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Microorganisms as indicators of soil health

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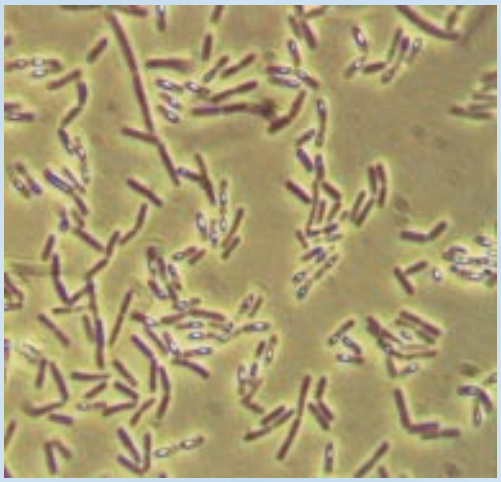

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2002

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Data sheet

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Abstract: Microorganisms are an essential part of living soil and of outmost importance for soil health. As such they can be used as indicators of soil health. This report reviews the current and potential future use of microbial indicators of soil health and recommends specific microbial indicators for soil ecosystem parameters representing policy relevant end points. It is further recommended to identify a specific minimum data set for specific policy relevant end points, to carefully establish baseline values, to improve scientific knowledge on biodiversity and modelling of soil data, and to implement new indicators into soil monitoring programmes as they become applicable.

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Preface

This report treats the use of microbial indicators in terrestrial monitoring programmes and provides recommendations for their implementation in a Danish terrestrial monitoring programme.

The report is divided into two parts: Part I presents the current knowledge on the use of microbial indicators in terrestrial monitoring with focus on monitoring of soil health. This includes advantages and disadvantages of using microorganisms as indicators, and considerations concerning data sampling, handling and evaluation. Finally, recommendations and research needs for implementation of microbial indicators in a terrestrial monitoring programme are presented. Part II is a detailed catalogue and description of i) microbial indicators already in use in some monitoring programmes and ii) potential new indicators that may provide more precise, detailed and integrated results necessary for a dynamic up-to-date monitoring programme.

Workshop on microbial indicators in soil health monitoring

Microbial indicators are used in some soil monitoring programmes in Europe. We found that an overview of these activities would be necessary before implementing a terrestrial monitoring programme in Denmark. A thorough understanding of the approaches and concepts used in these countries would allow us to adequately represent a state-of-the-art programme including the major strategies and implementation problems faced by others. Thus, a two-day workshop on "Microbial Indicators of Soil Health" was held in June 2001 at Department of Microbial Ecology and Biotechnology, National Environmental Research Institute (NERI), Roskilde, Denmark. Participants were Dr. Jaap Bloem (Alterra Green World Research, The Netherlands), Dr. Colin Campbell (Macaulay Land Use Research Institute, Scotland), Dr. Oliver Dilly (Kiel University, Germany), Dr. Paul Mäder (Forschungsinstitut für Biologischen Landbau (FiBL), Switzerland), MSc. Torben Moth Iversen (Deputy Director of NERI) and scientists from Department of Microbial Ecology and Biotechnology, NERI: Dr. Svend Binnerup, Dr. Bjarne Munk Hansen, Dr. Niels Bohse Hendriksen, Dr. Ulrich Karlson, Dr. Niels Kroer, Dr. Hap Pritchard and the authors of this report. Information from both the presentations and the following discussions are included in this report. The presentations dealt specifically with soil monitoring activities in which use of microbial indicators is included. The discussions mainly focused on the suitability of microbial indicators for soil monitoring activities (Part I chapter 4 and Part II), practical aspects of implementation and interpretation of data (Chapter 5).

Acknowledgements

We would like to thank the foreign scientists participating in the workshop together with Dr. Bo Stenberg (Swedish University of Agricultural Sciences, Sweden), and Dr. Heinrich Höper (Niedersächsisches Landesamt für Bodenforschung, Germany) for their inspiring presentations, discussions and contributions to this report. We also would like to acknowledge Dr. Hap Pritchard and Dr. Ulrich Karlson, Department of Microbial Ecology and Biotechnology, NERI, for critically reading the text. Department of Microbial Ecology and Biotechnology, NERI financed the report.

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Executive summary and recommendations

This report reviews current knowledge on the use of microbial indicators in terrestrial monitoring and gives suggestions for the implementation of new microbial indicators. It is our hope that the report will be a source of inspiration and guidance for the design of a Danish terrestrial monitoring programme.

Soil is part of the terrestrial environment and supports all terrestrial life forms. Soil health is the result of continuous conservation and degradation processes and represents the continued capacity of soil to function as a vital living ecosystem. A unique balance of chemical, physical and biological (including microbial) components contribute to maintaining soil health. Evaluation of soil health therefore requires indicators of all these components. The report specifically emphasises the important contribution by soil microorganisms to soil health and the pros and cons of using microorganisms as early warning indicators of environmental changes.

It is concluded that microorganisms appear to be excellent indicators of soil health because they respond quickly to changes in the soil ecosystem and have intimate relations with their surroundings due to their high surface to volume ratio. In some instances, changes in microbial populations or activity can precede detectable changes in soil physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil degradation. Since microorganisms are involved in many soil processes, they may also give an integrated measure of soil health, an aspect that cannot be obtained with physical/chemical measures alone.

Any monitoring programme will be based on indicators selected for specific purposes. We propose to direct these indicators towards policy relevant end points that cover different aspects of soil health. For the use of microbial indicators in a terrestrial monitoring programme the following is recommended:

- **Identification of specific minimum data sets for specific end points**

A minimum data set (MDS), that is a limited number of indicators, will be required in the development of a monitoring programme due to costs and labour. We recommend a specific MDS for each policy-relevant end point. For example, monitoring the leaching of nitrate or pesticides to groundwater requires a MDS composed of microbial indicators for N-cycling and bioavailability. On the other hand, monitoring ecosystem health, that is the overall state of the environment, requires a MDS composed of a broader range of indicators, e.g. microbial biomass, activity, and biodiversity. Recommendations for specific MDSs are summarised in Table 6.

- **Establishment of baseline values**

Baseline values on the selected microbial indicators, including information on both spatial and temporal variations, have to be known or

developed within the first year of monitoring to define reference and threshold values for repeated monitoring activities. Characterisation of the sampling sites by physical and chemical properties should be obtained simultaneously.

- **Improvement of the scientific basis**

It is recommended that further scientific knowledge should be developed through research activities included in the monitoring programme to provide part of the scientific base for new management policy at the national and international level. Specifically, research on microbial biodiversity should be in focus. This is consistent with recommendations made by the Wilhjelm committee, a working group nominated by the Danish government to formulate a national strategy for biodiversity and Nature conservation. We recommend that these research activities on microbial indicators should cover:

- relationship between genetic and functional biodiversity
- modelling of data as a way to predict soil health
- statistical considerations and modelling as means of optimising an up-to-date monitoring programme by identifying relevant indicators and evaluating number of samples, sampling areas, and frequency of sampling

- **Implementation of new indicators**

Implementation of new indicators is recommended as soon as these are applicable for soil monitoring purposes. These new indicators should be based on continuous development of microbial methods within the scientific community and will provide more precise, detailed and integrated results, and give a dynamic up-to-date monitoring programme. Implementation is recommended in parallel with existing measurements to assure the quality and comparability of the new indicator as the old indicators are phased out. The data sets of the new indicator can be used as the baseline for future monitoring activities.

Udvidet sammendrag og anbefalinger (Danish executive summary and recommendations)

Nærværende rapport gør status over brugen af mikroorganismer som miljøindikatorer i overvågning af den terrestriske natur. Rapporten giver ligeledes anbefalinger for implementering af mikrobiologiske indikatorer i et dansk terrestrisk overvågningsprogram. De levende organismer i jorden er en vigtig del af den terrestriske natur og er således vigtige for opretholdelse af jordens sundhed. Jordens sundhed er resultatet af kontinuerte nedbrydnings- og opbygningsprocesser og er karakteriseret ved jordens kapacitet som levende økosystem. En unik balance mellem kemiske, fysiske og biologiske (inkl. mikrobiologiske) faktorer bidrager til opretholdelse af jordens sundhed. En vurdering af jordens sundhed skal derfor baseres på alle disse faktorer. Denne rapport fokuserer på mikroorganismers betydning for jordens sundhed og fordele og ulemper ved at anvende mikroorganismer som indikatorer for ændringer i miljøet.

Mikrobiologiske indikatorer udmærker sig specielt ved at kunne varsle ændringer i jordmiljøet meget tidligt i forhold til for eksempel fysisk-kemiske faktorer. Dette skyldes primært at de har en tæt kontakt til jordmiljøet på grund af en stor overflade i forhold til deres volumen. Mikroorganismerne er desuden involveret i mange processer i jorden, hvilket medfører at én enkelt måling vil kunne afspejle flere processer. Mikrobiologiske målinger integrerer således jordens sundhed på en måde som ikke opnås ved brug af fysisk-kemiske målinger alene.

Indikatorer er et vigtigt redskab i ethvert overvågningsprogram og disse bør udvælges på baggrund af programmets formål. Denne udvælgelse bør endvidere baseres på politisk relevante målsætninger, som dækker forskellige aspekter af jordens sundhed. For brug af mikrobielle indikatorer i et terrestrisk overvågningsprogram anbefales følgende:

- **Identifikation af specifikke indikatorsæt for specifikke målsætninger**

Som følge af økonomiske og arbejdsmæssige omkostninger vil kun et begrænset antal indikatorer kunne indgå i et overvågningsprogram. Sådant et begrænset antal indikatorer, kaldet et minimum datasæt (MDS), anbefales for hver politisk relevante målsætning for jordens sundhed. For eksempel vil overvågning af nitrat- eller pesticidudvaskning kræve et indikatorsæt bestående af mikrobielle indikatorer for N-omsætning samt for den biologiske tilgængelighed af de pågældende stoffer. Overvågning af jord-økosystemets sundhed, dvs. jordmiljøets generelle tilstand, vil på den anden side kræve et bredere indikatorsæt. Dette kunne for eksempel være sammensat af indikatorer for den mikrobielle biomasse, aktivitet og biodiversitet. Anbefalinger af forskellige indikatorsæt findes i Tabel 6.

➢ **Fastlæggelse af baggrundsværdier**

Baggrundsværdier for de udvalgte indikatorer, herunder rum- og tidsmæssige variationer, bør kendes eller indsamles i løbet af overvågningsprogrammets første år for at definere referenceværdier og skadestærskler. Karakterisering af lokaliteternes fysisk-kemiske egenskaber bør registreres sideløbende.

➢ **Udbyggelse af videngrundlag**

Det anbefales, at et videngrundlag angående den mikrobielle biodiversitet opbygges gennem forskningsaktiviteter etableret i overvågningsprogrammet. Det vil udgøre et delelement af det videnskabelige grundlag for den fremtidige nationale og internationale naturforvaltning. Dette er i overensstemmelse med anbefalingerne fra Wilhjelm-udvalget, en arbejdsgruppe udpeget af den danske regering i forbindelse med udarbejdelsen af et grundlag for en national handlingsplan for biologisk mangfoldighed og naturbeskyttelse. Vi anbefaler, at forskningsaktiviteterne indenfor mikrobiologiske indikatorer dækker:

- sammenhængen mellem genetisk og funktionel biodiversitet
- modelberegninger til at forudsige jordens sundhed
- statistiske overvejelser og modelberegning til brug for udpegning af de mest optimale indikatorer samt vurdering af antal lokaliteter, prøver og indsamlingsfrekvens. Dette vil medvirke til at opretholde et tidssvarende overvågningsprogram.

➢ **Inddragelse af nye indikatorer**

Inddragelse af nye indikatorer på baggrund af den fortløbende videnskabelige udvikling af mikrobiologiske metoder anbefales at ske så snart disse er brugbare til overvågning. Inddragelse af nye indikatorer vil give mere præcise, detaljerede og integrerede resultater og være forudsætningen for et tidsvarende overvågningsprogram. Inddragelsen bør ske sideløbende med brug af den eller de eksisterende indikator(er) som skal erstattes, for at sikre kvaliteten og sammenligneligheden af den nye. Data for den nye indikator vil herefter kunne bruges som baggrundsværdier og dermed i udviklingen af referenceværdier og skadestærskler.

Part I
Microorganisms as indicators of soil health

1 Introduction

Intensification of agriculture is one of the major impacts to the Danish soil environment, as agriculture accounts for two-third of the land use (OECD 1999). Adverse impacts of agriculture include loss of biodiversity, nitrogen discharges into surface water, eutrophication of surface water, contamination of groundwater from pesticides and nitrate, and ammonia volatilisation due to over-fertilisation with manure (OECD 1999). These impacts are exacerbated by infrastructure development, increasing urbanisation, waste disposal and forestry practices (Ministry of the Environment 2000).

Healthy soils are essential for the integrity of terrestrial ecosystems to remain intact or to recover from disturbances, such as drought, climate change, pest infestation, pollution, and human exploitation including agriculture (Ellert *et al.* 1997). Protection of soil is therefore of high priority and a thorough understanding of ecosystem processes is a critical factor in assuring that soil remains healthy (Wilhjelm committee 2001).

National Monitoring of Water and Nature (NOVANA)

Protection of Nature and especially biodiversity is the main focus of the Rio Convention of 1992, which is agreed by Denmark and many other countries (Wilhjelm committee 2001). *Biodiversity* is defined as the variability among living organisms and include diversity within species, between species and of ecosystems. The term *ecosystem* covers a dynamic complex of plant, animal and microorganism communities and their non-living environment interacting as a functional unit. Protection of Nature and biodiversity in Denmark was subsequently recommended by OECD in 1999 to be covered by a nationwide monitoring programme for both terrestrial and aquatic environments (OECD 1999). This recommendation was adopted by the Wilhjelm Committee in 2001 (Wilhjelm committee 2001). The Wilhjelm committee is a working group nominated by the Danish government to establish the basis for a national strategy for biodiversity and Nature conservation. Parallel to this, the European Council has agreed on a directive on the conservation of natural habitats and of wild fauna and flora, the so-called Habitat Directive (Council Directive 92/43/EEC, 1992). This directive is directed to preservation of endangered habitats, animals and plants within the EU. A strategy for a Danish environmental monitoring programme called NOVANA (National Monitoring of Water and Nature) has been worked out (Iversen *et al.* 2001). In addition to the existing NOVA (National Monitoring of Water) programme, terrestrial monitoring will be included with the aim of meeting the obligations of the Habitat Directive and the recommendations by the Wilhjelm Committee. Thus, NOVANA will be integrated into the national environmental policy and become part of the strategic plans for Nature and environment in

Denmark. It is intended that NOVANA should be implemented by January 2004 (Iversen *et al.* 2001).

European soil monitoring programme

The need for a systematic approach to protect soil ecosystems within Europe has been described in the draft report of the Sixth Environmental Action Programme "Environment 2010: Our future, Our Choice", which was presented by the European Commission in the beginning of 2001 (Huber *et al.* 2001). A European monitoring and assessment framework on soil has subsequently been proposed to provide policy-makers with relevant information on soil and to bring together the wealth of soil information derived from current national soil monitoring programmes (Huber *et al.* 2001). Special emphasis will be on comparing biological properties with physical or chemical properties (Huber *et al.* 2001). Microorganisms as indicators of environmental impacts in soil monitoring is the objective of the EU COST Action 831 (www. isnp.it/cost/cost.htm), a cooperative project by scientists.

Danish legislation on soil protection

A variety of environmental protection programmes are implemented in Denmark (reviewed by OECD in 1999 (OECD 1999)). None of them, however, directly address soil. The current Nature Protection Act primarily addresses habitat protection, and the directives on nitrate, sewage sludge, and habitat preservation aim primarily at protecting other environmental compartments (water and the food chain), which individually may also result in protection of soil (Huber *et al.* 2001).

