 AM fungi are not only directly important to plants, they also interact synergistically with plant growth promoting rhizobacteria (PGPR) by changing root behaviour and through interactions between their mycelium and these bacteria in the rhizosphere soil [177].

4.101 The impact of declining mycorrhizal diversity on the soil ecosystem is not yet fully resolved. However, mycorrhizal diversity is considered likely to be important to the functioning of the soil system [184]. This is because diverse AM fungal communities are necessary to fulfil their activities and because many AM fungi are engaged in host specific interactions [184]. This combination suggests a low functional redundancy among the AM fungi and also that negative impacts of GM plants on the AM fungi could be of significance to the soil system. There is evidence correlating AM diversity with shoot phosphorus levels [182].

4.102 Agriculture-associated reductions in diversity have been identified and may be monitored to detect GMP effects either direct or through the interactions of the soil system. However, more research is needed to resolve the ecological significance of AM diversity to system function. The keystone role of the AM fungi is more likely to take the form of assays enumerating their numbers and the numbers of roots colonised.

Plant growth promoting rhizobacteria (PGPR)

4.103 Plant growth promoting rhizobacteria (PGPR) have been identified as having the potential to increase plant fertility by promotion of growth or protection from pathogens. The PGPR are a heterogeneous group of bacteria which are active via a number of diverse mechanisms including disease suppression mediated by iron-chelating siderophores; the release of plant growth regulators (auxins, giberellins, cytokinins) or their homologues; and the production of anti-fungals [185, 186]. In some cases the mechanism of plant promotion is speculative such as PGPRs which have been shown to produces blends of volatile organic compounds which can act as plant signals [187]. PGPRs may be applied to a wide range of crops where they increase yields and reduce disease [186].

4.104 The wide range of mechanisms by which PGPRs may operate, and the number of different taxa involved means that it would not be sensible to identify and assay all the plant beneficial bacteria within a specific soil. However, given the soil, the cropping, and other uses, it should be possible for a site to identify specific activities which are relevant and can be assayed.

4.105 This was illustrated in the studies monitoring the effects of transgenic antibacterial lysozyme production in potatoes [138]. Two plant pathogens, the bacteria *Erwinia carotovora* ssp*. atroseptica*, and the fungus *Verticillium dahliae* were used to enumerate the proportion of antagonists in the soil from transgenic and control treatments. Given the anti-bacterial nature of the transgenic product the researchers conducting the study also wanted a more general indicator of adverse effects on plant beneficial bacteria and thus assayed for the production of indole-3-acetic acid (IAA). Though this may be associated with some pathogenic strains it is a common indicator of PGPR activity and in the rhizosphere of this study was confirmed in 8-68 percent of bacteria isolated. This kind of approach can target key pathogens or processes and provide important information. It should be noted that it is not possible to identify and assay most of the classes/groups of PGPR. However, assays of plant productivity and disease (discussed below) provide complementary data when analysing data from experiments and field trials.

4.106 A PGPR activity with particular importance manifests itself in the occurrence of naturally suppressive soils where phytopathogens are held in check. Generally this occurs when the soil acquires a microbial population, or more probably a community, which is antagonistic toward and probably very competitive with the pathogen. Examples are soils suppressive to fusarium wilts which are known to benefit from non-pathogenic fusarium and fluorescent pseudomonads and soils suppressive of take-all in wheat, where *Gaeumannomyces graminis* var*. tritici* also has an antagonistic relationship with fluorescent pseudomonads.

4.107 Suppressive soil mechanisms are various and mixed and include resource competition, inducing plant resistance, and the production of antibiotics [188]. It is known that agricultural practices such as tillage and management of crop residues can affect the frequency of soil-borne plant diseases [145] and any interaction between a GM product and the soil suppressive activity would be of interest and potentially concern. Again this is a keystone process in which disease challenge assays will provide key data. However, a better understanding of the loss of suppressiveness and the bio-indicators of this would be useful.

Wood lignin decomposing fungi

4.108 In wood, the formation of lignin aromatic cross bonds by free radical reactions creates an irregular structure lacking regular targets for decomposer enzymes. The

residence time of lignin in soils is accordingly greater than most other plant residues. Biodegradation of lignins is pioneered by the white rot *Basidiomycotina* fungi which release highly diverse mixtures of extracellular enzymes. The energy costs of this enzyme mixture result in marginal energy benefits and slow progress in this degradation. Soil fauna can make an important contribution to this process through both mechanical attack exposing new surfaces, and the activity of gut microflora. Wood lignin decomposing fungi are proposed as suitable indicator species because they have low diversity, and the process of lignin decomposition has low functional redundancy is soil.

4.109 However, the low rate of lignin degradation in soil may mean that lignin decomposing fungi are not all that suitable as keystone indicators, except in long-term studies. They may of course also not be suitable for studying the effect(s) of crops modified for reduced lignin content [189, 190]. GM crops modified for lower lignin content may not support as high numbers of lignin decomposing fungi. However, this is only viewed as a significant effect, if the numbers do not recover following the removal of the GM plants.

Nitrogen fixation

4.110 Globally, atmospheric nitrogen fixed by a variety of soil bacteria makes a grater contribution to soil systems than that from agricultural fertilisers and atmospheric deposition [30]. Nitrogen fixation is an energy intensive activity requiring low oxygen tensions or anoxic conditions. It is carried out by a variety of free living soil bacteria, rhizosphere associated but free living N_2-fixers (e.g. *Azotobacter*), soil surface and lichen associated cyanobacteria, root associated actinomycetes (*Frankia*) which form nodules on some non-leguminous plants, and bacteria which form nodules on the roots of legumes. The latter group are bacteria from the family Rhizobiaceae and are globally the most important N_2 fixers. These are soil bacteria which infect the roots of legumes and form nodules. The bacteria provide the plant with fixed nitrogen and the plant provides the bacteria with nutrients and energy.

4.111 Nodulating bacterial strains infect specific legume species and vary in their nitrogen fixing efficacy. The long-term cultivation of nitrogen-fixing leguminous plants in a soil will have a substantial effect on the various rhizobial populations. These bacteria persist in soils at relatively low levels and displacement of these bacteria or reduction of their nodulating activity would be considered a cause for caution.

Nitrifying bacteria

4.112 Ammonia arises in soil as a result of nitrogen fixation, fertilisation, and the recycling of fixed nitrogen from plant and animal wastes. This ammonia is available for uptake and use as a nitrogen source by plants and bacteria. Alternatively the ammonia may

be oxidised by nitrifying bacteria to nitrite (NO_2^-) and on to nitrate. This two step oxidation is known as nitrification and the intermediate nitrite does not usually accumulate. The nitrate produced is available to plants and bacteria but may in anoxic conditions become an electron acceptor in anaerobic respiration and be reduced to nitrite (NO_2^-), to nitric oxide (NO), to nitrous oxide (N_2O) and on to N_2.

4.113 The nitrification process is ecologically significant in that is affects the flow of nitrogen in soil systems as the resulting nitrate may be lost through denitrification and leaching. The second nitrite-oxidising step is thought to be confined to a single genus in soil, Nitrobacter, but they appear to be ubiquitous and resilient and their activity has not been observed to limit nitrification, in contrast to the ammonia oxidizing bacteria (AOB). The AOB have been found, so far, to be taxonomically limited within the β-proteobacteria, mostly from the genera *Nitrosomonas* and *Nitrosospira*. This has facilitated PCR and DGGE based analysis of AOB communities and has been successfully applied to assay the impact of grassland agricultural practices on the diversity and spatial heterogeneity and enumeration of the AOB [74, 106, 191]. The analysis of *Nitrobacter* sp. in soil is more limited, although some studies have been reported to date (2004) using PCR [192, 193].

4.114 The low functional redundancy and ecological position of the AOB in an important and central nutrient cycle make this group of bacteria potential keystone indicators of soil system behaviour. This potential could be extended by understanding whether, or when, reductions in AOB diversity affect the soil system function.