Objective of Danish terrestrial monitoring

A long-term terrestrial monitoring programme with the objective to follow the state of the terrestrial environment in Denmark has been proposed (Iversen *et al.* 2001). It is proposed to include monitoring of important natural areas, biodiversity, and the impact of xenobiotics and climate changes. Monitoring activities will, according to present plans, primarily concentrate on vegetation, fauna and abiotic properties. Monitoring of soil is not explicitly mentioned, but as soil supports all life forms in the terrestrial environment, terrestrial monitoring without soil monitoring is incomplete. The monitoring strategy will consist of both extensive monitoring of many small areas and intensive monitoring of a few large areas with high priority. The monitoring activities will be designed to discriminate between natural variations and human induced changes, including impacts of policy management.

2 Soil health

To manage and maintain soil in a sustainable fashion, the definition of soil health must be broad enough to encompass the many functions of soil, e.g. environmental filter, plant growth and water regulation (Doran *et al.* 1997). Definitions of air and water quality standards have existed for a long time, while a similar definition does not exist for soil. There is, however, little if any parallel between air or water quality and soil health (Sojka *et al.* 1999). Air and water quality standards are usually based on maximum allowable concentration of materials hazardous to human health. A definition of soil health based on this concept would encompass only a small fraction of the many roles soil play (Singer *et al.* 2000). Soil health is the net result of on-going conservation and degradation processes, depending highly on the biological component of the soil ecosystem, and influences plant health, environmental health, food safety and quality (Halvorson *et al.* 1997; Parr *et al.* 1992).

Definition of soil health

Several definitions of soil health have been proposed during the last decades. Historically, the term *soil quality* described the status of soil as related to agricultural productivity or fertility (Singer *et al.* 2000). In the 1990s, it was proposed that soil quality was not limited to soil productivity but instead expanded to encompass interactions with the surrounding environment, including the implications for human and animal health. In this regard, several examples of definitions of soil quality have been suggested (Doran *et al.* 1994). In the mid-1990s, the term *soil health* was introduced. For example, a programme to assess and monitor soil health in Canada used the terms *quality* and *health* synonymously to describe the ability of soil to support crop growth without becoming degraded or otherwise harming the environment (Acton *et al.* 1995). Others broadened the definition of soil health to capture the ecological attributes of soil, and went beyond its capacity to simply produce particular crops. These attributes are chiefly associated with biodiversity, food web structure, and functional measures (Pankhurst *et al.* 1997). In 1997, Doran & Safley (Doran *et al.* 1997) proposed the following definition of soil health:

- *The continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health*

The definition encompasses a time component, reflecting the importance of continuous functions over time and the dynamic nature of soil. Soil health thus focuses on the continued capacity of a soil to sustain plant growth and maintain its functions regardless of the fitness for any certain purposes (Pankhurst *et al.* 1997). Examples of dynamic soil properties could be organic matter content, the number or diversity of organisms, and microbial constituents or products (Singer *et al.* 2000). We have adopted the definition by Doran & Safley in the present report.

Soil health and human health

Soil is a finite and non-renewable resource because regeneration of soil through chemical and biological weathering of underlying rock requires geological time (Huber *et al.* 2001). Deterioration of soil, and

thereby soil health, is of concern for human, animal and plant health because air, groundwater and surface water consumed by humans can be adversely affected by mismanaged and contaminated soil (Singer *et al.* 2000). As such, deteriorating soil health and the benefits of soil management has become of political concern. A healthy soil functions to buffer nutrients as well as contaminants and other solutes via sorption to or incorporation into clay particles and organic materials. The soil itself thus serves as an environmental filter for removing undesirable solid and gaseous constituents from air and water (Parr *et al.* 1992). The extent to which a soil immobilises or chemically alters substances that are toxic, thus effectively detoxifying them, reflects the degree of soil health in the sense that humans or other biological components of the system are protected from harm (Singer *et al.* 2000).

Physical, chemical and biological components of soil

Soil is dominated by a solid phase consisting of particles of different size surrounded by water and gases, the amount and composition of which fluctuate markedly in time and space. Water is normally discontinuous, except when the soil is water saturated. The pore space without water is filled with air and other gases and volatiles (Stotzky 1997). There is continual interchange of molecules and ions between solid, liquid and gaseous phases which are mediated by physical, chemical and biological processes (Doran *et al.* 1994). These processes represent a unique balance between physical, chemical and biological components (Doran *et al.* 1994). Maintaining this balance is of great importance to soil health.

Microorganisms have key functions in soil

The biological activity in soil is largely concentrated in the topsoil, the depth of which may vary from a few to 30 cm. In topsoil, the biological components occupy a tiny fraction (<0.5%) of the total soil volume and make up less than 10% of the total organic matter in soil. These biological components consist mainly of soil organisms, especially microorganisms. Despite their small volume in soil, microorganisms are key players in the cycling of nitrogen, sulphur, and phosphorus, and the decomposition of organic residues. Thereby they affect nutrient and carbon cycling on a global scale (Pankhurst *et al.* 1997). That is, the energy input into the soil ecosystems is derived from the microbial decomposition of dead plant and animal organic matter. The organic residues are, in this way, converted to biomass or mineralised to CO_2, H_2O, mineral nitrogen, phosphorus, and other nutrients (Bloem *et al.* 1997). Mineral nutrients immobilised in microbial biomass are subsequently released when microbes are grazed by microbivores such as protozoa and nematodes (Bloem *et al.* 1997). Microorganisms are further associated with the transformation and degradation of waste materials and synthetic organic compounds (Torstensson *et al.* 1998).

In addition to the effect on nutrient cycling, microorganisms also affect the physical properties of soil. Production of extra-cellular polysaccharides and other cellular debris by microorganisms help in maintaining soil structure, as these materials function as cementing agents that stabilise soil aggregates. Thereby, they also affect water holding capacity, infiltration rate, crusting, erodibility, and susceptibility to compaction (Elliott *et al.* 1996).

Microorganisms as indicators of soil health

Microorganisms possess the ability to give an integrated measure of soil health, an aspect that cannot be obtained with physical/chemical measures and/or analyses of diversity of higher organisms. Microorganisms respond quickly to changes, hence they rapidly adapt to environmental conditions. The microorganisms that are best adapted will be the ones that flourish. This adaptation potentially allows microbial analyses to be discriminating in soil health assessment, and changes in microbial populations and activities may therefore function as an excellent indicator of change in soil health (Kennedy *et al.* 1995; Pankhurst *et al.* 1995).

Microorganisms also respond quickly to environmental stress compared to higher organisms, as they have intimate relations with their surroundings due to their high surface to volume ratio. In some instances, changes in microbial populations or activity can precede detectable changes in soil physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil degradation (Pankhurst *et al.* 1995). An example is the turnover rate of the microbial biomass. This is much faster, e.g. 1-5 years, than the turnover of total soil organic matter (Carter *et al.* 1999). Observations in the Dutch Soil Monitoring Programme have shown that most microbial indicators indeed have discriminating power relative to different soil treatments (Schouten *et al.* 2000). This has also been shown for microbial biomass and basal respiration at a regional scale in the USA (Brejda *et al.* 2000c).

The bioavailability of chemicals, e.g. heavy metals or pesticides, is also an important issue of soil health because of its connection with microbial activities. The impact of such chemicals on soil health is dependent on microbial activities. For example, the concentration of heavy metals in soil will not change over small time periods, but their bioavailability may. It has thus been shown that the bioavailability of poly-aromatic hydrocarbons was lower in autumn compared to early spring due to a higher microbial activity after the growing season (H. Harms, pers. comm. 2001). Therefore, the total content of chemicals in soil is not a reliable indicator of its bioavailability (Logan 2000) and thereby soil health. Instead, bioavailability has to be measured in relation to bioassays and specific microbial processes. In context of this, microbial responses also integrate the effect of chemical mixtures, an information not obtained by studying the chemical mixtures themselves.

Definition of microbial indicator

In the present report, we have adapted the definition of environmental indicators by Christensen (1992) (Christensen *et al.* 2001) to also cover microbial indicators. A microbial indicator is thus in our context defined as:

- *A microbial parameter that represents properties of the environment (state variables) or impacts to the environment, which can be interpreted beyond the information that the measured or observed parameter represents by itself.*

Indicators of soil health have further been defined as measurable surrogates for environmental processes that collectively tell us whether the soil is functioning normally (Pankhurst *et al.* 1997). In the context of microbial indicators, these measurements will cover soil microbial processes and related parameters.

3 Framework for evaluating soil health

Evaluation of soil health should be considered relative to the many different land uses, e.g. agriculture, forestry, urbanisation, recreation, and preservation. The objective for evaluating soil health in an e.g. agricultural ecosystem may, consequently, be different from objectives used for assessing urban or natural ecosystems (Singer *et al.* 2000). Thus, in agriculture, soil may be managed to maximise production without adverse environmental effects, whereas in a natural ecosystem, soil may be managed by a set of baseline values against which future changes in the system may be compared (Karlen *et al.* 2000).

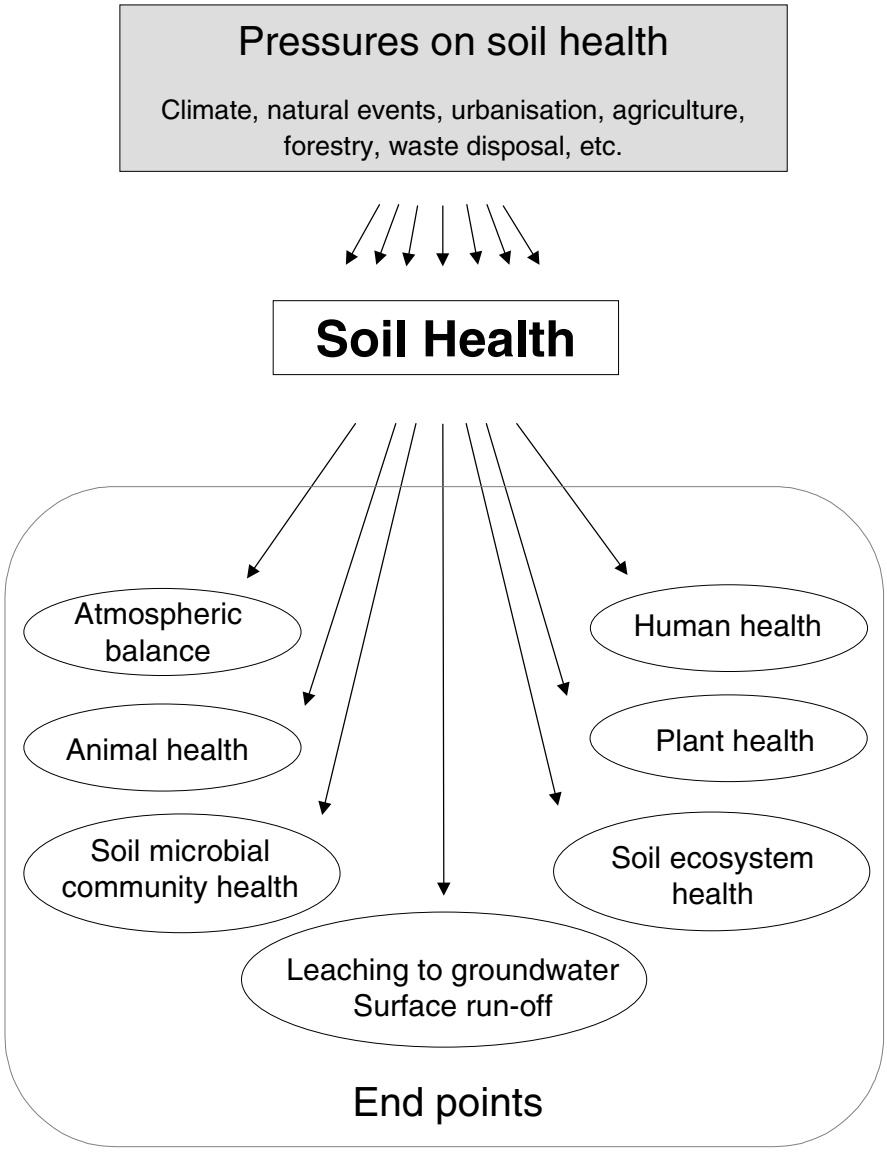


Figure 1. Policy-relevant end points of soil health monitoring. Several examples of pressures on soil health are presented (grey box) and this may impact several end points of soil health (elliptical boxes).

A framework for soil health evaluation is critical for the development of a useful monitoring programme covering the different functions and land-uses and it must identify priorities and relevant indicators relating to policy-relevant end points (Huber *et al.* 2001). An overall framework for soil health evaluation in Europe has recently been proposed by the European Environmental Agency (EEA), though it has yet to be implemented (Huber *et al.* 2001). The main objective of this is to provide policy-makers with relevant environmental parameters based on reliable and comparable data related to soil and to facilitate comprehensive reporting on the state of soils in Europe. It also provides consistent measuring and assessment at any site, from handling of soil samples to the evaluation and storage of data. A similar framework has been used for arable soils in Sweden (Torstensson *et al.* 1998; Stenberg *et al.* 1998b).

Policy-relevant end points of soil health

Definition of policy-relevant end points is very important as monitoring programmes are developed. End points should be pragmatic in the sense of providing logical categories for regulatory decisions and they should be integrated for indicators that are ecologically related. After reviewing environmental monitoring programmes, it is clear that end points for soil health need to be clearly specified and then used as guidance in the identification of indicators. As a consequence, we suggest an end point matrix (Figure 1) that, when integrated together, provide a comprehensive and effective assessment of soil health.

Integrated environmental assessment

Relevant indicators of specific end points can be identified using the Integrated Environmental Assessment method, which is based on the Driving force-Pressure-State-Impact-Response (DPSIR) assessment framework, that has been developed primarily for environmental issues (OECD 1993; Holten-Andersen *et al.* 1995). The DPSIR framework analyses the complex relationships between the environment and the impact of economic activities and societal behaviour. The driving force (D) lead to pressures (P) on the environment, affecting the state (S) and leading to impacts (I), which finally results in response (R) by the society. The DPSIR framework has recently been adopted by EEA specifically for soil issues (Figure 2) and is recommended for the Danish terrestrial monitoring programme (Iversen *et al.* 2001). It is used widely in the overall state evaluation of the environment in several countries, including Denmark (Bach *et al.* 2001), and in Europe (European Environment Agency 2001). A prerequisite for the use of the DPSIR framework is a clear definition of the problems and a scientific understanding of the causal mechanisms (Christensen *et al.* 2001). Further, the development of indicators for each of the PSI-elements is necessary (Huber *et al.* 2001). These indicators should relate to the policy-relevant end points of soil health.

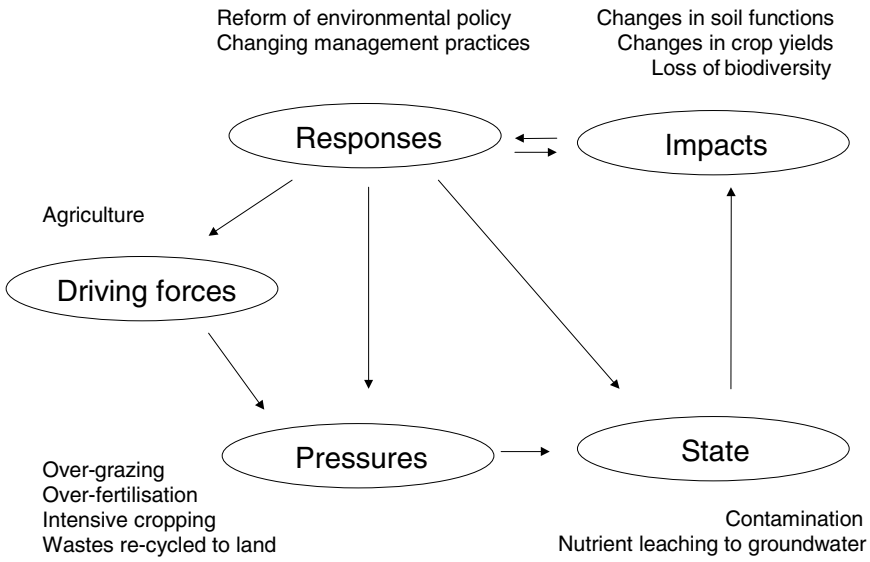


Figure 2. The DPSIR assessment framework applied to soil. Examples of different elements for agriculture are given. Modified from Huber *et al.* (2001).