Methods for evaluation of keystone communities

Mycorrhizal fungi

4.115 There are well established methods for the sampling enumerating and assaying of AMF diversity. Two sorts of samples may be taken:

(i) Soil cores (typical depth 10 cm). AMF spores are isolated from soil samples by wet sieving and sucrose density gradient centrifugation (see for example [194]). Spores are counted under low magnification and mounted on microscope slides and observed using a stereomicroscope at higher magnification (~ x400). Spores are assigned to species, to known morphospecies and to new (not recognised) morphospecies. This approach permits the frequency of AMF spore types to be estimated for each soil sample. As the persistence of spores may affect the efficacy of direct counts it is valuable in these studies to include trap cultures which provide fresh cultures; and

(i) Plant roots. AMF root colonisation is assayed using light microscopy and a stain (trypan blue) to detect mycorrhizal structures (see for example [195]). The primary data collected is the proportion of plants colonised by mycorrhizae and measurement of the root length colonised [183].

4.116 Further the diversity of root colonising AMF may be assayed using terminal restriction fragment length polymorphism (T-RFLP) [182].

4.117 Studies that have used these methods include [179, 181-183]. The soil spore evaluations provide base line data on the diversity of AMF present and can detect changes arising from altered field practices. While the soil spore evaluation gives information on the changing potential in the community; assays of root colonisation provide complimentary information about the realised colonisation. This is important because altered crops and field practices may affect the AMF root colonisation efficiency without necessarily directly changing the diversity of spores persisting.

Plant growth promoting rhizobacteria (PGPR)

4.118 The development of assays for the state of health of PGPR communities is in its infancy. It is not practical to use PGPR isolation methods to evaluate the numbers of PGPRs, which in many cases may anyway function as consortia. The wide range of traits which may be associated with plant growth have been considered above. However, individual traits may be selected and their frequency or expression assayed in control and treatment plots. The soil studied and its management may indicate specific PGPR traits which are relevant and can be assayed. This was the case in the studies reviewed above on the effect of transgenic antibacterial lysosyme producing potatoes [138]. These studies assayed the production of indole-3-acetic acid (IAA) which is a common indicator of PGPR activity. At the present time, the assaying of PGPR as keystone indicators is necessarily partial and specific to the field site. An alternative approach which should be considered is to make *in situ* or *in vitro* assays of PGPR activity by assaying seed germination and plant growth rates. This approach could be especially useful in assaying soil suppression of phytopathogenic diseases, an important PGPR activity.

4.119 A PGPR activity with particular importance manifests itself in the occurrence of naturally suppressive soils where phytopathogens are held in check. Generally this occurs when the soil acquires a microbial population, or more probably a community, which is antagonistic toward and probably very competitive with the pathogen. Examples are soils suppressive to fusarium wilts which are known to benefit from non-pathogenic fusarium and fluorescent pseudomonads, and soils suppressive of Take-all in wheat, where *Gaeumannomyces graminis* var. *tritici* also has an antagonistic relationship with fluorescent pseudomonads.

4.120 Suppressive soil mechanisms are various and mixed and include resource competition, inducing plant resistance, and the production of antibiotics [188]. It is known that agricultural practices such as tillage and management of crop residues can affect the frequency of soil-borne plant diseases [145] and any interaction between a GM product and the soil suppressive activity would be of interest and potentially concern. Again this is a keystone process in which disease challenge assays will provide key data. However, a better understanding of the loss of suppressiveness and the bio-indicators of this would be useful.

Wood lignin decomposing fungi

4.121 Changes in the wood lignin decomposing fungi are expected to reflect the low rate of lignin degradation in soil and are thus mainly suitable as keystone indicators in long-term studies. Further assessment of levels of these organisms will be important in crops modified for altered lignin content.

The use of specific indicators of soil system perturbation and damage

4.122 With some transgenic plants, the choice of one or more of the above keystone indicators will be as general indicators of perturbation. However, with some other transgenic plants the choice of keystone indicator will arise more directly from an assessment of the plant, transgene product, and field site. The identification and targeting of likely or important potential effects of GMPs is an important factor in resolving their testing and ultimately their field assessment.

4.123 This targeting has to be flexible and inevitably results in a wide range of specific assays. Examples include:

- when monitoring the effects of transgenic opine production by *Lotus corniculatus*, specific measures included enumeration of agrobacteria, mannopine utilisers and nopaline utilisers [134];

- the transgenic application of Bt insecticidal toxins has resulted in specific tests of the persistence, binding and activity of the toxin on soil, plant material and the guts and casts of earthworms; as well as specific tests on the biomass and mortality of earthworms [141, 162, 164];

- in the case of transgenic plants with potentially altered decomposition of residues, specific assays have included assays of decomposition, collembola and nematodes when transgenic and non-transgenic plant material were buried in litterbags [122], and C-13 nuclear magnetic resonance spectroscopy to assay lignin decomposition and decomposer activity [135]; and

- increased organic acid production in the rhizosphere by a transgenic alfalfa cultivar was monitored for affects on soil concentrations of P, K, Mn, Zn and Cu [156]. The availability of these nutrients having the potential to be altered by pH changes in the rhizosphere.

GM plant effects (2): broad measures of the soil system, diversity and activity

4.124 In addition to the monitoring of keystone groups and targeted areas of concern, many researchers continue to incorporate broad community assays (diversity, biomass, numbers and activity) in their approaches. Despite the use of keystone groups to detect perturbations to the soil ecosystem, there is a recognised need for broader assays of impacts on the soil microbial and faunal communities to improve sensitivity and detection of unforeseen effects.

4.125 In assaying for unforeseen effects, the ideal is to identify indicators with broad sensitivity. However, it is important that when effects are detected, the indicator chosen should provide some information on the nature of the possible causes or significance of the amplitude. In practice a mixture of complimentary measures is necessary to assay the soil system for impacts from GM plants. Field experiments over two seasons, reported by Donegan *et al.* (1999) [130] have shown that transgenic lignin peroxidase-producing alfalfa plants had:

- significantly reduced shoots,
- elevated N and P levels,
- produced distinct community level physiological profiles (CLPP) from Biolog plates,
- elevated aerobic spore-forming and cellulose-utilizing bacteria counts,
- reduced soil dehydrogenase and alkaline phosphatase activities, and
- elevated soil pH.

4.126 Despite observing these substantial effects of transgenic plants, no effects were observed on protozoan, nematodes and micro-arthropod counts, nor in soil bacterial DNA fingerprints (beta and gamma proteobacteria community DNA fingerprints). Lignin peroxidase is used industrially for large-scale lignin degradation and as a bleaching agent in biopulping processes. In the study of Donegan *et al.* (1999) [130] substantial perturbations in various aspects of the soil ecosystem were not reflected by perturbations in faunal counts or bacterial community structure despite the

detection of temporal changes. Similarly, Griffiths *et al*. (2003) [73] observed that drying and rewetting of intact grassland monoliths resulted in community changes that were detected by viable counts and substrate utilisation (Biolog-GN2) but were not detected by DGGE analyses of community rRNA genes and rRNA transcripts. Monitoring for non-specific effects requires a combination of measures responding to different types of change, such as bacterial, faunal, biomass/activity, taxonomic and physiological assays which will improve the detection of impacts.

4.127 When broad measure assays detect differences between transgenic and non-transgenic plant effects it is essential to have criteria to resolve whether results are satisfactory or whether they warrant further investigation. Differences within a range of values that may be attributed to cultivar-cultivar differences or field management factors may be considered within the normal scope of field management affects and thus not indicative of harm. This underlines the value of regular sampling over a reasonable period with sufficient replicates to establish a realistic measure of the natural variation in the system.

Population size and activity

4.128 The bottom-up influence of the rhizosphere effect and the top-down influence of predation result in microbial and faunal populations whose densities, biomass and activities are regulated by through complex interactions (Chapter 3). Bacterial levels may appear stable through top-down regulation (predation) and a number of studies reviewed have observed stable bacterial numbers despite otherwise substantial differences in predation [138, 141]. Where bacterial populations are regulated in this way their enumeration will be of limited value. Fungal population levels are considered likely to be more responsive to plant activity [60, 61]. The responses of the soil fauna to plant activity are decidedly mixed including combinations of bottom-up and top-down effects [61].

4.129 These complicated relationships may be partly illuminated by assays of activity including respiration [51], culturability or physiological indexes [196, 197], and soil enzymes such as dehydrogenase and alkaline phosphatase [130]. Despite the complexity involved these approaches remain popular because the standard measures of population size and activity that are determined are available, provide ecologically basic data and they have the potential to detect interesting or significant differences.

4.130 Despite recognised limitations, bacterial and fungal plate counts are popular methods for detecting fluctuations in culturable populations. In practice, plate media and incubation conditions are always selective for a subset of the community and limited by the distribution of physiological states within that subset. The choice of selective media allows specific types to be monitored. Plate counts are relatively quick,

inexpensive and provide replicated data on fluctuations within the microbial community. The use of most probable number (MPN) methods is less popular in soil ecology. Microscope and epifluorescence microscopy based counting methods are used to make total counts and with specific fluorescent dyes or probes (fluorescence in situ hybridisation, FISH) to assay physiological condition or enumerate higher level taxa.

Diversity

4.131 One of the most common broad measures of impact in the soil system is to assay for changes in biodiversity. Diversity is a favoured assay because:

- it is a useful sentinel, detecting changes arising from a wide range of causes;
- its conservation is considered a valuable aim in its own right;
- it is considered an important determinant of soil resistance and resilience; and
- molecular methods are improving the reliability and practicality of assaying diversity.

4.132 Loss of diversity is of concern because it may indicate a loss of stability by the soil system. Within this stability it is possible to distinguish resistance (the immediate capacity of a soil to resist a challenge) and resilience (the capacity to recover from a perturbation) [1]. Resistant soils display less perturbation for a given challenge and resilient soils recover more quickly from a given perturbation. Significant reductions in diversity may raise concern that either the resistance or the stability of a soil or both has been undermined. However, there is a lack of data relating indicating threshold values for the effects of declining diversity.

4.133 The resistance and resilience of field soils has been evaluated in an experiment that manipulated plant residues and assessed the impact of copper and heat stress on their decomposition [1]. Differences in respiration were used to calculate resistance and the change in resistance with time was taken as an estimate of resilience. The results showed that diversity assayed by substrate utilisation did not correlate with soil stability. The study also showed that while protozoan biomass could reflect changes in soil a soil, a taxonomic and functional analysis would be needed to develop them as bioindicators.

Diversity of soil fauna

4.134 The soil fauna operate at a variety of trophic levels including bacteriovores, fungivores, herbivores and predators to soil fauna. Assays of perturbations among the soil fauna have the potential to detect changes at a number of trophic levels and investigate impacts on this part of the biota which have important effects on the soil system. These affects of the soil fauna include strong influences of the microbial community, its activity and structure, nitrogen mineralisation and plant growth [61, 198].

4.135 It is known that environmental changes do result in detectable changes in nematode numbers and community structure. Nematodes, as a result, have been considered as candidate bioindicators [84, 85]. Diversity may be assayed by species, family or functional group (plant-feeding, fungal-feeding and bacterial-feeding). Different monitoring regimes can exploit the sensitivities of these families or functional groups to different pressures. The utility of nematode community structure as a bioindicator in environmental monitoring was reviewed by Bongers *et al*. (1999) [85]. The advantages of using nematodes as bioindicators include:

- simple observations can be used to identify nematode feeding methods and thus behaviour and soil system function. Characterisation to functional group or family from morphological characters generates data for the analysis of seasonal changes and differences in food webs, nutrient status, fertility, pH and pollutants in the soils studied;

- in soil, the nematode habitat is the capillary water where their permeable cuticle results in an intimate relationship with the microenvironment and where they do not migrate much. This results in a community structure which is responsive to circumstances within soil horizons;

- changes in the soil habitat, including microbial activity, result in prompt adjustments in the nematode community structure; and

- effective standardised methods, high nematode densities and high nematode diversities facilitate the processing of small sample sizes with many replicates producing significant information and data. Also nematodes are available for quick and standardised assessment in every soil type and climate, allowing timely reactions in land management.

4.136 Interestingly, this kind of monitoring may have the potential to go beyond pair-wise comparisons of transgenic-non-transgenic plots by using established grassland to supply a baseline for nematode diversity in a specific soil [84].

4.137 It has also been proposed [83] that soil microarthropod communities are candidates as bioindicators of changes in soil properties, especially forest soils. This is because these communities are characterised by stability, sedentary life styles, and intricate relationships with their soil niches. These high diversity soil microarthropod communities can be categorised in a variety of ways emphasising various aspects of soil ecology including structure, diversity of feeding types and life history patterns. Different populations exhibit different responses and sensitivities to specific challenges and the author concludes that a combination of physiotype classification and multivariate statistical analysis holds the greatest promise. This approach typically calibrates community response with a specific challenge such as acid rain. The calibration of community response against ecosystem function is more problematic [83].

Diversity of soil microbes

4.138 There is an intimate physical and nutrient flow association between microorganisms and both plant roots and plant residues. The microbial community is a sensitive indicator of changes in plant activity, responding with changes in activity, community structure and community composition. As primary consumers of plant exudates and plant material, soil microbial populations are likely to suffer negative effects from GM plants and are therefore good indicators of impacts associated with plant modification. Assaying for impacts in the microbial community is also prudent because of its key roles in nutrient cycling and soil quality.

4.139 Many of the methods of soil ecology are available for the detection of perturbations in the microbial community. Some of these methods, such as PCR-based detection of diversity, assay extended parts of the community and thus monitor for a wide range of unforeseen effects. A good review of the methods available for the analysis of the soil microbial community is to be found in the report of Bruinsma *et al.*, 2002 [172]. This review evaluates the sensitivities and relative strengths of these methods in assessing soil systems.

4.140 Unlike macro-ecology, microbial-ecology cannot produce thorough inventories of species present and their relative abundances. In practice all the assay methods are selective, defining dominant populations. These limitations are not fatal as the methods available are especially suitable for detecting changes in the community and progress is being made in understanding their relation to soil ecology.

4.141 When assays of microbial diversity detect differences a number of questions are posed regarding their ecological significance:

- what populations are coming and going and why?

- are the 'lost' populations still a part of (low numbers), or available to (dispersal) the system?

- what are the effects on soil functions? and

- what are the consequences for soil resilience?

4.142 In practice the scale of many affects will be judged unimportant when compared with various controls. However, there is a need to make better judgements based on a more informed understanding of the relationship between soil systems and their diversity. Though there is general agreement that diversity and resilience are linked, a lack of correlation between a specific measure of diversity (CLPP) and soil quality has been noted [1].

4.143 Bacterial and fungal diversities are investigated using a variety of techniques. The isolation of individuals on culture media followed by their taxonomic and or functional characterisation is recognised as only sampling a very limited and poorly defined subset of the community. This approach, though labour intensive, can provide quality data capable of detecting changes and relating taxonomic changes to functional changes. One study has identified differences in the bacterial community associated with the roots of non-transgenic oilseed rape cultivars and transgenic oilseed rape cultivars expressing glyphosate-resistance [31] (see also [168]). This study isolated 2300 bacteria and identities were assigned on the basis of fatty acid methyl ester (FAME) profiles. This approach can be focused by the choice of the initial isolation media.

4.144 Community diversity is also assayed by community level physiological profiling (CLPP), phospholipid fatty acid (PFLA) analysis and FAME analysis. In CLPP a sample of the soil or rhizosphere is assayed for utilisation of a number of carbon sources by the bacteria or fungi. The convenience of Biolog microtitre plates with up to 95 substrates and the use of plate readers to collect data for numerical analysis has resulted in extensive collection of data using this method. The sensitivity of the method is strongly influenced by the method of collecting and analysing the data [199], and variation in methods has limited comparison between results from different laboratories. Transgenic and non-transgenic alfalfa showed a clear separation of the untransformed and transgenic rhizospheres [156] with the rhizosphere bacteria of the transgenic alfalfa utilising significantly more substrates having a greater functional diversity than bacteria from the untransformed control soil. This method is popular in soil ecology and frequently provides information which can be related to changes in the soil habitat.