Requirements of indicators

According to OECD (OECD 1993), environmental indicators must fulfil the following three basic criteria. They should have:

- policy relevance and utility for users
- analytical soundness
- measurability

Criteria specific for soil health indicators have further been listed (Doran *et al.* 1997). They should be:

- linked and/or correlated with ecosystem processes
- integrated with soil physical, chemical, and biological properties
- selected relative to ease of performance and cost effectiveness
- responsive to variations in management and climate at an appropriate time scale
- compatible with existing soil data bases when possible

Because of the multi-functionality of soil, it is difficult to identify one single property as a general indicator of soil health (Paterson 1998). Instead, end points can be characterised by several soil ecosystem parameters (Table 1), which again can be characterised by several microbial indicators (Table 2):

End point ↔ soil ecosystem parameters ↔ microbial indicators

A list of microbial indicators relating to end points of soil health is shortly presented in the next chapter, while a more detailed description of these is presented in Part II.

Table 1. End points of terrestrial monitoring and corresponding soil ecosystem parameters.

End point	Soil ecosystem parameter
Atmospheric balance	C-cycling
Soil ecosystem health	Biodiversity C-cycling N-cycling Microbial biomass Microbial activity Key species
Soil microbial community health	Biodiversity C- cycling Microbial biomass Microbial activity Bioavailability
Leaching to groundwater or surface run-off	N-cycling Bioavailability
Plant health	N-cycling Key species
Animal health	Microbial biomass Bioavailability
Human health	Key species Bioavailability

4 Microbial indicators of soil health

Microbial indicators of soil health cover a diverse set of microbial measurements due to the multi-functional properties of microbial communities in the soil ecosystem (Table 2). In this report, microbial indicators cover bacteria, fungi and protozoa. The indicators are grouped according to the different soil ecosystem parameters. It is not a complete list of all possible microbial indicators, but it includes a vast number of available and future methods. Both traditional methods and modern, often molecular-based methods are included, while methods that would not be suitable for a monitoring programme or which are overtaken by new technologies are not included. The suitability of the specific microbial indicators for a soil monitoring programme was discussed at the workshop "Microbial indicators of soil health" (see Preface).

4.1 Guidelines for selection of microbial indicators

Minimum data set (MDS)

Inclusion of all the microbial indicators listed in Table 2 in a monitoring programme is not feasible. Instead, a minimum data set (MDS) consisting of the smallest number of indicators needed to address the specific end point should be defined. Besides microbial indicators, a MDS for soil health monitoring should also include physical, chemical and biological indicators.

A MDS is based on the objective of the monitoring programme and may very well be different for different end points. Furthermore, the optimal MDS vary for different soil types and regions, since indicators vary due to climate, topography, parent material, vegetation and land use practices (Brejda *et al.* 2000b). Representatives of both inherent and dynamic soil components should be included in a MDS. Inherent soil properties are determined by the basic soil forming factors, including the geological material, climate, time, topography and vegetation (Karlen *et al.* 2000). Dynamic soil properties are based on biological activity and include microbial indicators (Singer *et al.* 2000). In the following, only microbial indicators will be dealt with as a part of a MDS. Full soil profile descriptions together with data of a range of physical and chemical properties are available for Danish soils (Madsen *et al.* 1986).

Generally, indicators of a MDS should be selected on the basis of their ease of measurements, reproducibility, and their sensitivity towards key variables controlling soil health (Larson *et al.* 1994). Each microbial indicator, however, represents slightly different aspects of soil health and has its advantages and disadvantages. Some kind of guiding of this selection is therefore needed and several ways to select are presented below.

Table 2. List of microbial indicators for soil health monitoring. See Part II for more details of the specific indicators.

Soil ecosystem parameter	Microbial indicator	Ready-to-use methods	Future methods
Biodiversity	Genetic diversity	PCR-DGGE	T-RFLP
	Functional diversity	BIOLOG™	Enzyme patterns Diversity of mRNA Oligo-/copiotrophs
	Marker lipids	PLFA	
C-cycling	Soil respiration	CO_2-production or O_2-consumption	
	Metabolic quotient (qCO_2)	$C_{resp}/C_{biomass}$	
	Decomposition of organic matter	Litterbags	Wood sticks
	Soil enzyme activity	Enzyme assays	
	Methane oxidation	Methane measurements	
	Methanotrophs	MPN PLFA	FISH
N-cycling	N-mineralisation	NH_4^+-accumulation	
	Nitrification	NH_4^+-oxidation assay	
	Denitrification	Acetylene inhibition assay	
	N-fixation: Rhizobium	Pot test	Molecular methods
	N-fixation: Cyanobacteria	MPN Nitrogenase activity	
Microbial biomass	Microbial biomass: Direct methods	Microscopy PLFA	
	Microbial biomass: Indirect methods	CFI,CFE SIR	
	Microbial quotient	C_{micro} / C_{org}	
	Fungi	PLFA Ergosterol	
	Fungal-bacterial ratio	PLFA	
	Protozoa	MPN	MPN-PCR
Microbial activity	Bacterial DNA synthesis	Thymidine incorporation	
	Bacterial protein synthesis	Leucine incorporation	
	RNA measurements		RT-PCR FISH
	Community growth physiology	CO_2-production or O_2-consumption	
	Bacteriophages		Host specific plaque assay
Key species	Mycorrhiza	Microscopy Pot test	Molecular methods
	Human pathogens	Selective plating	Molecular / immunological methods
	Suppressive soil	Pot test	
Bioavailability	Biosensor bacteria	Remedios™, Microtox®	New genetic constructions
	Plasmid-containing bacteria	Gel electrophoresis	
	Antibiotic-resistant bacteria	Selective growth	Molecular methods
	Incidence and expression of catabolic genes	Selective growth	Activity Molecular methods RNA measurements

Broad or detailed measurements

The selection of indicators should be broad enough to give policy-makers and/or the general public an overview of the state of the environment, while detailed indicators are needed to better understand underlying trends (Huber *et al.* 2001). For microbial indicators, the overview will be accomplished by measurements at the ecosystem level (e.g. processes), which have been proposed to offer the best possibilities for rapidly assessing changes in soil health (Visser *et al.* 1992). Resulting data will allow decisions to be made at the community (e.g. biomass and biodiversity) or population (e.g. species or functions) levels and allow these detailed studies to be planned more precisely.

Ranking of the indicators

Ranking of the indicators according to applicability, economy, ease of interpretation, development needs, sensitivity etc. has also been proposed as a way to select the optimal indicator of soil health (Pankhurst *et al.* 1997). It is our experience that ranking is very subjective. We found ranking of applicability and development needs to be straightforward, while ranking of the interpretation, sensitivity, and economy was more complicated. In Table 2, a ranking with respect to applicability and needed development of the microbial indicators is attempted.

Methodological requirements

Methodological requirements are included in the selection of indicators in the Swiss Soil Monitoring Programme (Oberholzer *et al.* 2001). The methods thus should (i) have a high degree of standardisation, (ii) have a high practicability and be labour extensive, (iii) have a high reproducibility, (iv) be statistically evaluated, (v) have a satisfactory experience so far, and (vi) be broadly accepted internationally.

Laboratory versus field measurements

Indicators can also be selected on basis of whether they are laboratory (*in vitro*) or field (*in situ*) measurements. *In vitro* measurements may involve incubation of a soil sample in the laboratory under standardised conditions and thus provide an estimate of the potential of the soil. Interpretation of *in vitro* measurements in relation to soil health can be difficult, since the results depend on the incubation conditions, which may not be comparable to field conditions. Examples of *in vitro* measurements are soil respiration, CFI/CFE, SIR, N-mineralisation, nitrification, denitrification, MPN and other growth-based methods (Table 2). *In situ* measurements are based either on direct measurements in the field or fixed samples analysed in the laboratory. They give a "snap-shot" measurement of the conditions in the soil. *In situ* measurements, however, are often very sensitive to spatial and temporal variation (see 5.1) and this may over-ride the variability in soil health status. Examples of *in situ* measurements are gas emissions, PLFA, organic matter decomposition, thymidine and leucine incorporation, short-term enzyme assays and most molecular methods (Table 2).

Integrated measurements

Integration of more indicators into one single method may be a way to reduce the number of indicators. At present, only few methods provide such integrated information. The phospholipid fatty acid (PLFA) analysis provides information about soil microbial biomass, fungal-bacterial ratio, biodiversity and occurrence of key species (see Part II for more details) in one analysis. Substrate induced respiration (SIR) provides measurement of basal respiration and soil biomass.

Finally, the carbon utilisation pattern (BIOLOG™) provides a profile of the microbial community and information on potential metabolic capacity, which together comprise functional diversity.

Microbial indicator MDS in soil monitoring programmes

It has recently been noted that measurements relating to early changes in organic matter and biological and microbial attributes are particularly underrepresented in existing soil monitoring networks world-wide, although these are emerging areas of interest to the scientific community (Huber *et al.* 2001; Wilhjelm committee 2001). Experience with the use of microbial indicators in soil monitoring is available in some European countries, where the most commonly used indicators are microbial biomass and soil respiration (Table 3). A recent report on new molecular tools for soil monitoring activities recommend BIOLOG™ and PLFA analysis as future methods for biodiversity measurements in ecotoxicological analysis (Chapman *et al.* 2000). Two research programmes in Sweden (1993 to 1997) studied several microbial indicators of C-, N- and P-cycling (Torstensson *et al.* 1998), although Sweden does not have a national soil monitoring programme at present (Bo Stenberg, pers. comm. 2001). In the United States, comprehensive investigations on microbial indicators are implemented at many monitoring sites that are part of The International Long-Term Ecological Research (ILTER) network (Castle 1998).

Table 3. Minimum data sets (MDS) of microbial indicators in European soil monitoring programmes. [a]ICP-IM: The International Co-operative Programme on Integrated Monitoring. See Part II for detailed information of the methods.

Monitoring programme	MDS (microbial indicators)
Germany *Niedersachsen*	Soil respiration Microbial biomass (SIR)
Germany *Schleswig-Holstein*	Soil respiration Microbial biomass (SIR) Metabolic quotient (qCO_2) Soil enzymes
The Netherlands	Microbial biomass (direct microscopy) Potential C-mineralisation Potential N-mineralisation Bacterial growth rates Microbial diversity (DGGE, BIOLOG™)
Switzerland	Microbial biomass (SIR, CFE) Soil respiration Potential N-mineralisation
The Czech Republic	Microbial biomass (SIR) Soil respiration N-mineralisation, nitrification Soil enzymes (cellulase, catalase)
The United Kingdom *UK SS Network* *(1994-2006)*	Microbial biomass Soil respiration Microbial diversity (BIOLOG™) *Rhizobium*
The United Kingdom *Sewage sludge project* *(1998-2006)*	Microbial biomass Soil respiration Microbial diversity (BIOLOG™) Biosensor bacteria (heavy metals)
The United Kingdom *Countryside Survey 2000*	Microbial diversity (BIOLOG™)
The United Kingdom *Scottish Soil Transects* *(1990, 1999)*	Microbial diversity (PLFA)
Estonia, Finland, Germany, Italy, Latvia, Russia, Sweden *ICP-IM*[a]	Soil respiration Organic matter decomposition Soil enzyme (phosphatase) Potential N-mineralisation
Austria *ICP-IM (extended MDS)*	Microbial biomass (SIR and ergosterol) Soil enzymes (dehydrogenase, xylanase,arylsulfatase, protease) Nitrification Bacteria and fungi, e.g. Mycorrhiza

References: Germany ((Höper *et al*. 2001); Dilly, pers. comm. 2001), Netherlands ((Schouten *et al.* 1997; J. Bloem, pers. comm. 2001), Switzerland (P. Mäder, pers. comm. 2001), United Kingdom (C. Campbell, pers. comm. 2001), ICP-IM (www.vyh.fi/eng/intcoop/projects/icp_im/im.htm).

5 Practical considerations

During establishment of a monitoring programme constraints exist between number of indicators of the MDS, soil variability and sampling intensity due to practical and financial considerations. Also standardisation is an important factor to consider along with data evaluation procedures.

5.1 Spatial and temporal variation

The spatial and temporal variation of microbial properties in a soil can be very large and this has to be considered when selecting the indicators used to assess soil health (Singer *et al.* 2000). In general, soil attributes that are subject to temporal variation (e.g. soil microbial activity, soil moisture and soluble nutrients), are often also subject to a high spatial variability (Halvorson *et al.* 1997). This variability often limits our ability to accurately quantify microbial populations and processes in soil.

Spatial variation

The spatial variability of microbial processes differs with spatial scale (Parkin 1993). Key variables at the regional scale are climatic factors, land use patterns, vegetation and land surface characteristics. At the landscape level, they are soil type, surface topography and water distribution. The main contributors at the plot scale level are the rhizosphere, application of fertilisers and pesticides, and other soil management practises.

Temporal variation

Temporal variations of microbial indicators are non-systematic, periodic, cyclic or trend changes (Stenberg 1999). Only trend changes are the focus of monitoring and these may be addressed selectively with *in situ* measurements (Paterson 1998). High temporal variability of such measurements suggests that samples need to be collected more often. Alternatively, standardised laboratory (*in vitro*) measurements, excluding the natural changes in temperature and moisture, would be more appropriate for large-scale, long-term sampling of soil variables (Halvorson *et al.* 1997; Visser *et al.* 1992).

Sampling methods and pre-treatment of samples are important considerations in the attempt to minimise the variability in soil health assessment. Together with baseline data on spatial and temporal variability of individual microbial indicators these considerations will help to establish the most appropriate sampling strategies.

5.2 Sampling strategies

A sampling strategy includes plans for site selection, sampling methods, sampling frequency, and pre-treatment of samples and is intimately connected to the purpose of the programme. Generally, the biggest challenge in soil sampling strategies is to reduce the number of samples to an acceptable level based on scientific output and analytical costs (Dick *et al.* 1996b).

5.2.1 Site selection

There are two main approaches for site selection in a soil monitoring programme: the regional and the plot approach (Billett 1996). The regional approach involves hundreds or thousands of sites and generates large amounts of data on different land-use types, thereby overriding inter-site variability. The plot approach is more site-specific and involves a smaller number of sites. The data generated is generally more intensive and of greater scientific value, especially for understanding ecological relationships between the soil attributes (Stenberg 1999). The plot approach is therefore useful for basic research studies, while the regional approach is useful for monitoring purposes. However, the plot approach is recommended in Scotland for a future soil monitoring programme since a comprehensive soil database already exists (Paterson 1998).

It is important that the sites are large enough and that the time periods are long enough to identify trends at the scale of the habitat (Halvorson *et al.* 1997). Managed soil ecosystems constitute two-third of the area in Denmark and must be considered. Undisturbed soils are also of value for monitoring because they provide a baseline to which the influence of land use and soil management on natural soil processes can be compared (Paterson 1998).

5.2.2 Sampling methods

Different sampling methods are available (Wollum 1994; Dick *et al.* 1996b) and basically the selection is a matter of precision level compared to costs. *A priori* information about the variation within the sampling area and preliminary field inspection are of great help in determining the sampling method (Dick *et al.* 1996b).

Composite sampling

Composite sampling is a way to reduce the cost of analysing samples in the laboratory, since individual samples, obtained from the area, are bulked together and mixed. The method requires that the sampling units are the same and that no significant interactions exist among the individual sampling units. The use of field-scale composite samples has been claimed to be an insensitive strategy for the purpose of monitoring undisturbed sites, since it does not say anything about the distribution of variation (Stenberg 1999). Composite sampling should be avoided, since it greatly reduces the variability (Wollum 1994).

Systematic sampling

By systematic sampling, samples are obtained at predetermined points, usually along sets of parallel lines (transects) or in a grid. This method ensures that the entire site being sampled is well represented by the individual samples. The approach is effective in characterising contaminated soil and advantageous for geostatistical methods (see below) and for identifying high and low values of the indicator (Dick *et al.* 1996b). Systematic sampling is used in the Scottish Soil Transects Programme (C. Campbell, pers. comm. 2001).

Random sampling

Random sampling uses random sample points within a grid and is completely unbiased. The method provides limited information on the spatial distribution of the soil property being measured (Dick *et al.* 1996b) and deviating sub-areas are generally underrepresented by

this sampling method (Stenberg 1999). Random sampling is unsuitable for a monitoring programme, as the aim of fully categorising the site is of a higher priority than that of having a completely unbiased site selection (Paterson 1998).

Stratified random sampling

Stratified random sampling takes deviating sub-areas into account, because the area to be sampled is divided into smaller sub-areas according to specific habitats and/or land use patterns. Each sub-area is sampled following the random sampling procedure. This sampling method is probably the most suitable for soil monitoring and is consistent with the ecosystem and land use boundary concept used in the definition of soil health (see Chapter 2) (Paterson 1998). Stratified random sampling is used by The Dutch Soil Monitoring Programme (J. Bloem, pers. comm. 2001), the Swiss Soil Monitoring Network (P. Mäder, pers. comm. 2001), the Countryside Survey in United Kingdom (www. cs2000.org.uk) and the National Soil Inventory in The United States (Brejda *et al.* 2000ab).

Selection of sub-areas may play a significant role in soil monitoring programmes due to the need of specific habitats or land uses being included, and practical considerations such as accessibility, ownership etc. (Paterson 1998). Stratified random sampling further allows the researcher to make statements about each of the sub-areas separately, which greatly increases the precision of estimates over the entire sampling area. Division into sub-areas may, however, also be a disadvantage since it depends on an individual judgement. This can be counteracted by the use of soil maps.

Geostatistical analysis

Geostatistic is a modern statistical tool designed to determine spatial patterns and predict values of non-sampled locations (Rundgren *et al.* 1998). A comprehensive review of this method for characterisation of microbial soil properties is published by Goovaerts (Goovaerts 1998). The analysis is based on the assumption that points situated close to one another in space share more similarities than those farther apart. The first step is to develop a mathematical model, a variogram, which describes the spatial relationship of sampling points. The second step is kriging, which uses the model to estimate each value in the non-sampled area and use these to produce detailed interpolation maps of specific parameters. Geostatistical analysis is also a tool for estimating number of samples for a given precision (Bouma 1997) and have improved the sensitivity of forest soil monitoring (Bringmark *et al.* 1998). The practical use of this method for a national-scale monitoring programme has, however, been questioned by Paterson (Paterson 1998), because a minimum of 200 sample points may be required to estimate a variogram.

5.2.3 Sampling frequency

The required sampling frequency depends on the degree of variation within the sampling area (Dick *et al.* 1996b) and financial limitations. Sampling frequencies in several European soil monitoring programmes are summarised in Table 4 and vary from one to ten years depending on the microbial indicator. This frequency fits well with the identification of microbial properties as dynamic indicators,

which is recommended to be analysed within these time intervals (Stenberg 1999; Halvorson *et al.* 1997).

Due to their dynamic nature, microbial indicators are highly variable and it is recommended to measure at a time of the year when the climate is stable and when there has been no recent soil disturbances (Dick *et al.* 1996b). Late autumn or early spring are proposed as appropriate time periods in northern Europe (Stenberg 1999). It has been shown that there is less variability and low yearly variation at that time of year (O. Dilly, pers. comm. 2001; Pfiffner *et al.* 1999). Time of sampling is usually early spring before plant growth and when the soil is not frozen and not too wet (50-60% WHC). Transferring this observation to Denmark suggests that sampling in February and March would be appropriate.

Table 4. Sampling time and frequency of soil samples in some European soil monitoring programmes. n.a.: data not available.

Monitoring programme	Sampling time	Sampling frequency
Germany *Niedersachsen*	Spring	1 year
Germany *Schleswig-Holstein*	March	3 years
The Netherlands	May-June	5 years
Switzerland	Early spring	5 years
The Czech Republic	n.a.	6 years
The United Kingdom *UK SS Network (1994-2006)*	Autumn/spring	2 years
The United Kingdom *Sewage sludge project*	Spring	2 years
The United Kingdom *Countryside Survey 2000*	n.a.	6 years
The United Kingdom *Scottish Soil Transects 1999*	n.a.	Every year
ICP-IM	August-October	1-5

References: Germany ((Höper *et al.* 2001); Dilly, pers. comm. 2001), Netherlands ((Schouten *et al.* 1997; J. Bloem, pers. comm. 2001), Switzerland (P. Mäder, pers. comm. 2001), United Kingdom (C. Campbell, pers. comm. 2001), ICP-IM (www.vyh.fi/eng/intcoop/projects/icp_im/im.htm).

5.2.5 Pre-treatment of soil samples

Pre-treatment of soil samples for analysis in the laboratory includes packing in the field, transporting, and possibly sieving, storage and incubation before analysis. It is generally recommended that soil samples for microbial analyses are packed in plastic bags and placed on ice for transport to the laboratory and subsequent use (Wollum

1994). The microbial analyses should be carried out, as quickly as possible. International standards for pre-treatment of soil samples for microbiological analyses do exist (see below).

Sieving

Sieving is used to obtain homogenous soil samples free of plant residues and soil animals. A mesh size of 2 to 4 mm is recommended, the larger mesh size for moist clay soil (Stenberg 1999). A mesh size of 5 mm is used in some monitoring programmes (Table 5). If the soil is too wet, careful drying is necessary before sieving to avoid smearing of aggregates. It is recommended to sieve before freezing of the samples (Stenberg 1999).

Storage

Storage of soil samples for microbial analysis is performed differently in the reviewed soil monitoring programmes (Table 5). Storage time varies between one and six months, depending on storage temperature and microbial indicator. It is generally recommended to store soil samples for microbial analysis at 2-4°C (Wollum 1994). Experiments in Switzerland have shown that soil samples for microbial biomass determination can be stored up to six months at 2-4°C, however, analysis of some soil enzyme activities allows only a very short storage period, because of rapidly decreasing activity with time. Storage of moist soil at –20° C for up to one year was found to be the best method for determination of microbial biomass and several microbial processes in Swedish soils (Stenberg *et al.* 1998a; Breure *et al.* 2001). Fast thawing and a subsequently short pre-incubation period has further been shown to be important, especially for studies on N-mineralisation and basal respiration (Stenberg *et al.* 1998a).

Table 5. Soil storage and pre-treatment in European soil monitoring.

Monitoring programme	Storage		Sieving (mm)	Pre-incubation		
	time (months)	temp. (°C)		time (days)	temp. (°C)	WHC (%)
Germany *Niedersachsen*	<6	4 or -21	2	7	22	50
Germany *Schleswig-Holstein*	<6	4 or -21	5	3-5	Room temp.	40-60
The Czech Republic	0	Field temp.	n.a.	n.a.	n.a.	n.a.
The Netherlands	1-2	12	5	28	12	50
Switzerland	3-6	2-4	2 or 5	2-28	n.a.	n.a.
The United Kingdom (several)	1-2	4 or -20	5	7	22	40-60
ICP-IM	0-2	4	2-4	12	20	60

References: Germany ((Höper *et al.* 2001; Dilly, pers. comm. 2001), Netherlands ((Schouten *et al.* 1997; J. Bloem, pers. comm. 2001), Switzerland (P. Mäder, pers. comm. 2001), United Kingdom (C. Campbell, pers. comm. 2001), ICP-IM (www.vyh.fi/eng/intcoop/projects/icp_im/im.htm). n.a.: data not available.

Pre-incubation

Pre-incubation of soil samples for *in vitro* analyses is often used to condition the samples before analysis. Applied pre-incubation conditions may vary. The time of pre-incubation varies from 3 to 28 days, the temperature from 12°C to 22°C or room temperature and the soil moisture from 40 to 60% WHC (Table 5).

5.3 Standardisation of methods

Sampling method standardisation

Soil monitoring programmes may run for many years, different people are likely to perform the sampling and different laboratories will be involved. This calls for standardisation of sampling strategies. Furthermore, the sampling phase is the most important source of error in the whole procedure of soil monitoring (Hortensius *et al.* 1996) and standardisation is needed to obtain comparable results as a function of time and location. International standards for sampling procedures (collection, handling and storage) and pre-treatment of soil samples exist within the ICP-IM network (www.vyh.fi/eng/incoop/projects/icp_im/im.htm) and as ISO standards (ISO 10381-6) (ISO 1994).

Analytical method standardisation

The analytical variability between laboratories can be controlled by inter-laboratory investigations as done within the Swiss Soil Monitoring Programme (Paul Mäder, pers. comm. 2001) or by analysing all samples by one specific method within one specific laboratory as done in the Dutch Soil Monitoring Programme (J. Bloem, pers. comm. 2001). It is very important to standardise indicator methodology before implementation in a monitoring programme. Harmonisation of protocols is proposed by EEA (Huber *et al.* 2001) and a handbook is under preparation by the COST Action 381 (www.isnp.it/cost/cost.htm). ISO standards exist however for determination of microbial biomass by SIR (ISO 14240:1:1997) and CFE (ISO 14240-2:1997) and for N-mineralisation and nitrification (14238:1997) (www.iso.org).

5.4 Data evaluation and interpretation

Evaluation and presentation of the multiple data obtained in a monitoring programme are important, since the results will be used in political decision-making on environmental management strategies. As such, it may be necessary to express the results in an easily interpretable form.

5.4.1 Soil Health Index

A soil health index is an integration of several (microbial) measures of soil health into one number by weighing the individual measures relative to each other. This results in a single-digit index. Threshold values can be established for the index rather than for the individual indicators. The drawbacks of the index approach are that all information on the relationships between indicators are lost and that weighing of the individual indicators may be subjective (Stenberg 1999). Furthermore, there is no direct relationship between an index value and a specific function or indicator, which may cause problems when

interpreting the reasons for e.g. a high or low index (Stenberg *et al.* 1998b; Sojka *et al.* 1999).

Multivariate statistical tools

Multivariate statistical tools simplify the interpretation of the large amount of data and can be used in the development of a soil health index. Principal component, discriminant, factor and covariance analyses are examples of such multivariate statistical tools. By these analyses, the data are reduced into a small number of indices (principle components, factors) which are linear combinations of the original values, representing most of the variation in the data set. These indices can be combined into a soil health index by the multiple variable indicator transformation (MVIT) procedure (Smith *et al.* 1993). By this procedure, data on several soil variables at one location are combined together into a single binary indicator value, the MVIT. Combined with geostatistics and kriging (see 5.2.2), soil maps can be calculated based on specified threshold values of each individual indicator. If the threshold values adequately reflect soil health then the kriging can produce maps of the probability of a soil being of good or bad health. The procedure has been evaluated by Halvorson et al. (Halvorson *et al.* 1996) using soil chemical variables, microbial biomass and enzyme activities as indicators of soil health and these indicators were shown to co-vary spatially across the landscape in a systematic pattern.

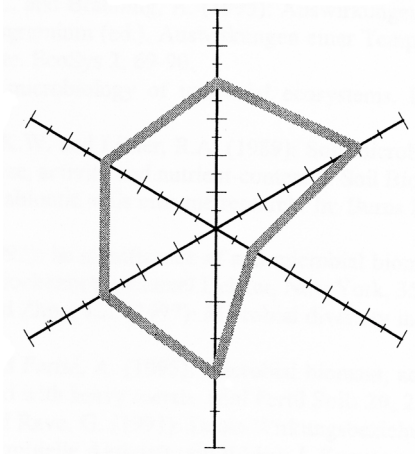
5.4.2 Graphical presentation methods

Results can be integrated and evaluated using graphical methods, which gives a relatively simple visual presentation of the complicated results. Several variations of such data presentation exist, including orientor stars (Dilly *et al.* 1998), AMOEBA presentations (Schouten *et al.* 2000) and cobwebs (Stenberg 1999; Gomez *et al.* 1996) (Figure 3), which are all based on the same principle. All indicator variables are plotted into the graph, either as raw data or scaled against a desired reference situation. The reference or threshold values (100%) are also plotted into the graph, and thereby yielding a reference or threshold line. The interpretation of the data is based on the shape of the graphics and comparisons with the reference or threshold line. A changed shape may thus be the result of either spatial or temporal changes (Schouten *et al.* 2000). The choice of reference or threshold values (see also below) is obviously very important, since these methods rely on deviations from the reference values. Establishment of proper reference or threshold values, probably per soil type and per land use, are part of future efforts in The Netherlands (Schouten *et al.* 2000).

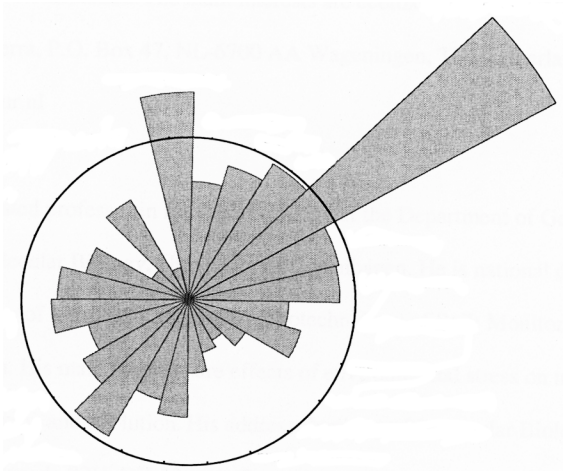
5.4.3 Reference values

Reference values may be defined on the basis of existing sustainable habitats or predicted by modelling. In many cases, no reference value is available and the initial measurements may be the best reference value for future measurements. Measuring soil parameters in a specific soil system over time rather than in comparison with other systems is recommended as a dynamic assessment approach (Larson *et al.* 1994).

ORIENTOR STAR. Each axes represent a soil ecosystem parameter on a scale of 0-100%. The actual values of a soil are plotted on the axes and combined by a heavy line. (Dilly *et al.* 1998).



AMOEBA. The values of 24 indicators of a grassland soil are presented in relation to the reference value (100%) shown by the circle (Schouten *et al.* 2000)



COB WEB. The threshold value (dotted line) of six selected soil health indices shown together with actual values of two soil types (stippled and full lines) (Gomez *et al.* 1996)

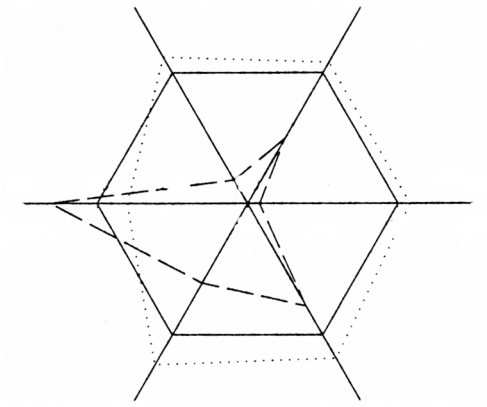


Figure 3. Examples of graphical methods used for presentation of multi dimensional results. The shape of the graphics and comparisons with the reference or threshold line assist interpretation of data.