4.145 PLFAs extracted from soil and rhizosphere samples can be enumerated providing information on biomass, community structure and at high taxonomic levels, community composition. Analysis of PLFA profiles has been used detect community changes associated with time, soil depth and tillage [76]. Though not as common as PLFA analysis, FAME profiles have been applied to detecting community level changes, including oilseed rape where transgenic related differences were ducted by this method [169].

DNA technologies

4.146 The growth of DNA technologies and methods exploiting the phylogenetic nature of ribosomal RNA sequences has transformed microbial ecology. It is now possible to assay community patterns and assign identities well beyond the limits of the very partial subset of bacteria that are cultured on media. This report will briefly review the applications of the DNA technologies in the monitoring of soil systems. For the reader who wishes more detail we recommend the report of Bruinsma *et al*., (2002) [172] and a review of DNA based methods which are available for opening the black box of soil microbial diversity [200].

Percentage guanine plus cytosine content (%G+C)

4.147 This method, involves the extraction of total microbial DNA from soil samples and the use of a thermal melting to assay the guanine plus cytosine content (%G+C) of the DNA. Soil samples can be contrasted with respect to their %G+C profiles and by their hybridisation in pairs to compare their similarity with respect to community DNA [201, 202].

4.148 This method was used to compare the microbial populations of three upland site. The %G+C profiles detected differences with one of the sites, and spatial differences at each of the sites in semi-improved grasslands [203]. The pair-wise hybridisation assays detected significant differences between improved, semi-improved and unimproved grasslands and between replicates at all three sites.

4.149 This approach is distinctly broad, detecting coarse similarities and differences (above genera) in the proportions of bacteria sharing similar %G+C values. Unfortunately, when differences are detected, no information is available regarding their nature. Given this, the low sensitivity of this method and the labour involved, it is unlikely to be a monitoring method of choice.

Clone libraries

4.150 The cloning and sequencing of 16S rDNA libraries provides culture-independent data on the composition and, potentially, the relative frequencies of taxa within the

community. The impact of transgenic alfalfa over-expressing malate dehydrogenase on the rhizosphere bacterial community was investigated by sequencing of 240 16S rDNA clone sequences from PCR-based 16S ribosomal rDNA clone libraries [156]. This study identified eleven bacterial phyla, their major subdivisions and differences in community rhizosphere composition.

4.151 The assay of taxa within a sample by cloning and sequencing is laborious. In studies with a number of treatments and replicates, the effect is, for practical reasons, to limit the number of clones assayed per sample. This weakens the assessment of changes in relative frequencies and limits the role of clone libraries in monitoring the impacts of GM plants.

PCR fragment patterns

4.152 Typically PCR fragments are those generated from taxonomic specific primers facilitating the targeting of broad or narrow taxa. These fragments may be visualised to generate community fingerprints in gel electrophoresis (DGGE - denaturing gradient gel electrophoresis or TGGE - thermal gradient gel electrophoresis).

4.153 DGGE finds wide application in bacterial ecology including studies of the diversity of the soil fungal community in moorland and forest soil [204], assays (16S rDNA) of ammonia-oxidizing bacteria and ammonia monooxygenase genes along an agronomic transect [205], and monitoring (16S rRNA) for changes in diversity of bacteria in the potato rhizosphere and geocaulosphere arising from transgenic modification to express T4 lysozyme [139]. DGGE of 16S rDNA fragments amplified by PCR from rhizosphere samples was used to assay the effects of transgenic glufosinate-tolerant oilseed rape and herbicide application on eubacterial and *Pseudomonas* communities [123]. The method detected a slight difference in the rhizosphere bacterial community of transgenic plants but this was minor compared to the differences detected between plant growth associated effects.

4.154 Another method resolves community fingerprints with electrophoretic gel separation, exploiting differences in the folding of single-stranded PCR products. This single-strand conformation polymorphism (SSCP) method was used by Schmalenberger *et al*. (2003) [171] to study the affect of the transgenic herbicide resistance on the rhizosphere bacterial community. The SSCP patterns contained around fifty bands and could detect differences associated with positions in the field and with season. No differences arising from the transgenic modification were detected. Another method, amplified ribosomal DNA restriction analysis (ARDRA), generates a community fingerprint from the variable lengths of fragments generated by restriction digest of PCR products.

4.155 These methods DGGE, TGGE, SSCP and ARDRA are all capable detectors of changes in community structure which can be taxonomically or functionally broad or focused by the choice of PCR primers. These methods can be made routine, but they provide no information of the taxonomic identities in the fingerprint. Though it is possible to excise some bands and gain information by sequencing. For assessing changes in community structure these methods are limited by their lack of, or poor, quantification of relative abundances.

4.156 A method which is regarded as overcoming some of the deficiencies of these methods is terminal amplified ribosomal DNA restriction analysis (TRFLP) [172, 200]. This method is similar to ARDRA but records only the length of one (terminal) fragment from a restriction digest of the PCR products. The fragments are fluorescently labelled so they can be identified (length) and quantified using readily available DNA sequencing technology. Each fragment can be regarded as a specific ribotype whose length can be related to lengths for taxa in the ribosomal database and whose quantification provides an improved, though limited, estimation of relative abundances.

4.157 TRFLP has been used to study the diversity of arbuscular mycorrhizal (AM) fungi in a limestone grassland soil with differing plant communities [182], and changes in wheat rhizosphere bacterial communities following infection with take-all [77]. Another similar method is length heterogeneity PCR (LH-PCR) which does not digest the PCR products first. This provides less detailed information, detecting changes in higher taxa only but is quick and reliable.

GM plant effects (3): soil quality

4.158 The ultimate aim of targeted keystone and assays of diversity and activity is to alert ourselves to potential negative impacts. A further approach is to assay more directly for the outcome of negative impacts.

4.159 As was reviewed in Chapter 2, there is considerable scientific and technical expertise in the field of land management which has a well developed capacity to assess and evaluate soils and prescribe remedial measures. Chapters 3 and 4 considered how plants and their transgenic derivatives may influence soil systems. These influences would be of particular concern if they were to reduce soil quality. The genetic modification of plants has the potential to influence soil quality. GM plants may, for example, have altered root architecture, altered decay of residues or altered root exudation. These types of differences can have effects on soil quality. While monitoring regimes would prefer to detect the negative impacts of GM plants before the degradation of soil quality, the definition and testing of soil quality provides an important arbiter of affects, impacts and damage.

4.160 Chapter 2 listed characteristics which are considered key soil functions:

- biomass production;
- regulation of water quality and quantity;
- regulation of the recycling of nutrients and other elements;
- provision of mechanical support for living organisms and their structures;
- carbon sequestration and regulation of carbon balance; and
- bioremediation of waste.

4.161 By assessing the impacts of transgenic plants on soil quality an assessment is made as to whether the functions of a particular soil, such as plant fertility and bioremediation of agrochemicals are being maintained in a sustainable manner. Unlike some assays of impact considered above, soil quality includes the development of criteria for distinguishing positive and negative effects.

4.162 Again summarising from Chapter 2, generally, an assessment of the impacts or processes on soil quality [3, 14, 17] proceed by:

- identification of soil functions or services, such as the regulation of water quality;
- identification of the soil attributes that influence or determine soil function;
- selection of a minimum data set (MDS) of indicators that may be used as measurable surrogates of the soil attributes; and
- quantification of change in the soil quality indicators. Changes in soil quality indicators are assessed by comparison against agreed threshold values.

4.163 It is recognised that a generic set of indicators is not attainable and that the choice of MDS indicators will include both generic and site specific considerations. An Environment Agency report [11] on the identification and development of a set of national indicators for soil quality lists examples of MDS variables. These variables are organised under the headings of site characteristics, soil type, vegetative cover, nutrients, organic carbon, soil chemistry, soil water characteristics, soil structure, soil

biology, contamination and soil management. Key indicators applied in many studies are pH, aggregate stability, organic matter level, soluble phosphorus, mineralisable nitrogen and electrical conductivity [21].