Reference values have been proposed for specific purposes e.g. reference samples for specific soil types or use of a local reference sample for pollution gradient assessment. In the Dutch Soil Monitoring Programme ten organic farms are used as reference in agricultural soil monitoring, counteracting the lack of a proper reference (J. Bloem, pers. comm. 2001). In the Swiss and German monitoring programmes, threshold values are used as references in soil monitoring (Oberholzer *et al.* 2001). Similarly, an "ecological dose value", that represents the inhibitory effects of heavy metal on the kinetics of soil biological properties is proposed in New Zealand (Speir *et al.* 1995)

Indicators that have some form of "internal reference", e.g. biomass as a percentage of soil organic matter, have also been proposed (Brookes 1993). Finally, to accommodate changes in soil density, it has been recommended to express biological attributes on a soil volume basis, rather than on a concentration basis (Doran *et al.* 1994).

Data obtained through national monitoring activities are recommended by EEA to be stored in a future European soil database, since information on accepted reference values is necessary for the correct interpretation of the data obtained (Huber *et al.* 2001).

5.4.4 Modelling

Modelling is a way to evaluate the indicators in use, besides the use in estimating other indicators. A MDS may thus be extended by estimated indicators, which simulate indicators that are too costly or difficult to measure. One example of modelling is the pedotransfer function (PTF), which is a mathematical function that predict difficult-to-obtain properties from already available basic soil properties (Larson *et al.* 1994). The accuracy of PTFs may, however, only be appropriate at regional scales and not for specific locations, in which case direct measurement is the only option (Wösten 1997). Furthermore, PTFs are based on a synthesis of our current knowledge, which may be far from perfect (Paterson 1998) and they are only as good as the original measured data from which they were derived (Wösten 1997). Food web modelling has been proposed as a supplement to indicator measurements within the Dutch Soil Monitoring Programme (Schouten *et al.* 1997), but more baseline data are needed at present (J. Bloem, pers. comm. 2001).

Results from multivariate statistical analyses can be used for modelling, since these analyse and predict the most variable indicators. These results have further been used for selection of the most variable indicators of soil health for a MDS (Johansson *et al.* 2000; Stenberg *et al.* 1998b; Brejda *et al.* 2000ab).

6 Conclusion

Soil microorganisms appear to be very suitable and sensitive early-warning indicators or predictive tools in soil health monitoring. Soil health monitoring programmes may thus benefit considerably by including microbial indicators. Measurements relating to early changes in organic matter and biological and microbial attributes, however, are among the least monitored parameters at national levels in Europe (Huber *et al.* 2001).

6.1 Recommendations of microbial indicators for a Danish terrestrial monitoring programme

- **Specific MDS are required for specific end points**

We recommend that a specific MDS for each policy-relevant end point is defined. A MDS should, as a minimum, consist of one microbial indicator for each soil ecosystem parameter. Examples of this are given in Table 6. If, for instance, leaching of nitrate or pesticides to groundwater is the end point, the MDS should be composed of microbial indicators of N-cycling and bioavailability, e.g. nitrification and biosensor bacteria. On the other hand, monitoring ecosystem health generally requires a MDS covering several parameters, e.g. microbial biomass, activity, diversity and key species. Furthermore, the MDS for ecosystem health may have a different composition depending on the ecosystem of interest. For example, N-cycling would be relevant to measure at moorland, which is characterised by a general N-deficiency, but is subject to N-deposition from the atmosphere. Bacterial diversity, on the other hand, might be more relevant to measure in arable land. Another example is the occurrence of human pathogens, which is more critical to arable soil and urban areas than to moorland.

- **Baseline data**

Development of baseline information on the selected microbial indicators, including information on both spatial and temporal variation, is recommended within the first years of monitoring to define reference and threshold values for repeated monitoring activities. Characterisation of the sampling sites by physical and chemical properties should be obtained simultaneously. These data may also provide information on specific ecosystems of interest.

- **Implementation of new improved indicators**

Implementation of new improved indicators is recommended as soon as these are applicable for soil monitoring purposes to provide more precise, detailed and preferably, more integrated results. This will result in a dynamic up-to-date monitoring programme. Abrupt changes in data series within a MDS are undesirable. Implementation of new methods is thus recommended to parallel measurements of the indicator to be replaced during a certain time period. This will

provide a quality assurance of the new method. Data obtained for the new indicator during this time period can then be used as baseline data.

➢ **Research needs**

An improved understanding of microbial processes, community structure, and natural temporal and spatial variation is needed before the use of microbial indicators will assist in the establishment of long-term strategies for better management practices and determination of soil health (Parkin 1993; Sojka *et al.* 1999; Turco *et al.* 1994). In line with this, the Wilhjelm committee has recommended that further scientific knowledge should be developed through research activities included in the monitoring programme to provide a scientific base for new management policies at the national and international level (Wilhjelm committee 2001). We fully agree and recommend that this specifically should include research on biodiversity and the use of modelling.

- Microbial biodiversity

The Wilhjelm committee has specifically noted that there is a need for methodological development within microbial biodiversity measurements (Wilhjelm committee 2001), and we fully support that. Such development specifically involves research concerning the relationship between functional and genetic diversity (DGGE, rRNA, enzymes, PLFA analysis), which will have a significant scientific output. Further, the interpretation of biodiversity and its effect on resilience, robustness and soil health is important.

- Modelling of data

No matter what strategy is used for evaluating monitoring results, a decision has to be made as to whether the soils are healthy or not (Stenberg 1999). This decision will, to a large extent be political, but improved interpretation of data in the context of soil health will provide the scientific base. As of today we have a wealth of analytical tools for characterising a healthy soil, but we lack the means to integrate these tools to quantify soil health (Kennedy *et al.* 1995). A scientifically sound MDS followed by qualified interpretation are the tools available today for such quantification.

Mathematical models describing relationships of several indicators can be a useful tool in evaluating obtained data and provide new directions for monitoring and research. Models will predict soil health and up-coming changes. Furthermore, modelling will aid in reducing the number of sampling locations, decisions of sampling frequency and of indicators within a MDS. Modelling has been proposed as a supplement to indicator measurements within the Dutch Soil Monitoring Programme (Schouten *et al.* 1997) and within the coming NOVANA programme.

Table 6. Recommended microbial indicators in a Danish terrestrial monitoring programme.

End point of soil health	Soil ecosystem parameter	Proposed microbial indicator included in a MDS for a specific end point
Atmospheric balance	C-cycling	Methane oxidation
Soil ecosystem health	Biomass	Microbial biomass (direct method)
	C-cycling	Decomposition of organic matter
	N-cycling	N-mineralisation
	Biodiversity	Genetic diversity Functional diversity Structural diversity
	Key species	Mycorrhiza
Soil microbial community health	C-cycling	Decomposition of organic matter
	Microbial activity	Bacterial DNA / protein synthesis
	Biodiversity	Genetic diversity Functional diversity Structural diversity
	Bioavailability	Biosensor bacteria
Leaching to groundwater or surface run-off	N-cycling	N-mineralisation
	Bioavailability	Biosensor bacteria
Plant health	N-cycling	N-mineralisation
	Key species	Mycorrhiza
Animal health	Biomass	Protozoa biomass
	Bioavailability	Antibiotic-resistant bacteria
Human health	Bioavailability	Antibiotic-resistant bacteria
	Key species	Human pathogens

Part II
Catalogue of microbial indicators of soil health

Microbial indicators of soil health encompass a diverse set of microbial measurements due to the multi-functional properties of microbial communities in the soil ecosystem. In the present catalogue, bacteria, fungi and protozoa indicators are considered. They are grouped according to the different soil health parameters of the ecosystem, that is biodiversity, carbon cycling, nitrogen cycling, biomass, microbial activity, key species and bioavailability. The indicators relate to the ecosystem (e.g. processes), community (e.g. biomass and biodiversity) or population (e.g. species or functions) levels and this relationship is noted together with relations to policy-relevant end point (see Part I Chapter 3). The catalogue presents a comprehensive list of microbial indicators, some of them are ready-to-use in a monitoring programme, while others have to be developed for that purpose. Only a limited number of indicators will be implemented in a monitoring programme and a selection of indicators is necessary. The discussions of each indicator in relation to soil health and suitability for monitoring purposes in this catalogue will be helpful in this selection procedure.

1 Indicators of biodiversity

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Soil microbial community health	**Biodiversity**	Genetic diversity Functional diversity Structural diversity

Information about microbial community structure and diversity has been noted as important for understanding the relationship between environmental factors and ecosystem functions (Torsvik *et al.* 1996). Microbial diversity measurements have thus been recommended in soil health monitoring programmes (Turco *et al.* 1994) and represents measurements at the community level. The diversity of a community is expressed as the species richness and the relative contribution each species makes to the total number of organisms present. Diversity of a microbial community is often described by the Shannon-Weaver index (H') (Shannon *et al.* 1949). The number of species has traditionally been determined by taxonomic classification studies, but as these are sub-optimal for microorganisms, molecular and biochemical techniques of estimating abundance and number of each species must be applied. The benefit of a high genetic diversity is currently under debate because it is not always correlated to functional diversity

(Griffiths *et al.* 2000; Griffiths *et al.* 2001). Furthermore, the correlation between soil health and biodiversity is not completely understood, although a medium to high diversity is generally considered to indicate a good soil health.

1.1 Microbial genetic diversity

The genetic resources present in the environment are the basis of all actual and potential functions. The genetic diversity of soil microorganisms is an indicator of the genetic resource. Methods for determination of the genetic microbial diversity include several molecular methods of which a few may be implemented into a soil monitoring programme.

Bacterial genetic diversity

Genetic diversity of bacteria is most commonly studied by diversity of the 16S rDNA genes, which occur in all bacteria and which show variation in base composition among species. 16S rDNA genes are thus used for phylogenetic affiliation of *Eubacteria* and *Archaea* and large databases exist on sequences of 16S rDNA (e.g. (www.ncbi.nlm.nih.gov/BLAST/) and http://rdp.cme.msu.edu/html/). It consists of variable and conserved regions, and this has facilitated the design of primers in the conserved regions for targeting the majority of members of defined groups of bacteria. Two methods have been developed to examine the diversity of 16S rDNA sequences in total DNA extracted from soil microbial communities, namely PCR-DGGE and T-RFLP.

Denaturing Gradient Gel Electrophoresis (PCR-DGGE) (Muyzer *et al.* 1993) and Temperature Gradient Gel Electrophoresis (PCR-TGGE) (Heuer *et al.* 1997) are based on variation in base composition and secondary structure of fragments of the 16S rDNA molecule. By PCR with primers principally targeting all eucaryotes or selected subgroups, a fragment of 16S rDNA of known size can be amplified. Following PCR, the products are separated by gel electrophoresis. By PCR-DGGE the gel itself contains a chemical-denaturing gradient, making the fragments denature along the gradient according to their base composition. By PCR-TGGE a temperature gradient is created across the gel, resulting in the same type of denaturation. The number and position of fragments reflect the dominating bacteria in the community.

For the PCR-DGGE and PCR-TGGE methods, the low resolution of gel electrophoresis compared to the high diversity of bacterial communities can be a problem. Soil communities may easily contain several hundred bacterial strains, while the resolution of more than 20-50 bands on a gel is difficult (Heuer *et al.* 1997; Johnsen *et al.* 2001). For a visible band on the gel, a bacterial species has to comprise approx. 1% of the entire population (Casamayor *et al.* 2000), of course dependent upon many practical circumstances. Sequencing and identification of the visible bands on the gel following PCR-DGGE or PCR-TGGE (e.g. Riemann *et al.* 2001) may further improve the resolution of the important players of a microbial community. PCR-DGGE has recently been implemented in the Dutch Soil Monitoring Programme. Results from the first year showed that the mean number of DNA bands was found to be about 50 independent of the season. Furthermore, no sig-

nificant difference was found between a dairy farm on clay soil and a horticultural farm on sand (Bloem *et al.* 2002).

Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Liu *et al.* 1997) is an alternative method for examining diversity of 16S rDNA sequences of microbial communities. It is also based on PCR amplification of 16S rDNA with specific primers. The primers are labelled with a fluorescent tag at the terminus resulting in labelled PCR-products. The products are cut with several restriction enzymes, one at a time, which result in labelled fragments that can be separated according to their size on agarose gels. As the PCR products are labelled at the terminus, only restriction enzyme fragments containing either of the terminal ends of the PCR product will be detected. The digested PCR products are subsequently loaded on a sequencer. The output includes fragment size and quantity.
Recently, the potential of the T-T-RLFP method to discriminate soil bacterial communities in cultivated and non-cultivated soils has been demonstrated (Buckley *et al.* 2001). As data accumulate and become accessible (e.g. http://rdp.cme.msu.edu/html/analyses.html) the method will allow comparison between different soils analysed in different labs. The method, however, requires delicate and expensive instruments along with very pure DNA (Liu *et al.* 1997; Tiedje *et al.* 1999).

Fungal genetic diversity

The classical method for estimating the fungal diversity of soil has been number and morphology of fruiting bodies. However, the majority of fungi in soil are present either as resting stages (spores) or mycelium. Both spores and mycelium can be isolated from soil, but if a fruiting body is not formed, identification of the organisms is difficult at best, and generally impossible (Bridge *et al.* 2001). Further, the isolation step may be selective to specific fungal groups, e.g. the fast growing ones. Molecular methods based on 18S rDNA provide tools that can overcome these problems. However, a major limitation is the limited number of fungal nucleic acid sequences presently available in databases (Bridge *et al.* 2001; Smit *et al.* 1999). Diversity measurements within the fungal community in soil can also be measured by PCR-DGGE (Kowalchuk *et al.* 1997; Pennanen *et al.* 2001b) and PCR-TGGE (Smit *et al.* 1999). The methodologies are described above in relation to bacterial genetic diversity.

Protozoan genetic diversity

Protozoae is a phylum of single cell eucaryotic organisms and as such may resemble and better represent higher organisms than prokaryotes (Foissner 1994). Protozoa are a paraphyletic group primarily consisting of naked amoebae, testate amoebae, ciliates and heterotrophic flagellates (Foissner 1999). Protozoa are very abundant in soil, like bacteria, and exist in very diverse and harsh environments. They also resemble bacteria in that they are important for soil health and fertility, react quickly to environmental changes, are ubiquitous and do not easily move around in soil. Protozoa form an essential part of all soil ecosystems and have been proposed as early warning indicators (Foissner 1994). Protozoan bioassays, for example, have been used in the United Kingdom as a discriminating indicator of heavy metal contamination in soil amended with sewage sludge (Campbell *et al.* 1997b).

Determination of the diversity of protozoa is normally carried out by taxonomic affiliation to species, groups or families based on morphological features. This method is very time consuming, requires specialists and is further complicated by the incomplete taxonomic description of protozoa (Fredslund *et al.* 2001). Alternatively, protozoan diversity can be determined by molecular methods. The diversity of protozoa has been characterised by PCR-DGGE targeting an 18S rDNA fragment (van Hannen *et al.* 1999). The PCR-DGGE method is described above in relation to bacterial genetic diversity. Recently, a method of PCR-DGGE specific to *Kinetoplastida*, a monophyletic group of protozoa, has been published revealing a relatively high diversity in freshwater sediments (Rasmussen *et al.* 2001). As protozoa form a paraphyletic group, specific primers for the various important protozoan groups have to be designed (Fredslund *et al.* 2001). The technique is being developed in these years and further development is necessary before implementing into a monitoring programme.

1.2 Microbial functional diversity

The diversity of functions within a microbial population is important for the multiple functions of a soil. The functional diversity of microbial communities has been found to be very sensitive to environmental changes (Kandeler *et al.* 1999; Kandeler *et al.* 1996; Zak *et al.* 1994). However, the methods used mainly indicate the potential *in vitro* functionality. Functional diversity of microbial populations in soil may be determined by either expression of different enzymes (carbon utilisation patterns, extra-cellular enzyme patterns) or diversity of nucleic acids (mRNA, rRNA) within cells, the latter also reflecting the specific enzymatic processes operating in the cells. Indicators of functional diversity are also indicators of microbial activity and thereby integrate diversity and function.

Carbon utilisation patterns (BIOLOG™ assay)

Carbon utilisation patterns can be measured by the BIOLOG™ assay (Garland *et al.* 1991). In this assay, a soil extract is incubated with up to 95 different carbon sources in a microtiter plate and a redox-dye is used to indicate microbial activity. Sets of specific carbon sources have been selected specifically for studies of soil microbial communities (Insam 1997; Campbell *et al.* 1997a). The result of the assay is a qualitative physiological profile of the potential functions within the microbial community. Differences in the profiles can be analysed by multivariate statistics.

The BIOLOG™ assay is dependent on growth of cells under the specific conditions in the microtiter plate and thereby indicating only potential functional diversity. However, the technique has gained widespread use, primarily due to the ease of use and the capacity to produce comprehensive data sets. The data are analysed by multivariate statistics and experience with data interpretation is still developing (Garland *et al.* 1991; Winding *et al.* 1997; Winding 1994). Considerable data are, however, available for a future reference database and this may facilitate data interpretation. The BIOLOG™ assay has been shown to be more sensitive than microbial biomass and respiration measurements to impacts of soil management practices and of

sewage sludge amendments to soil (Bending *et al.* 2000; Burgess *et al.* 2001). The assay is currently implemented in the Dutch Soil Monitoring Programme where it has been shown to be discriminatory to different types of soil and management practices (Schouten *et al.* 2000; Breure *et al.* 2001; Rutgers *et al.* 1999). The assay is also recommended for soil monitoring in Scotland and Northern Ireland (Chapman *et al.* 2000). However, caution should be taken when using a commercial assay, as the product may go off the market or change composition. The utility of the assay can be extended by the Pollution-Induced Community Tolerance (PICT) approach, where a range of concentrations of a specific heavy metal can be added into the plate and the tolerance of the community be estimated (Rutgers *et al.* 1999).

Enzyme pattern

The enzymatic activity in soil is mainly of microbial origin, being derived from intracellular, cell-associated or free enzymes. Only enzymatic activity of ecto-enzymes and free enzymes is used for determination of the diversity of enzyme patterns in soil extracts. Discrimination between free and cell-associated enzyme activity can be obtained by a simple filtration step to separate microbial cells from the soil extract. The enzyme activity is quantified by incubation of the soil extract with commercial fluorogenic enzyme substrates (4-methylumbelliferin (MUF) and 4-methylcoumarinyl-7-amide (MC) (Hoppe 1993)) or colometric substrates (remazol brilliant blue (Wirth *et al.* 1992), p-nitrophenol or tetrazolium salt) coupled with specific compounds of interest (e.g. cellulose or phosphate). The data are typically analysed by multivariate statistics.

If incubation times are kept short, cell growth and synthesis of new enzymes are prevented. It has been recommended that a diverse set of enzyme activities are measured, since a few dominating organisms expressing a high enzyme activity may give a biased result (Miller *et al.* 1998). Similar diversity patterns were obtained when various soil types were tested by a set of 14 MUF-substrates and by the BIOLOG™ assay (N. B. Hendriksen & A. Winding, unpublished result). Compared to the BIOLOG™ assay, this enzyme activity assay is closer to *in situ* functions, since it is independent of growth and enzyme synthesis. However, fewer functions are generally measured.

Diversity of mRNA

mRNA molecules are gene copies used for synthesis of specific proteins by the cell. The nucleotide sequences of mRNA molecules reflect the type of enzymes synthesised. Concentration of mRNA is correlated with the protein synthesis rate and as such with the activity of the microorganism. Therefore, the content and diversity of mRNA molecules will give very accurate pictures of the *in situ* function and activity of the microbial community. Detection and quantification of a specific mRNA molecule can be done by reverse transcription PCR (RT-PCR), which is a very sensitive method (Pfaffl *et al.* 2001). A prerequisite of this technique is knowledge of the nucleic acid sequence of the mRNA for a specific gene. For certain genes, this information is available. However, the technique of quantifying mRNA is still in its developmental stage. Sensitivity of the method has though been improved by associating a magnetic capture system (Lleo *et al.* 2001).

1.3 Structural diversity

Phospholipid fatty acids (PLFAs) are stable components of the cell wall of most microorganisms. They are polar lipids specific for subgroups of microorganisms, e.g. gram-negative or gram-positive bacteria, methanotrophic bacteria, fungi, mycorrhiza, and actinomycetes (Zelles 1999). Individual PLFAs can thus be related to microbial community structure. The method gives a fingerprint of the relative PLFA composition of the resident microbial community.

PLFAs are extracted from soil samples and subsequently analysed by gas chromatography (Zelles 1999; Frostegård *et al.* 1993). Specific PLFAs are subsequently identified and/or quantified and the result is evaluated by multivariate statistics.

PLFA profiles of soil samples offer sensitive reproducible measurements for characterising the numerically dominant portion of soil microbial communities without cultivating the organisms (Zelles 1999). The technique gives estimates of both microbial community composition and biomass size (see chap. 4.1), and the results represent the *in situ* conditions in soil. The method is, however, time-consuming, although the extraction procedure may be automated (Macnaughton *et al.* 1997). PLFA analysis has been used to detect a pollution gradient in soil (Dahlin *et al.* 1997; Colin Campbell, pers. comm.) and found to be more discriminatory than BIOLOG™ measurements for characterising soil microbial communities (Pennanen 2001a); Colin Campbell, pers. comm. 2001). The method has recently been recommended for soil monitoring in Scotland and Northern Ireland (Chapman *et al.* 2000).

Ratio of oligo- and copiotrophic bacteria

The ratio of oligotrophs (bacteria that require a low nutrient input) to copiotrophs (bacteria that require a high nutrient input) has been proposed to reflect the nutrient stress tolerance of the species present in soil (van Bruggen *et al.* 2000; Klappenbach *et al.* 2000; De Leij *et al.* 1993; Hattori 1985). A high ratio, e.g. dominance of oligotrophs, may indicate stable environmental conditions with low substrate availability. A low ratio, e.g. dominance of copiotrophs, may, in contrast, indicate an environment regularly receiving input of organic rich substrate, e.g. addition of sewage sludge or pesticides.

The ratio of oligotrophs to copiotrophs can be determined by either colony appearance on agar substrates, the rRNA-gene copy number in isolated bacteria or rRNA-expression in bacterial microcolonies. The appearance of colonies on agar substrates may simply be determined by counting colony forming units (CFUs) at specific time intervals (De Leij *et al.* 1993). The counts are complemented by calculation of mean lag-phases and absolute numbers of bacterial subpopulations (Hattori 1985). Early appearing CFUs represent copiotrophic bacteria, while late appearing CFUs represent oligotrophic bacteria. The number of rRNA copies in isolated bacteria, determined by molecular techniques (Klappenbach *et al.* 2000), has recently been shown to correlate with the expression of the rRNA gene (Binnerup *et al.* 2001). The rRNA gene expression can be determined during growth in bacterial microcolonies (mCFUs) by measuring the 16S

rRNA concentration by fluorogenic *in situ* hybridisation (Amann *et al.* 1995). A low rRNA-copy number or a low rRNA expression during growth indicate dominance of oligotrohic bacteria and hence a high ratio.

The CFU method is a simple and inexpensive method and is ready to use upon standardisation of incubation and counting procedures. The molecular methods are more comprehensive and time consuming and still need considerable testing before implementation into a monitoring programme. However, they have the potential for specifying the interacting groups of organisms depending on the specificity of the hybridisation probes in use.

2 Indicators of carbon cycling

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Soil microbial community health Atmospheric balance	**Carbon cycling**	Soil respiration Organic matter decomposition Soil enzymes Methane oxidation

A major activity of soil microorganisms is decomposition of organic matter. Soil microorganisms are in general heterotrophic and rely on input of carbon energy from outside the microbial community. Organic matter in soil is largely derived from higher plants consisting of cellulose (15-60%), hemicellulose (10-30%) and lignin (5-30%). Indicators of carbon cycling represents measurements at the ecosystem level.

2.1 Soil respiration

Soil respiration, which is the biological oxidation of organic matter to CO_2 by aerobic organisms, notably microorganisms, occupies a key position in the C cycle of all terrestrial ecosystems. It provides the principal means by which photosynthetically fixed carbon is returned to the atmosphere. The metabolic activities of soil microorganisms can be quantified by measuring CO_2 production and/or O_2 consumption.

Measurement of soil respiration is one of the oldest, but still most frequently used techniques for quantification of microbial activities in soil (Zibilske 1994; Alef 1995). Soil respiration is positively correlated with soil organic matter content, and often with microbial biomass and microbial activity (Alef 1995). Soil respiration measurements are included in most soil monitoring programmes (see Part I Chapter 4)

and have been found to discriminate between different soil types and land uses within the Dutch Soil Monitoring Programme (Bloem *et al.* 2002).

Soil respiration can be determined by either CO_2 production or O_2 consumption. Measurement of CO_2 concentration is more sensitive, because the atmospheric concentration of CO_2 is only 0.033% versus 20% for O_2. Determination of CO_2 production from soil samples can be made in the laboratory by simple and inexpensive techniques based on alkaline CO_2 traps followed by chemical titration or by more sophisticated automated instruments based on electrical conductivity, gas chromatography or infrared spectroscopy (Alef 1995). Combined with automated sampling from test soil samples, automated instruments make it possible to determine CO_2 production as a function of time for several days (Zibilske 1994). An ISO standard is presently at the level of discussion (ISO/DIS 16072; H. Höper, pers.comm. 2001).

Respiration is highly influenced by temperature, soil moisture, nutrient availability and soil structure (Alef 1995). Pre-conditioning and standardisation of the soil before measuring respiration is necessary to minimise the effect of these variables. Field measurements of soil respiration are less often used due to the high sensitivity to environmental conditions, although such measurements have been shown to discriminate between different soil management practices (Pankhurst *et al.* 1995). Finally, soil respiration measurements have been used as an indicator of pesticide and heavy metal toxicity (Brookes 1995).

Metabolic quotient

The metabolic quotient (qCO_2), also called the specific respiratory rate, is defined as the microbial respiration rate (measured as evolution of CO_2) per unit microbial biomass (Anderson *et al.* 1990; Coleman *et al.* 1995). Microbial biomass for this purpose is often determined by substrate induced respiration (see chap 4.1), and the respiratory activity is determined concomitantly using the same instruments. The qCO_2 is included in the soil monitoring programme in Schleswig-Holstein, Germany (O. Dilly, pers. comm. 2001).

The qCO_2 has been used to study soil over time and, generally, the quotient decreases as the soil ages (Insam *et al.* 1988; Insam *et al.* 1989; Anderson *et al.* 1990). Furthermore, the qCO_2 has been used in effect studies of environmental conditions, such as temperature and pH, soil management, soil texture and compaction and heavy metals (Anderson 1994). Generally, the qCO_2 is found to be highest when ecosystem stress level is high. Caution, however, should be taken when interpreting qCO_2, since a high quotient may infer stress, an immature ecosystem or a more respirable substrate (Sparling 1997). Threshold values of $qCO_2$for different soil texture classes of conventional agricultural soils have been elucidated within the soil monitoring programmes in Germany (Lower Saxony) and Switzerland (Oberholzer *et al.* 2001).

2.2 Organic matter decomposition

Any disturbance in microbial activity will result in a change of the organic matter (OM) decomposition rate and hence the availability

and cycling of the most important organic bound nutrients within the ecosystem, such as carbon, nitrogen, sulphur and phosphorus. Knowledge about rates of OM decomposition is thus a prerequisite for understanding the availability and recycling of all these nutrients.

Field incubation of different types of plant litter or more standardised pieces such as cotton strips and wood sticks, are the most commonly used methods for studying OM decomposition rates.

Litter bags

Decomposition of plant litter can be measured by placing the litter in so-called litterbags in the field. Litterbags are made of inert nylon with a defined mesh size allowing a free exchange of air, water and nutrients and access for organisms. The mesh size defines the groups of organisms that can contribute to the decomposition within the litterbag. The decomposition rate of the litter is determined as weight loss per time interval (Verhoef 1995).

The advantage of using plant litter for studying decomposition rates is the natural origin of the litter, which provides a direct correlation to naturally occurring processes within the soil ecosystem. The disadvantage of the method is the difficulties in obtaining uniform litter from year to year. Changes in cellulolytic and ligninolytic enzyme activities in litterbags have recently been shown to explain changes in litter decomposition upon nitrogen deposition (Carreiro *et al.* 2000). A protocol for litterbag decomposition studies is included in the ICP-IM manual (www.vyh.fi/eng/intcoop/projects/icp_im/im.htm).

Cotton strips and wood sticks

Decomposition of cotton strips and wood sticks can be measured by direct placement into the soil. Decomposition rate of the cotton strips is determined as reduction in tensile strength per time interval, while the rate for the sticks is determined as simple weight loss (Harrison *et al.* 1988). The advantage of using cotton strips and wood sticks is the ease of obtaining standardised material. The disadvantage is the fact that both substrates are surrogates for the natural occurring processes and hence, results that may be difficult to interpret. The decomposition rate of cotton, which consists of pure cellulose, is much faster than the rate of wood sticks. The cotton strip method is however dependent on specialised equipment for tensile strength measurements. Wood sticks inserted into the soil have recently been recommended for decomposition studies in the Environmental Change Network in UK (Parr *et al.* 1999).

All three types of OM tests make it possible to determine and compare the decomposition rates between different sites, ecosystems, and time. Vertical position in the soil horizon and the time intervals between samplings must be standardised.

2.3 Soil enzymes

Enzymes are the direct mediators for biological catabolism of soil organic and mineral components. Thus, these catalysts provide a meaningful assessment of reaction rates for important soil processes. Soil enzyme activities (i) are often closely related to soil organic matter, soil physical properties and microbial activity or biomass, (ii)

change much sooner than other parameters, thus providing early indications of changes in soil health, and (iii) involve simple procedures (Dick *et al.* 1996a). In addition, soil enzyme activities can be used as measures of microbial activity, soil productivity, and inhibiting effects of pollutants (Tate 1995). Disturbance of the soil microbial activity, as shown by changes in levels of metabolic enzymes, can serve as an estimate of ecosystem disturbance. This relationship has been clearly shown when soil is polluted with heavy metals (Kandeler *et al.* 1996).

Easy, well-documented assays are available for a large number of soil enzyme activities (Dick *et al.* 1996a; Tabatabai 1994). These include dehydrogenase, β-glucosidases, urease, amidases, phosphatases, arylsulphatase, cellulases and phenol oxidases (Table 7). A standard method for determination of acid phosphatase activity exists within the ICP-IM soil monitoring network (www.vyh.fi/eng/intcoop/projects/icp_im/im.htm). Hydrolysis of the fluorescent fluorescein diacetate is thought to broadly represent soil enzyme activity, because it is hydrolysed by a number of different enzymes, such as proteases, lipases and esterases (Schnürer *et al.* 1982). These enzymatic activities are widely distributed in soil, where they mainly originate from microorganisms, but also from plants or animals.

Table 7. Soil enzymes as indicators of soil health.

Soil enzyme	**Enzyme reaction**	**Indicator of**
Dehydrogenase	Electron transport system	Microbial activity
Beta-glucosidase	Cellobiose hydrolysis	C-cycling
Cellulase	Cellulose hydrolysis	C- cycling
Phenol oxidase	Lignin hydrolysis	C- cycling
Urease	Urea hydrolysis	N- cycling
Amidase	N-mineralisation	N- cycling
Phosphatase	Release of PO_4^-	P- cycling
Arylsulphatase	Release of SO_4^-	S- cycling
Soil enzymes	Hydrolysis	General OM* degradative enzyme activities

* OM: organic matter.