4.164 Chapter 2 reports a number of relevant indicators and the characteristics sought when selecting assays. Organic matter content is often a key assay providing an indicator of nutrient cycling, pesticide and water retention, soil structural stability, crop water availability and erosion control [206].

4.165 While this approach can detect areas of specific benefit or concern (the single indicator level) it may also integrate a variety of measures of beneficial and detrimental change into an evaluation in a weighted additive model [17, 19].

4.166 The evaluation of quantitative data, the role of critical limits, development of mathematical models, and the integration of several measures into a common assessment of soil quality and the development of pedotransfer function are areas identified in Chapter 2 as areas where progress would facilitate assessing impact on overall soil function.

4.167 Whether specific indicators or an integrated approach is taken, the essence of this aspect of monitoring is to ask whether the soil system retains its capacity to function, that is, is it healthy?

4.168 A common soil service which is important and potentially vulnerable is fertility. The vulnerability of fertility was tested in an evaluation of the non-target effects of transgenic potato lines expressing lectins to discourage feeding by invertebrate pests [132]. This study observed within season effects on soil protozoa, microbial activity (soil dehydrogenase) and on CLPP. This study went on to test for the effects of transgenic plants on the fertility of a subsequent winter barley sown on the plots previously used for two years to grow transgenic and non-transgenic potatoes. Some of the transgenic plots received an additional supplement of transgenic plant residues. This test was observed to integrate the effects of residue quality, decomposition, phytotoxicity and nutrient supply [132]. One transgenic cultivar was observed to have no negative effect on the barley crop. Another transgenic cultivar significantly reduced barley yield and this was assessed not to be a direct toxic effect of transgenic residues but an indirect effect of the physiology of the cultivar. The fertility of the barley crops were assayed by measurements of plant height, and length of the flag leaf (at flag leaf emergence stage (May)). These characteristics, together with ear size were measured during grain filling (July). The number and weight of grain was determined at senescence (August).

4.169 Microorganisms maintain soil quality through the biodegradation of pollutants, transforming a highly diverse collection of substrates, and often achieving their

mineralisation. In agriculture this activity degrades and detoxifies agrochemicals which would otherwise accumulate in the environment. Biodegradation, like many bacterial activities in soil, may be influenced by plant activity and its affects on microbial activity.

4.170 It is also known that the classes of chemicals released by roots can predispose bacterial populations to degrade homologous pollutants [207]. Poor degradation arising from low bacterial activity, a lack of suitable bacteria or recalcitrant pollutants may result in long residence times or escape of pollutants into water flows. Ideally, keystone assays, targeted assays and assays of diversity and activity should detect such an effect. However, it may be difficult to deduce this class of change from assays of bioactivity and biodiversity. Furthermore, the accumulation of contaminants may take some time to be significant. This type of effect involving diverse taxa and slow cumulative effects may be better addressed within the framework of periodic soil quality audits.

Observations on soil quality, risk assessment and monitoring of GM plants

4.171 A complication in risk assessment and monitoring is that a GM cultivar may have been produced to be grown with substantially different field management, for example altered times of sowing and different herbicide or pesticide applications. This limits the comparison of effects of transgenic plants and non-transgenic plants on soil systems. While some experiments can be devised to detect differences arising from the genetic modification, ultimately it is necessary to monitor and assess the GM cultivar when grown 'as intended'. By many measures, the soil system is likely to be noticeably different to any 'control'. In these circumstances it is necessary and useful to generate independent criteria for monitoring and judging the health of the soil system.

4.172 In terrestrial habitats such as agriculture and silviculture, modifications to plants and management practices may have impacts which are slow to accumulate or become apparent only in exceptional seasons. In these circumstances there is a requirement to be able to monitor the effects in real systems in many soils, crop rotations etc, for which there will often not be a 'true' control. The soil quality approach seeks an integrated definition of 'healthy soils' and 'degraded soils' recognising the ecosystem and other services provided. This approach then seeks to monitor the condition of the soil system services as directly as is practical.

EFFICACY OF THE METHODS FOR THE EVALUATION OF IMPACT

4.173 Assessment of the effect of GM plants and their associated field management will usually involve the identification and targeting of specific populations to assay for impacts and perhaps loss of function. It is also important to have broader assays of

the impacts on the soil bacterial communities to improve both the scope of the assessments and for the detection of unforeseen effects. However, no methods are available for exhaustive or even substantial inventories of soil bacterial community diversity with respect to taxa (species to 16S OTU levels), traits or physiology. The various measures of diversity used, including DNA-based and trait-based assays have different strengths and weaknesses. Some are more reliable measures of the actual community while others are subject to more bias, but may be more sensitive indicators of certain classes of change within community structure. Finding an appropriate level of sensitivity is important to avoid excessive noise or failure to indicate real change.

4.174 When considering the impacts of GM plants via changes in bacterial diversity it has to be understood that differences in community diversity/structure inevitably will be influenced by two processes which cannot be adequately separated. These are:

(i) Changes in the presence/absence of a taxa, traits or functions, i.e. the outcome of loss (extinction) and colonisation (inward dispersal). Given the limitations attendant on sampling these very diverse communities, an additional complication arises from those populations which appear to disappear but which just fall below the radar of detection. In fact the majority of populations (species, 16S OTUs for example) are not detectable for the inclusion in assays of community structure; and

(ii) Changes in the environmental conditions and cell physiologies result in altered levels of activity and in the altered probabilities of populations (or traits) being detected. It is obviously of some significance when detecting such impacts of GM plants whether the community changes reflect the plasticity within the community adjusting to conditions or whether the changes are more persevering or reflecting a more substantial change.

4.175 From some perspectives, it may be felt that these complex measures of community structure which simultaneously reflect presence/absence, relative numbers and relative activity are appropriate when relating whole communities to ecological process and function. The difficulty arises with not knowing the relative contribution of these factors to specific plant-soil situations, especially when change is observed.

4.176 Despite their limitations, general measures of bacterial diversity remain popular for assessment of the impacts of GM plants because:

(i) There is a spatially intimate association between soil bacteria and plants (roots and residues) involving, ecologically key, nutrient exchanges.

(ii) The bacterial community is a sensitive indicator of changes in plant activity, responding with changes in activity, community structure and community composition. Bacteria are useful sentinels, responding to a wide range of changes.

(iii) The conservation of diversity is considered a valuable aim in its own right;

(iv) Bacterial diversity is considered an important determinant of soil resistance and resilience; and

(v) Molecular methods are improving the reliability and practicality of assaying diversity.

Methods

4.177 Drawing on the review of methods and studies in the previous sections, the report will briefly re-cap on the methods used to assay diversity in soil bacterial communities and address their relative merits and limitations.

4.178 The gold standard for assaying community structure is the construction of clone libraries and sequencing or typing [156]. This approach no doubt has some problems with DNA extraction and cloning and other molecular biases. However, it probably comes the closest, at present, to estimating the real diversity distributions in nature. The difficulty with this approach arises with the processing effort required to accumulate the data needed from a even a small field trial.

4.179 Methods such as DGGE, TGGE, SSCP, LH-PCR and ARDRA are fairly responsive (but not sensitive) to changes which, by selection of primers, may be taxonomically or functionally broad or narrow. These methods do not provide reliable estimates of community structure and are limited by their lack of, or poor, quantification of relative abundances and are generally limited to pattern comparisons. Detection of treatment effects may none-the-less be improved using a quantitative approach to band content. Methods such as TRFLP [172, 200] are improving these approaches and provide some moderately quantitative abundance data.

- *Percentage guanine plus cytosine content (%G+C)* - this is a very broad approach, detecting only coarse similarities and differences in the proportions of bacteria sharing similar %G+C values [201, 202], [203]. Little or no data is available relating differences observed to soil community composition and function and this method therefore has limited utility in impact assessment.

- *Community level physiological profiles* - CLPP methods are common in soil ecology, providing information which can be related to changes in the soil habitat. This information is interpreted variously as useful for contrasting the catabolic potential of soils, measuring a notional community functional diversity (independent of taxonomic diversity) or indicating changes in (taxonomic) community structure [73, 130]. Generally these methods prove more sensitive to change than PCR-DNA fingerprint methods.