Enzyme activities can be measured as *in situ* substrate transformation rates or as potential rates if the focus is more qualitative. An important parameter is whether decisions are made relative to *in situ* or to maximum enzyme activities. For comparisons of soil enzyme activities, the natural choice is the maximum activities (Dick *et al.* 1996a). Measurements of soil enzyme reaction are usually based on the addition of an artificial, soluble substrate at a concentration sufficient to maintain zero-order kinetics, thus achieving a reaction rate proportional to enzyme concentration. Long incubation periods have to be

omitted to avoid substrate depletion and microbial growth. Enzyme activities are usually determined by a dye reaction followed by a spectrophotometric measurement.

2.4 Methane oxidation

Methane (CH_4) is found extensively in Nature and is a greenhouse gas in the atmosphere. Methane is produced in anoxic environments by methanogenic *Archaea* and consumed by aerobic methane-oxidising bacteria, the methanotrophs (Ritchie *et al.* 1997)(see below). Important terrestrial sites for methane oxidation are wetland areas receiving a high input of organic material. Furthermore, landfills containing high amounts of organic wastes are a source of methane and the habitat of many methanotrophs (Ritchie *et al.* 1997).

Net production of methane can be considered as an indicator of greenhouse gas emission and may further be linked to monitoring of the atmospheric balance. Methane oxidation is measured by spiking a soil sample with methane and incubate the sample in a closed jar in the laboratory. Loss of methane is subsequently determined by gas chromatography.

Number of methanotrophs

The number of methanotrophs is an indicator of potential greenhouse gas consumption. Methanotrophs can be quantified directly in soil by fluorescent *in situ* hybridisation (FISH) (Bourne *et al.* 2000) or standard growth-dependent MPN counts. Analyses of methanotrophic communities can be done with PCR-DGGE (see chap. 1.1) using methanotrophs-specific 16S rDNA primers (Ritchie *et al.* 1997).

3 Indicators of nitrogen cycling

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Plant health Leaching to groundwater Surface run-off Atmospheric balance	**Nitrogen cycling**	N-mineralisation Nitrification Denitrification N-fixation

The mineralisation of soil organic nitrogen (N) through nitrate to gaseous N_2 by soil microorganisms is a very important process in global N-cycling. This cycle includes N-mineralisation, nitrification, denitrification and N_2-fixation (Figure 4). Indicators of nitrogen cycling represent measurements at the ecosystem level.

Organic N is mineralised to ammonium (NH_4^+) by a wide variety of soil microorganisms and it reflects the turnover of organic material in soil and the available indigenous N-pools to plants. Ammonium is

subsequently either immobilised by soil microorganisms (that is, assimilated into new biomass) or oxidised to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-) by aerobic nitrification. Chemoautotrophic bacteria, the nitrifier population, carry out this process. At this step, leaching of N to the groundwater may occur due to the negative charge of the nitrate ion. Under normal circumstances, however, nitrate is subsequently reduced to gaseous nitrogen (N_2) via nitrous oxide (N_2O) by anaerobic denitrification.

Denitrification is represented by a variety of soil bacteria (Zumpft 1992). Nitrification and denitrification together lead to losses of bioavailable N since nitrous oxide and gaseous N_2 may be lost to the atmosphere. N_2 can be re-fixed into the soil by N_2-fixing microorganisms. Nitrous oxide is a greenhouse gas when lost to the atmosphere.

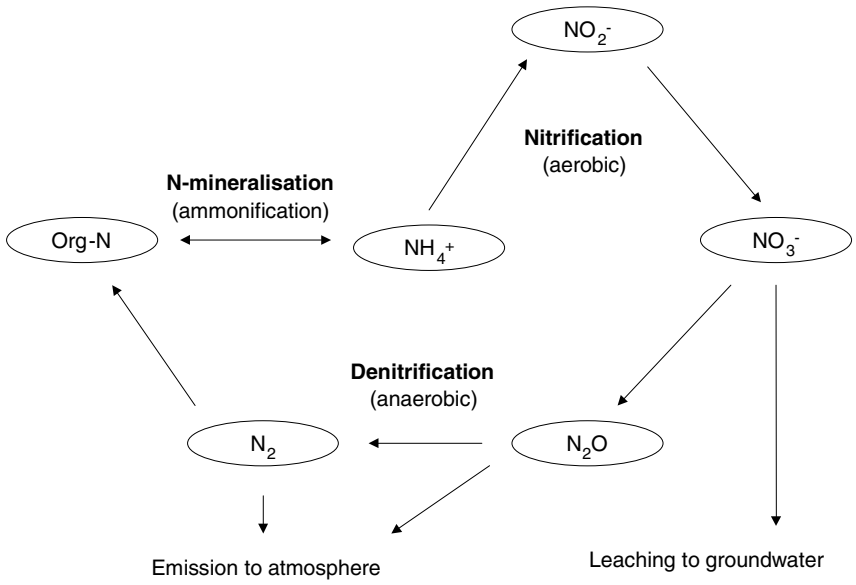


Figure 4. Global cycling of nitrogen. See text for further explanation.

3.1 N-mineralisation

Ammonification is actually a measure of the net N-mineralisation, since immobilisation of NH_4^+ by soil microorganisms into new biomass occurs simultaneously with the mineralisation process. The measurement thus reflects the potential N-mineralisation in soil and is measured by the accumulation of NH_4^+ in soil slurry under aerobic conditions over a period of several weeks (Hart *et al.* 1994). Anaerobic incubation is sometimes preferred because there is less microbial immobilisation under anaerobic conditions and nitrification is inhibited (Stenberg 1999).

Measurement of potential N-mineralisation (either aerobic or anaerobic) is included in soil monitoring programmes in Austria (Kandeler *et al.* 1999), the Czech Republic (Zbíral 1995), the Netherlands (Bloem *et al.* 2002), New Zealand (Schipper *et al.* 2000) and Switzerland (Maurer-Troxler 1999), where it has been shown to discriminate be-

tween different soil management practices and land uses. Aerobic N-mineralisation measurements are further included in the ICP-IM protocol (www.vyh.fi/eng/intcoop/projects/icp_im/im.htm) and exist as an ISO-standard (14238:1997). Compared to other measurements of N-cycling, the N-mineralisation is relatively insensitive to disturbances because a wide variety of microorganisms are involved in the process.

3.2 Nitrification

Nitrification is believed to be a more sensitive parameter than N-mineralisation, because only a small number of bacteria, the nitrifiers, are involved in the process (Visser *et al.* 1992). Nitrification measurements are included in soil monitoring in Austria (Kandeler *et al.* 1999), the Czech Republic (Sanka *et al.* 1995) and an ISO-standard is available (ISO 14238:1997). Nitrification measurements have, however, been reported to be no more sensitive than N-mineralisation (P. Mäder, pers. comm. 2001) and, as a result of this, nitrification measurements have recently been replaced by N-mineralisation measurements in the Dutch Soil Monitoring Programme (J. Bloem, pers. comm. 2001). Nitrification measurements reflect the population size of the nitrifiers since ammonium is an essential substrate for these organisms (Bock *et al.* 1992). Furthermore, these measurements together with denitrification measurements may indicate deposition of ammonia on N-limited habitats.

Nitrification is measured by the ammonium oxidising assay. With this method, a soil slurry is incubated with excess ammonium and chlorate, the latter inhibiting the oxidation of nitrite to nitrate (Belser *et al.* 1980). The oxidation of ammonium to nitrite is measured by gas chromatography.

3.3 Denitrification

The denitrifying capacity is a widespread feature among soil bacteria and therefore denitrification can be used as a representative for microbial biomass (Stenberg 1999). Since denitrification is an anaerobic process the amount of denitrification found in soil is very dependent on abiotic factors such as precipitation and soil compaction. Thus, soil management practices readily influence the amount of denitrification found in agricultural fields. Denitrification measurements may, together with nitrification measurements, indicate deposition of ammonia in N-limited habitats.

Measurement of denitrification is carried out by the acetylene inhibition technique (Smith *et al.* 1979), in which the reduction of N_2O to N_2 is inhibited by acetylene and accumulated nitrous oxide is measured by gas chromatography. Nitrate must be available in surplus. The method is often used to measure the potential denitrification where nitrate and carbon are added and anaerobic conditions are established. However, interpretation of denitrification data is complicated, because the denitrification enzymes are synthesised only under anaerobic conditions and the enzymes are not functional under aerobic

conditions, even though they persist in the microbial community. The denitrification assay may thus reflect historical anaerobic situations and not necessarily the size of the actively denitrifying biomass.

3.4 N-fixation

Gaseous nitrogen (N_2) is a product of the anaerobic denitrification of nitrate. N_2 is lost to the atmosphere or consumed by N_2-fixing *Rhizobium* or cyanobacteria due to their nitrogenase enzyme.

Rhizobium

Bacteria of the genera *Rhizobium* are abundant in soil, where they form symbiotic associations with legume roots. The bacteria reside in nodules where they fix N_2 and provide the plant with nitrogen for growth. In return, the plant provides the bacteria with organic substrates for growth. The *Rhizobium*-legume symbiosis is characterised by high host specificity. Numbers of *Rhizobium* has previously been proposed as an indicator of soil health (Brookes 1995; Visser *et al.* 1992) based on the organisms sensitivity to pesticides (Domsch *et al.* 1983) and heavy metals (McGrath *et al.* 1988; Chaudri *et al.* 1993). The abundance of *Rhizobium* has been included in the UK Sewage Sludge Network as a microbial indicator of heavy metal contamination in agricultural soils (Chambers *et al.* 1999).

The frequency and diversity of *Rhizobium* in soil can be determined by a simple pot test, where a diverse set of legume seeds are sowed in the test soil and number of nodules formed are determined after a specific growth period. Alternatively, the bacteria may be quantified by direct isolation from soil using selective growth media (Laguerre *et al.* 1993; Bromfield *et al.* 1995; Tong *et al.* 1994) together with morphological and physiological characterisations (Hungria *et al.* 2001). A number of molecular methods have also been applied for diversity measurements of these bacteria. These include plasmid profiles and insertion sequence fingerprints (Hartmann *et al.* 1998), 16S-23S rDNA spacer sequences (Tan *et al.* 2001), PCR detection of specific genes (Tesfaye *et al.* 1998), colony hybridisation (Laguerre *et al.* 1993), RFLP (Laguerre *et al.* 1994) and RAPD (Baymiev *et al.* 1999).

Detection of *Rhizobium* by growing legumes in the test soil and determining root nodule-formation is a rather simple method. The molecular methods, on the other hand, are more technically demanding. Although it relies on the development of specific probes for the different *Rhizobium*-subgroups, the colony hybridisation procedure is probably the best way to detect *Rhizobium*. A combination of quantitative and diversity measurements will allow a screening of the soil potential for *Rhizobium*-legume mediated nitrogen fixation.

Cyanobacteria

The cyanobacteria, or blue-green algae, are photoautotrophic bacteria. In contrast to *Rhizobium*, they are non-symbiotic. They form microbiotic crusts in intimate association with surface soil, which contribute significantly to the stabilisation of soil towards erosion (Eldridge *et al.* 1994).

Cyanobacteria have mainly been used as indicators of heavy metal contamination (e.g. from sewage sludge application) in soil. Most

experiments have shown a negative correlation between the number of cyanobacteria or nitrogenase activity and the concentration of heavy metals (Brookes 1995; Lorenz *et al.* 1992; Dahlin *et al.* 1997; Scherr *et al.* 2001). It has been noted that cyanobacteria may be too sensitive to experimental conditions to provide a robust indicator of heavy metal contamination (Brookes 1995; Lorenz *et al.* 1992). Measurement of the potential N_2-fixation under standard laboratory conditions has, therefore, been suggested as a better alternative (Brookes 1995). Nevertheless, the number of cyanobacteria is recommended as an early indicator of heavy metal pollution in the Swiss soil monitoring network (Scherr *et al.* 2001).

The number of cyanobacteria can be determined either by MPN methods (Scherr *et al.* 2001) or determinations of nitrogenase activity using light as energy source (Olson *et al.* 1998). Nitrogenase activity is measured by the acetylene reduction assay, where the reduction product, ethylene, easily can be measured by gas chromatography.

4 Indicators of soil biomass

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health	**Soil biomass**	Microbial biomass Protozoan biomass

In this report, soil biomass includes bacterial, fungal and protozoan biomass. Biomass is fundamental for soil processes to occur and quantification of microbial biomass is as such a measurement at the ecosystem level (Visser *et al.* 1992).

4.1 Microbial biomass

Soil microbial biomass represents the fraction of the soil responsible for the energy and nutrient cycling and the regulation of organic matter transformation (Gregorich *et al.* 1994; Turco *et al.* 1994). A number of studies has reported a close relationship between soil microbial biomass, decomposition rate and N-mineralisation (Jenkinson 1988; Smith *et al.* 1990; Carter *et al.* 1999). Microbial biomass has also been shown to correlate positively with grain yield in organic, but not in conventional farming (Mäder *et al.* 2001). Finally, soil microbial biomass contributes to soil structure and soil stabilisation (Fließbach *et al.* 2000; Smith *et al.* 1990). Soil microbial biomass has also been recommended as indicators of soil organic carbon (Carter *et al.* 1999).

Several methods have been used for the estimation of microbial biomass in soil. The methods can be divided into direct (e.g. microscopy or determinations of specific membrane phospholipid fatty acids (PLFAs)) and indirect (e.g. chloroform fumigation (CFE/CFI) or substrate induced respiration (SIR)). Microbial biomass measurements are used in several soil monitoring programmes: microscopy in the

Dutch Soil Monitoring Programme, CFE or SIR in monitoring programmes in Germany, Switzerland, United Kingdom and The Czech Republic (Part I chapter 4).

Direct methods (microsopy, PLFA)

Determination of soil microbial biomass by direct methods (microscopy or PLFA analysis) gives results that very closely represent the *in situ* soil conditions. Although the methods are time-consuming, they are currently used for soil monitoring purposes (Bloem *et al.* 2002; C. Campbell, pers. comm. 2001). The automation of PLFA extraction has reduced analysis time to some extent (Macnaughton *et al.* 1997).

Direct counts or bio-volume estimations using conversion factors can estimate microbial biomass. Different soil preparation methods and staining techniques in combination with epifluorescens microscopy are available (Bloem *et al.* 1995). A Danish standard for epifluorecens microscopy (DS 2212:1990) is further available. Combined with automated image analysis, direct counts can be used routinely for the determination of soil microbial biomass in many samples of different origin.

The total amount of PLFAs in soil is an alternative method to microscopic counting (Petersen *et al.* 1991; Zelles 1999). PLFAs are found only in membranes of bacteria and fungi. Individual PLFAs are specific for specific subgroups of microorganisms. Using extraction of soil samples and analysis by gas chromatography (Zelles 1999; Frostegård *et al.* 1993), the total amount of PLFAs can be quantified. It is also possible to quantify different groups of microorganisms by this method (Schloter *et al.* 1998; Zelles 1999). PLFA analysis hereby provides information on biodiversity (see chap. 1.3) and the fungal-bacterial biomass ratio (see below).

Indirect methods (CFI, CFE, SIR)

Indirect methods are generally cheaper, faster and easier to use than the direct methods. Results obtained by the indirect methods have been documented to be very close to the direct measurements (Carter *et al.* 1999), thus providing confidence in the utility of indirect methods.