- *PFLA and FAME analysis* - PLFAs are used regularly in soil ecology to provide data on biomass, community structure and at high taxonomic levels, community composition [76]. FAME analysis may also be used to indicate gross changes in community structure [169].

- *Cultured bacteria* - although not so popular today in soil ecology, bacterial diversity can be investigated by the isolation of individuals on culture media followed by their taxonomic and or functional characterisation. Despite the constrained diversity which will grow on any medium, the method does provide quality data capable of detecting changes and relating taxonomic changes to functional changes [31, 168].

Are these methods useful?

4.180 Without repeating comments made in the above review of methods and GM plant impact studies it should be noted that all the methods available for assaying community structure have selective biases and that in a variety of conditions different community measures proved variously sensitive in detecting change. These studies show there is a lack of identified equivalence between these methods which are probably sensitive to different, but overlapping, changes in community structure. It will be easier to assess the differences if more researches referenced these methods against clone libraries. Given that different community measures will be sensitive to somewhat different combinations of components of community structure, it is sensible to use them in combination to improve their efficacy in detecting changes in populations and community structure. This corresponds to the need in GM impact assessment for a combination of measures responding to different types of change, such as bacterial, biomass/activity, taxonomic and physiological assays.

4.181 The gap between the diversity *in situ* and the diversity assayed may not matter too much if the majority of cells/biomass, (either totally, or associated with a specific group/function), in fact belongs to a relatively small number of taxa, for example 30-50 OTUs. This is conceivable and would mean that assays of a small but dominant set of populations would encompass the major part of ecosystem activities such as carbon flow. It is highly probable that these measures do assay an ecologically significant portion of the community. An element of doubt hangs over the large

number of numerically rare types which may make important contributions to the repertoire of community responses and hence resilience of a soil or make as yet unrecognised contributions to community interactions and functions.

4.182 An unexplored aspect of assessments of soil ecosystem function is the contribution of inward dispersal to community structure, function and responsiveness. This process clearly has the potential to buffer many impacts on bacterial communities. This dispersal may be genetic, via bacterial horizontal gene transfer, and results in a horizontal gene pool in which many key functions for soil health (especially the degradative and decontaminating activities) are mobile and not limited to specific species.

4.183 A serious weakness is apparent when these broad measures do detect significant transgenic plant effects and as yet no criteria are available to resolve whether harm is indicated or further assessment is necessary. Data from control plants, seasonal studies and different cultivars provides some indication of the scale of change expected within normal operating parameters. There is however no work calibrating these measures against defined models of harm.

A final observation

4.184 In assays of GM plant impacts, it may be that there is an imbalance between, on the one hand, assays of community diversity, which are common, and, on the other hand, assays of soil function, which are much less common. Often, assays of soil function (quality) would provide direct measures of the outcomes which community diversity is being used to assess. Co-collection of soil community and soil health data will also improve the 'at the time' assessment of the drivers and significance of any observed impacts.

5. SUMMARY

REVIEW OF SOIL SYSTEMS AS PROVIDERS OF ECOSYSTEM SERVICES

5.1 Different ecosystems and land uses have different requirements from soil functions. Six key function or services that are regarded as essential from all soils are:

- biomass production;
- regulation of water quality and quantity;
- regulation of the recycling of nutrients and other elements, both within the soil itself and the Earth's biosphere as a whole;
- provision of mechanical support for living organisms and their structures. This is viewed by some studies as including the support of man-made structures;
- carbon sequestration and regulation of carbon balance; and
- bioremediation of waste (the filtration, buffering, degradation, immobilisation and detoxification of organic and inorganic substances.

5.2 The capacity of a soil to provide and sustain these functions defines 'soil quality'. Soil quality assessment can involve unifying the multifunctional roles of soil in a single concept. Key indicators of soil quality include pH, aggregate stability, organic matter level, soluble phosphorus, mineralisable nitrogen and electrical conductivity.

Evaluation of soil quality

5.3 A number of indicators or properties are used to evaluate soil functions. Generally the stages are;

- identification of soil functions, such as the regulation of water quality;
- identification of the soil attributes that influence or determine soil function;

- selection of a minimum data set (MDS) of indicators that may be used as measurable surrogates of the soil attributes; and

- quantification of change in the soil quality indicators.

5.4 Changes in soil quality indicators are assessed against agreed threshold values and may be analysed at the specific indicator level or by integrated assessment incorporating all the indicators into the assessment via some form of weighted additive model.

5.5 Soil attributes are identified which are essential for the maintenance of particular soil functions. Soil attributes are themselves ascertained by the measurement of soil quality indicators. The MDS indicators chosen will include both generic indicators and specific indicators appropriate to the soil and functions assessed. These indicators are physical, chemical and biological. Common soil indicators and their relationship to soil function are presented and considered. One ubiquitous indicator of special merit is soil organic matter (SOM) which is the 'organic fraction of the soil exclusive of undecayed plant and animal residues'. The maintenance of SOM content is considered paramount in sustaining the quality of the soil and is reviewed in greater depth than other indicators.

REVIEW OF SOIL ECOLOGY AND THE INFLUENCE OF PLANTS

Plants are drivers

5.6 Plants have a key influence on the soil ecosystem, with organic material from plants root exudates and plant litter the major drivers. The type and quantity of these organic nutrients affects the numbers, activity and diversity of soil microbial communities. The influence of plants on soils is most acute in the rhizosphere, a zone surrounding roots where root exudates result in elevated numbers and activity of microbes (the rhizosphere effect).

The affects of plant productivity on the decomposer microflora and the soil fauna biomass

5.7 Despite the expected influence of plant productivity and rhizosphere effects there is evidence that bacterial levels in soil are frequently observed to be "top-down" regulated by predation. Fungal biomass is more likely to reflect the plant activity as these populations are more typically regulated by fungi-fungi competition. Both bottom-up regulation (availability of primary nutrients) and top-down regulation (predation) have been observed to affect varieties of soil fauna. However the relationship between plant activity, microbial biomass and soil fauna is multifaceted and often does not follow predictable patterns.

5.8 It is to be expected that changes in the plant community or in plant activity will affect changes to the soil system and that changes in nutrient flow from plants will affect the decomposers and detritivores. Nutrient cycling by microbes may be specifically affected by plants (in response to conditions such as herbivory) releasing nutrients and signals affecting bacterial gene expression and promoting the release of nutrients via protozoan predation. The complexity of plant microbe faunal interactions has implications for assessing and monitoring the impact of GM plants on soil ecology.

5.9 Consideration of the interactions between plants and three nutrient cycles (nitrogen-cycle, phosphorus-cycle and sulphur cycle) indicate some general features of nutrient recycling by mineralisation in soil systems. Notably, plant influences on nutrient cycling is mediated by a myriad of interactions in which the health (success) of the system is dependent on both generalist and specialist microbial populations which play key roles in resolving the availability of these essential nutrients.

Diversity is influenced by plants and soil management

5.10 The influence of plants on the diversity of soil biota may be direct (e.g. driven by exudates), or less direct as with cultivar associated changes to field management practices. The diversity (species diversity and functional diversity) of microbes in soil communities is influenced by and sensitive to many factors including plant species, root zone, water stress, fertilisation, field management, tillage, fungal disease, grassland improvement, nitrification and soil depth. The diversity of soil fauna are also influenced by soil and plant factors including nutrients, tillage, acid rain, fertilisers, biocides and heavy metals.

The role of diversity in ecosystems is controversial

5.11 Biodiversity is a prominent issue with current concerns that declining diversity will undermine ecosystems. However, the role of biodiversity in soil ecosystem function is controversial with some evidence supporting a strong role for diversity in ecosystem function and other evidence that soil function can be maintained with a relatively low diversity of species present. There is consensus that some minimum number of species is essential for ecosystem functioning under constant conditions and that a larger number of species is essential for maintaining the stability of the ecosystem process in changing environments.

The role of diversity in soil systems

5.12 There are a lack of reports supporting a causal link between diversity in soil communities and function of the soil system. Generally, there is no predictable relationship between microbial diversity or faunal diversity and function in soils.

Species richness is not an important factor in the overall function of the soil. However, understanding diversity remains a major focus of soil ecology.

5.13 The poor relationship between diversity and function in soils is often attributed to the substantial functional redundancy prevailing in the soil biota. Redundancy occurs when a number of species may fulfil the same ecosystem roles and some species may be lost without detrimental affects. Experimental reductions in diversity indicate that substantial diversity loss must be achieved to impair the function or resilience of a soil. Soil systems with high redundancy are considered likely to be more resilient when challenged. Experiments manipulating diversity have found some relationship between diversity and resilience.

5.14 It is increasingly thought that soil systems are regulated more by dominant species, their traits and the complex species interactions that arise, rather than the diversity of organisms present. Some researchers are now more concerned with the linkages between key species or functional groups and ecosystem function, rather than relationships between total soil diversity and ecosystem function.

Conclusions

- Soil diversity is primarily a result of the structure and conditions prevailing in a soil.
- There is no consistent relationship between diversity and ecosystem function in soils.
- The dominant species and food web structures, with their complex interactions, are probably the key biotic factors in the function of soil systems.
- Changes in diversity are sensitive indicators of perturbation.
- Diversity *per se* may be valuable as a source for resilience (more research needed).

REVIEW OF THE POTENTIAL IMPACTS OF GM CROPS ON THE SOIL ECOSYSTEM AND APPROACHES TO THEIR ASSESSMENT AND MONITORING

5.15 A review has been made of studies of the impacts of transgenic plants on soil systems. The observations of this review are summarised here:

- Considerations of the transgene product, its activity, site of expression and persistence are important guides to developing, on a case by case basis, lines of investigation into likely or important potential effects.

- Most transgenic plants have detectable effects on the soil system which are relatively minor compared with differences between cultivars or those associated with weather and season. Assays of the natural variation in the system provide valuable baseline references.

- The response of soil systems when transgenic plants are removed provides an important measure of impact. Studies generally find a quick return of many soil parameters to match those of the control soils.

- Many apparent losses of taxa observed in field monitoring are probably changes in the structure (relative numbers) rather than the composition of the community.

- Regular sampling is important as changes in community structures through the season and plant development confer an added level of complexity to comparing transgenic-non-transgenic effects. Many of these effects are context dependent and not systematic in character through the season.

- Arising from the case by case approach, specific targets for monitoring are established which have intrinsic and clear definitions of damage. However, there is a lack of monitoring activity which is linked to a concept of damage to the system.

Approaches for the assessment of the impact of GM plants on soil ecology

5.16 The development of environmental risk assessments for GMPs has been reviewed. The basis of any assessment approach is that it is not possible to assay all aspects of a soil system receiving GM plants. Generally this process will start by taking account of whether the introduced genes are expressed (as opposed to regulatory), the activity of their products and when and in what plant tissues are they expected to be active. Whilst risk assessment and monitoring must proceed on a case by case basis there is need for a systematic approach, rooted in soil ecology, which can guide the making of assessments and establish relevant research targets. In practice, risk assessments and monitoring are tiered, starting in the laboratory and scaling up to the variety and complexity of conditions in full field trials. Field trials are important as observed behaviours do not always 'scale up' from the laboratory and greenhouse to the open environment.

5.17 The approaches and methods available for assessing and monitoring the risk to the soil ecosystem are presented, under the following three categories:

1. GM plant effects: keystone and important groups and processes

2. GM plant effects: broad measures of the soil system, diversity and activity

3. GM plant effects: soil quality

1. GM plant effects: keystone and important groups and processes

5.18 The use of keystone indicators is sensible as it is inconceivable to assay all components in a soil system, and it allows the assessment strategy to focus on those organisms whose loss is likely to have a significant effect on soil ecosystem function. A number of criteria for choosing these indicators are reported; ecological significance, susceptible to change, pertinent, low redundancy, practical and validation.

5.19 A number of keystone groups are reviewed; mycorrhizal fungi, plant growth promoting rhizobacteria (PGPR), wood lignin decomposing fungi, nitrogen fixation, and nitrifying bacteria. In some cases, the type of transgenic plant (plant or modification) will influence the choice of more specific keystone indicators, in addition to (or instead of) those listed. The identification and targeting of likely or important potential effects of GMPs is an important factor in resolving their testing and ultimately their field assessment.

2. GM plant effects: broad measures of the soil system, diversity and activity

5.20 Despite the use of keystone groups to detect perturbations to the soil ecosystem, there is a recognised need for broader assays of impacts on the soil microbial and faunal communities to improve sensitivity and detection of unforeseen effects. Such monitoring requires a combination of measures responding to different types of change including; microbial, faunal, biomass, activity, and diversity to improve the detection of impacts.

5.21 When different effects are detected from transgenic and non-transgenic plants, it is essential to have criteria to resolve whether results are satisfactory or whether they warrant further investigation. Differences typical of cultivar-cultivar or field management effects may be considered to be within a ‘normal’ range and therefore not indicative of harm. This underlines the value of regular sampling over a reasonable period with sufficient replicates to establish a realistic measure of the natural variation in the system.

Population size and activities

5.22 Population size and activity of bacteria, fungi and fauna are complex and sometimes insensitive as ecosystem measures. Despite this, these approaches remain popular because;

(i) standard measures of population size and activity are available,

(ii) they provide ecologically basic data, and

(iii) they have the potential to detect interesting or significant differences.

Biodiversity

5.23 Biodiversity is one of the most common broad measures of impact in the soil system and is a favoured assay because:

- diversity is a useful sentinel, detecting changes arising from a wide range of causes;
- the conservation of diversity is considered a valuable aim in its own right;
- diversity is considered an important determinant of soil resistance and resilience; and
- molecular methods are improving the reliability and practicality of assaying diversity.

Diversity of soil fauna

5.24 Diversity of soil fauna provides an opportunity to assay at distinct trophic levels including bacterivores, fungivores, herbivores and predators to soil fauna. GM plant effects on the soil fauna have the potential to affect the microbial community, nitrogen mineralisation and plant growth. The utility of nematode and soil microarthropod community structures as bioindicators in environmental monitoring is considered. These have the capacity to be characterised and analysed at functional group levels and taxonomic levels.

Diversity of soil microbes

5.25 This is sensitive to change and is established in intimate physical and nutrient flow association with plant roots and plant residues. As primary consumers of plant

exudates and plant material, microbes are good indicators of impacts associated with plant modification. Assaying for impacts in the microbial community is also prudent because of its key roles in nutrient cycling and soil quality.

5.26 Bacterial and fungal diversities are investigated using a variety of techniques which are considered. These techniques define diversity in various ways (functional, physiological, taxonomic, by isolate, by sample and various others). The techniques also provide a variety of assays varying in sensitivity and level of focus, and typically a small number of these are chosen to extend the scope of the assay.

3. GM plant effects: soil quality

5.27 The ultimate aim of targeted keystone assays and assays of diversity and activity is to anticipate or detect negative impacts. A further approach is to assay more directly for changes to the ecosystem and other services provided by the soil, that is, changes to soil quality. These services include biomass production, regulation of water quality and quantity, regulation of the recycling of nutrients and other elements, provision of mechanical support for living organisms and their structures, carbon sequestration and regulation of carbon balance, and bioremediation of waste. Whilst monitoring regimes would prefer to detect the negative impacts of GM plants before the degradation of soil quality, the definition and testing of soil quality provides an important arbiter of affects, impacts and damage.

5.28 By assessing the impacts of transgenic plants on soil quality an assessment is made as to whether the functions of a particular soil, such as plant fertility and bioremediation of agrochemicals are being maintained in a sustainable manner. Soil quality includes the development of criteria for distinguishing positive and negative effects. Usually, a minimum data set of both generic and site specific indicators are chosen. Common indicators include pH, aggregate stability, organic matter level, soluble phosphorus, mineralisable nitrogen and electrical conductivity. Data collection and analysis can focus on specific indicators or generate integrated models of soil condition.

5.29 The soil quality approach may have specific utility in long term monitoring, in post commercialisation monitoring, in monitoring where non-transgenic controls are not good equivalents, and in refocusing these issues on agricultural practice in general.

6. ASSESSMENT OF ISSUES WHICH NEED TO BE ADDRESSED BY FUTURE RESEARCH

6.1 A broad range of plant traits are targets for modification which will affect factors such as plant growth, herbivory, agrochemical applications, and include the expression of novel products. As discussed, plants maintain a variety of intimate interactions with the soil ecosystem, and it should therefore not be unexpected that GM plants are likely to have some effect on the soil system. The challenge is to anticipate, monitor and understand the effects of GM plants on soil ecosystems.

DIVERSITY

6.2 While diversity *per se* is no longer regarded as a determinant of soil function, it remains at the centre, both conceptually and practically, of soil ecology, risk assessment and monitoring. It is intriguing that a soil under two regimes (different crops or crops-fallow) will display significant differences in diversity, yet will reconverge to a common pattern of diversity when brought under a common regime. This implies something more conserved about patterns of diversity in soils.

6.3 There is still an inadequate understanding of how community diversity is assembled and the role of factors such as dispersion. Many monitored changes in community diversity are structural (relative proportions rather than species counts) and are evaluated using complex measures or indices. These measures provide a convenient way of comparing soils but there is a paucity of data relating their magnitude to ecosystem measures. The monitoring of GM plants would benefit from a better and more predictive understanding of the operation of diversity within soil systems. Key issues are:

- What causes the observed changes in soil diversity?
- What is the relationship between biodiversity and soil resistance and resilience?
- What is the relationship between reductions in diversity and soil damage and degradation?

- Can threshold values be established for acceptable reductions in diversity?
- What is the significance of diversity for suppressive soils?
- Can indicator groups be identified (ability to measure key biological indicators is essential for a monitoring strategy)?
- Better standardisation of data collection to facilitate models of soil similarities and differences.

SOIL ECOSYSTEM FUNCTION

6.4 Risk assessments and the monitoring of the effects of GM plants need to be rooted in strong models of soil ecosystem function. Improvements in the conceptual and methodological tools of soil ecology are continuous and a variety of methods have accelerated the progress of microbial ecology, providing the tools for the investigation and dissection of soil communities. Though many of these techniques are too detailed for use in a monitoring context, they are improving our understanding of soil ecology and thus improving the confidence with which risk assessment and monitoring decisions are taken.

6.5 Two research areas in soil ecology (soil food web interactions and soil spatial heterogeneity) can be identified which have the most potential to improve both our understanding of soil ecology and our assessment and monitoring of GM plants. These areas of study should lead to improved models of function and vulnerability, sampling strategies and identification of keystone groups or processes.

(i) In models of seven community food webs using empirical data from native and agricultural soils, the patterns of top-down and bottom-up interactions were resolved to play an important role in community stability [208]. Food web structure is considered a key determinant of ecosystem productivity in which the effects of soil fauna on soil system function are reasonably predictable and the effects of reductions in diversity can be anticipated from their trophic positions and the functional redundancy within trophic levels [209]. Food web studies have been hampered below ground by difficulties in assigning functional roles within very diverse communities of microorganisms, though the availability of stable isotope methods shows promise for the analysis of food web interactions in soil [210]. There is evidence that soil ecosystem function is resolved primarily by dominant species and the complex interactions of soil food webs, and it has been proposed that these interactions and their sensitivity to environmental change should be research priorities [68].

(ii) Integrating studies of spatial heterogeneity into soil ecology is thought to have serious potential to improve our basic understanding of soil ecology. It is recognised that spatial soil ecology can provide insights into the regulation of soil biodiversity, coexistence and the effects of soil communities on plant growth and plant diversity [211]. Spatial approaches to soil ecology, the study of spatial heterogeneity in the distribution of populations and activities of soil organisms at scales ranging from the bacterial to the field or larger will underpin a better understanding of the behaviour of diversity and strategies for sampling.

Keystones

6.6 There are many examples of specific groups or processes being targeted in the monitoring of the impacts of a GM plant. There is, however, little evidence of the incorporation of keystone groups simply as indicators of ecological perturbations in the soil ecosystem. The situation may be improved by the development of keystone assays yielding more ecologically interesting information. Proposals for additional keystones groups and processes are needed and will probably emerge from progress in the fields of soil system ecology and soil quality.

Damage and degradation

6.7 In the compilation of this report, very few examples of damage to a soil system function arising from the introduction of a GM plant and subsequent perturbation of the microbial community were encountered. Similarly lacking are examples of cases where a reduction in microbial diversity has led to a functionally significant outcome. However, Hirsch *et al*., (1993) [212] have reported that soils treated with sewage and thus contaminated with heavy metals lost their genetically diverse rhizobial populations which were efficient nodulators and fixers of nitrogen for clover growing in the soil. The depleted rhizobial population that did persist in the sewage-contaminated soil comprised ineffective nodulators, resulting in a loss of nitrogen fixation to the system. Generally, however, there is a lack of studies relating perturbations in the soil community to soil system function and damage.

6.8 Arising from the case by case approach, some specific targets for monitoring have been established (e.g. loss of plant pathogen antagonists) which have intrinsic and clear definitions of damage. However, there is generally a poor understanding of the relationship between changes in the factors measured (e.g. CLPP, DGGE etc) and changes in soil function. Whether at the keystone level, or at the soil quality level a key task is defining damage, degradation, or losses which leave a soil vulnerable to future damage.

6.9 Research is needed to provide:

- GM plant relevant definitions of damage, degradation, resistance and resilience.
- A better understanding of the relationship between soil factors (diversity, activity, etc) and soil function.
- Baseline or threshold values, where possible, for assessing damage to the soil system.
- Standardised assays of resilience and resistance.
- Experimental data relating the indicators in use to examples of real damage.
- Guidance on the appropriate timescale for monitoring.
- Studies of the experiences of predicting effects - scaling up from laboratory to greenhouse, to field plots and more extensive plantings.

SOIL QUALITY

6.10 The soil quality approach asks whether a soil is functional in providing the ecosystem services and other services required. Soil quality monitoring recognises that the impacts of GM plants in terrestrial habitats such as agriculture and silviculture may have impacts which are slow to accumulate or become apparent only in exceptional seasons. There is a need for monitoring criteria which can be extended beyond the transgenic/non-transgenic control experiment to make short term and long term assessment of soil system outcomes.

6.11 Harris (2003) [213], concerned with land restoration, has proposed that measuring specific soil microbial community characteristics can be used to assess the status of the microbial ecosystem and with it, a soil's quality and potential for restoration. The key measurements proposed were the size, composition and activity of the soil microbial community especially profiles of PLFAs and substrate induced respiratory responses to different carbon substrates

6.12 It has also been proposed [214] that the application of soil quality measures to sustainable land management would be increased by research to:

- Demonstrate causal relationships between soil quality and ecosystem functions.
- Increase the power of soil quality indicators to predict response to disturbance.
- Increase accessibility of monitoring systems to land managers.
- Integrate soil quality with other biophysical and socio-economic indicators.
- Place soil quality in a landscape context.

STANDARDISATION AND IMPROVING CRITERIA

6.13 Monitoring of soil systems is marked by high specificity with distinct approaches, a lack of standardised assay procedures and a low profile for theory and ecological models. A subset of standardised assays is needed to improve the utility and our understanding of the monitoring process. It has been proposed for example that US researchers using standardised assays would improve the quality of the biosafety data, particularly if it were also collected after commercial release [215]. The monitoring process and the study of soil ecology should be in symbiosis here.

6.14 Considerable expertise and skill resides within the communities of soil scientists, soil ecologists, agronomists and land managers. This resource could provide the multidisciplinary approach needed to establish criteria for the risk assessment and monitoring of the release of GM plants. Such an exercise, however, may ultimately be better contained within a wider assessment of the influence of agronomic and land management practices.

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