Chloroform fumigation is the most commonly used indirect method. This method is considered to measure most of the soil microbial biomass, e.g. both dead and alive, though some microorganisms (e.g. spores) are insensitive to the fumigation process (Toyota *et al.* 1996). Determination of microbial biomass by chloroform fumigation covers two indirect methods: the chloroform fumigation incubation method (CFI) and the chloroform fumigation extraction method (CFE) (Carter *et al.* 1999). In both cases, the chloroform vapour kills the microorganisms in the soil, and subsequently the size of the killed biomass is estimated either by quantification of respired CO_2 over a specified period of incubation (CFI) or by a direct extraction of the soil immediately after the fumigation followed by a quantification of extractable carbon (CFE; ISO-standard 14240-2:1997). The release of CO_2 after fumigation is the result of germinating microbial spores utilising the C substrate provided by the killed microbial cells.

Another common indirect method is substrate induced respiration (SIR). This method measures only the metabolically active portion of the microbial biomass (Carter *et al.* 1999). SIR (ISO-standard 14240:1:1997) measures the initial change in the soil respiration rate as a result of adding an easily decomposable substrate (e.g. glucose) (Anderson *et al.* 1978). The technique has been automated (Heinemeyer *et al.* 1989) and is used in soil monitoring in several countries, e.g. Germany (Höper *et al.* 2001)). Soil microbial biomass is subsequently calculated using a conversion factor (Kaiser *et al.* 1992).

Microbial quotient

The amount of microbial biomass carbon (C_{micro}) may be related to the total carbon (C_{org}) content by the microbial quotient (C_{micro}/C_{org}). This quotient provides a measure of soil organic matter dynamics and can be used as an indicator of net C loss or accumulation (Anderson *et al.* 1986). Using the quotient avoids the problems of comparing trends in soils with different organic matter content (Sparling 1997).

Fungal biomass

Living fungal biomass can be estimated by quantification of fungal-specific membrane molecules such as ergosterol (Stahl *et al.* 1996) or specific phospholipids (PLFAs) (Frostegård *et al.* 1996) (see above). The procedure for determination of ergosterol content in soil is simpler compared to determination of PLFAs. However, an important disadvantage of this method is that oomycetous fungi and a number of yeasts do not produce ergosterol (Stahl *et al.* 1996). Additionally, it is recommended that total hyphal length is measured simultaneously for precise estimations of only living fungal biomass (Stahl *et al.* 1996), but this is a very laborious and cumbersome technique. Quantification of enzyme activities such as fluorescein diacetate hydrolytic activity (FDA) or N-acetyl-beta-glucosaminidase (Nag) activity have been proposed as alternative, semi-quantitative measures of soil fungal biomass (Miller *et al.* 1998; Gaspar *et al.* 2001).

Fungal-bacterial biomass ratio

The fungal-bacterial biomass ratio can also be determined directly from measurements of fungal-specific and bacterial-specific PLFAs (Bardgett *et al.* 1996; Frostegård *et al.* 1996; Bardgett *et al.* 1999). More information is thus obtained from one single PLFA-analysis. The ratio has been used in soil management studies as a microbial indicator. A higher ratio is typical of long-term unfertilised or organic managed grasslands compared to fertilised grasslands of the same soil type (Bardgett *et al.* 1996; Yeates *et al.* 1997; Bardgett *et al.* 1999).

4.2 Protozoan biomass

Protozoan biomass is determined by extracting a soil sample and counting directly by use of an inverted microscope (Foissner 1994). This yields the number of active protozoa. However, the vast majority of protozoa are encysted (inactive). An alternative method is thus to extract protozoa from the soil followed by a MPN counting based on a growth medium (e.g. Rønn *et al.* 1995) that causes protozoa to excyst. Both methods are very laborious and limited by the problems of extraction efficiency. The MPN approach further possesses the problems of culturability; not all cysts will excyst and not all protozoa grow under the laboratory conditions in liquid culture (Rønn *et al.* 1995). A newly developed molecular method, MPN-PCR, has been

used to quantify a specific group of soil flagellates directly in a gnotobiotic soil system and higher but corresponding numbers was found compared to traditional MPN counting based on culturing (Fredslund *et al.* 2001). The application of MPN-PCR assays for soil protozoa is, however, currently limited by the scarcity of molecular data (Fredslund *et al.* 2001). Bioassays based on a 24 h growth response of common ciliates have been developed (Forge *et al.* 1993; Pratt *et al.* 1997) and successfully applied to heavy metal toxicity testing (Campbell *et al.* 1997b).

5 Indicators of microbial activity

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Soil microbial community health	**Microbial activity**	Bacterial DNA synthesis Bacterial protein synthesis RNA measurements Bacteriophages

Indicators of microbial activity in soil represent measurements at the ecosystem level (e.g. processes regulating decomposition of organic residues and nutrient cycling, especially nitrogen, sulphur, and phosphorus). Measurements at the community level include bacterial DNA and protein synthesis. Frequency of bacteriophages is a measurement at the population level.

5.1 Bacterial DNA synthesis

Synthesis of DNA is a prerequisite for bacterial cell division and, as such, an indicator of bacterial growth. DNA is unique in the way that it only participates in cell division. DNA synthesis can be determined by incorporation of ^{3}H- or ^{14}C-thymidine into bacterial DNA as thymidine is a unique nucleoside, which only participates in DNA synthesis. The method has several requirements: (i) DNA synthesis has to be linearly correlated with the cell growth (balanced growth); (ii) all bacteria must take up thymidine through the cell membrane, which has been shown not to be the case (Michel *et al.* 1993); (iii) thymidine should not be metabolised and (iv) the radioactive label (^{3}H) should not exchange with other molecules, e.g. proteins. It has been shown that only 5-20% of the ^{3}H-thymidine incorporated into total macromolecules is incorporated into DNA (Bååth 1998).

A soil extract is incubated with radiolabelled thymidine for a short time period and then filtered to measure the amount of radiolabel in the cells. A thorough extraction and purification of DNA from the cells can solve the problem with unspecific incorporation of radiolabel. The method is extensively used in aquatic environments (Kemp *et al.* 1993). During the last decade it has been adopted to soil (Bååth 1992; Christensen *et al.* 1992; Alden *et al.* 2001; Michel *et al.* 1993; Har-

ris *et al.* 1994), but the use is not as widespread as in aquatic environments. The method is used routinely in the Dutch Soil Monitoring Programme and has been shown to discriminate between different soil types and land uses, e.g. grassland on clay and horticultural farm on sand (Schouten *et al.* 1999).
Bacterial growth rate (number of cells formed per unit time) is calculated by use of a conversion factor (Michel *et al.* 1993). This conversion factor is based on many assumptions, including estimates of the number of cells present and the amount of radiolabelled thymidine incorporated in relation to GC content of the total DNA content of cells.

5.2 Bacterial protein synthesis

Bacterial protein synthesis is directly correlated to bacterial activity and can be determined by incorporation of ^{3}H or ^{14}C leucine, as this amino acid is incorporated into proteins only. The method for leucine incorporation (Bååth 1994) is the same as for thymidine incorporation (see above) and the incorporation of both precursors can be carried out in a single assay if different radiolabels are used (Bloem *et al.* 2002). Incorporation of ^{14}C leucine is routinely measured in the Dutch Soil Monitoring Programme in combination with ^{3}H-thymidine incorporation (Bloem *et al.* 2002) and has been shown to possess discriminative power (Schouten *et al.* 1999).

The advantages and drawbacks of the method are the same as for radiolabelled thymidine incorporation, although balanced growth is not a prerequisite. Furthermore, most bacteria take up leucine, although the incorporation efficiency may differ between soils (Bååth 1998). Measurements of protein synthesis are supposed to be more accurate than that of DNA synthesis, because of a relatively higher protein content in cells (Bååth 1998).

5.3 RNA measurements

The RNA molecules, ribosomal RNA (rRNA) and messenger RNA (mRNA), play key roles in the protein synthesis. The amount of RNA in individual cells or in a community may, therefore, be taken as an indicator of protein synthesis and, thus, microbial activity.

The number of active cells can be detected by fluorescent *in situ* hybridisation (FISH) (Amann *et al.* 1995). By this method, individual cells carrying high concentrations of rRNA, situated on ribosomes, are quantified by fluorescence microscopy. The amount of rRNA in a community can also be detected by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), where rRNA extracted from soil is detected by creating a DNA copy and separating by gel electrophoresis (Duineveld *et al.* 2001). Quantification of activity by either method is still problematic (Felske *et al.* 2000) and comprehensive method development is needed before implementation into a monitoring programme. In the future this will also include implementation of microarrays with simultaneous measurements of numerous genes.

mRNA molecules are gene copies used for synthesis of specific proteins by the cell. Determination of mRNA can be taken as equivalent to the expression of a specific gene in soil. Such measurements can be done by real time quantitative RT-PCR, which detects and quantifies low amounts of mRNA in environmental samples including soil (Pfaffl *et al.* 2001; Lleo *et al.* 2000; Mendum *et al.* 1998). A prerequisite for using this method is knowledge of the sequence of the mRNA. At present, this method is probably too advanced for use as a microbial indicator in a monitoring programme, but with further method development it may prove useful.

5.4 Bacteriophages

A bacteriophage is a virus, which infects and multiplies in a specific host bacterium. Bacteriophages are abundant in the soil environment and have been isolated for nearly every known species of soil bacteria (Angel 2000). Most phages isolated from soil are temperate phages, e.g. phages that can lie dormant in bacterial cells after infection (Angel 2000). The multiplication of bacteriophages strictly depends on the activity of the host bacteria (Ashelford *et al.* 2000; Marsh *et al.* 1994; Pantasticocaldas *et al.* 1992; Germida 1986). As such, monitoring of the frequency and host specificity of free bacteriophages in soil is an indicator of the activity of specific soil bacteria. This is in contrast to the other microbial activity indicators, which measure the activity of whole microbial communities.

Determination of free bacteriophages in soil can be carried out by a standard method of extraction followed by a plaque-assay (e.g. (Hu 1998)) with specific host bacteria, e.g. *Pseudomonas* (Cambell *et al.* 1995), *Bacillus* (Pantasticocaldas *et al.* 1992), *Rhizobium* (Radeva *et al.* 2001). A high number of plaques are presumed to indicate a recent high activity of similar host bacteria in the test soil assuming a direct correlation between the number of bacteriophages and bacterial activity. Such a correlation has indeed been shown for *Azospirillum brasilense* (microcosm study; (Germida 1986)) and *Serratia liquefaciens* (field study; (Ashelford *et al.* 2000)), but has to be confirmed for other bacterial groups.

The selection of host bacteria should be representative for the soil type to be investigated. Furthermore, the bacteriophage sensitivity to the host bacteria should be known. The frequency and persistence of the bacteriophages in different soil types should be estimated *a priori* in order to standardise the method. Generally, temperate bacteriophages survive for long periods of time within the host bacteria. Without host bacteria, the survival of bacteriophages depends on abiotic parameters, e.g. clay content, soil moisture, temperature and pH (Angel 2000; Vettori *et al.* 1999; Marsh *et al.* 1994).

6 Key species

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Plant health Animal health Human health	**Key species**	Mycorrhiza Suppressive soil Human pathogens

Microbial key species in soil are here defined as organisms that possess important functions in the soil ecosystem (e.g. nutrient cycling, plant pathogenesis) or are of human health concern (e.g. human pathogens/zoonoses). A number of criteria has to be fulfilled for key species to be useful in a monitoring programme. For example, they should be (ecologically) relevant, preferably abundant, and easy to enumerate and identify (Oberholzer *et al.* 2001). Key indicator species represent measurements at the population level.

6.1 Mycorrhiza

The majority of higher plants exist in natural symbiosis with mycorrhizal fungi. The group of mycorrhizal fungi includes ectomycorrhizal (mainly forest trees), arbuscular mycorrhizal (terrestrial plants) and ericoid mycorrhizal (heather) fungi (Allen *et al.* 1995). They colonise plant roots and provide the plant with nutrients, especially phosphorus, due to the increased nutrient availability caused by the extra-radical mycelium. Furthermore, mycorrhizal associations can have a positive influence on plant diversity (Allen *et al.* 1995), plant stress and disease tolerance, and on soil aggregation (Kling *et al.* 1998). Only arbuscular mycorrhiza (AM) will be dealt with here. Colonisation by AM has been shown to be highly dependent on the presence of host plants, on land use and on soil management practices (Kling *et al.* 1998). Spore abundance and diversity have been shown to discriminate between extensively and intensively managed soils (Oehl *et al.* 2001) and AM diversity has been reported to be sensitive to heavy metal contamination, organic pollutants and atmospheric deposition (Siciliano *et al.* 1999; Cairney *et al.* 1999; Egli *et al.* 2001; Egerton-Warburton *et al.* 2000). Quantitative analysis of AM based on spore morphology is implemented as a microbial indicator in the Swiss soil monitoring network, where it is used to indicate heavy metal contamination in soil (Egli *et al.* 2001). Colonisation of AM in soil has been proposed as an important indicator of plant and ecosystem health (Stenberg 1999; van der Heijden *et al.* 1998).

Abundance and diversity of AM is determined by extraction of spores from soil samples and subsequent counting in a microscope (Oehl *et al.* 2001). An alternative method is to use the test soil in a plant bioassay and harvest either the spores (Oehl *et al.* 2001) or the roots (Kling *et al.* 1998; Egli *et al.* 2001). The determination of spore numbers is, however, poorly correlated to the actual colonisation potential of the soil (Kling *et al.* 1998) and molecular tools for detection

of AM in roots are the future needs within the Swiss Soil Monitoring Programme (Redecker *et al.* 2001). Methods for direct detection and quantification of AM in soil samples or in roots have been developed. These include 18S rDNA PCR (Chelius *et al.* 1999), nested PCR at the species level (Jacquot *et al.* 2000; Redecker *et al.* 2001) and AM-specific PLFAs (see chapter 1.3) (Olsson 1999).

6.2 Suppressive soil

Many of the proposed soil health indicators focus on the presence of beneficial rather than the absence of detrimental organisms, although both are important (Singer *et al.* 2000). The presence of plant pathogens (e.g. fungi) in soil may indicate the existence of other soil health problems, e.g. nutrient imbalance (Hornby *et al.* 1997). A suppressive soil is able to suppress specific plant diseases by inherent biotic and abiotic factors (Alabouvette 1999; Murakami *et al.* 2000; Toyota *et al.* 2000; Dominguez *et al.* 2001; Stone *et al.* 2001). The suppressiveness of a certain soil may thus be an indicator of plant health.

Several methods are available for determining soil suppressiveness as reviewed by van Bruggen & Grünwald (1996). It can be determined by inoculation of target-plant seeds directly into the test soil or into a pathogen-infested test soil (Toyota *et al.* 1995; Knudsen *et al.* 1999; Persson *et al.* 1999; Murakami *et al.* 2000). The frequency of diseased plants and/or pathogenic propagules in soil is scored after incubation for about 3 to 4 weeks and compared to a reference soil.

The plant bioassay is a conventional technique and a positive correlation between the plant bioassay and the actual field measurements has been shown for suppressiveness of pea root rot (Persson *et al.* 1999). A specific test plant system has to be selected for a monitoring programme and the correlation between bioassay and field measurements has to be confirmed on a diverse set of soils. The assay requires a relatively long time (e.g. weeks) before the results can be obtained, but it is simple and cheap.

6.3 Human pathogens

Human pathogens can enter agricultural soils through amendment with manure and sewage sludge. The presence of human pathogenic bacteria in soil is an indicator of potential human infection and as such an indicator of human health. Presence of *Escherichia coli*, have traditionally been used as an indicator of faecal contamination (of e.g. coastal waters) and hence as an indicator of the possible presence of other more pathogenic bacteria (Rhodes *et al.* 1988). Since the ability of the pathogenic bacteria to survive in the environment may not necessarily be equal to that of *E. coli* (Morales *et al.* 1996), it would be advantageous if the pathogens were enumerated directly. Zoonotic bacteria and antibiotic resistant bacteria are presently monitored in livestock, food products of animal origin and humans by the Danish Integrated Anti-Microbial Resistance Monitoring and Research Programme (Anonymous 2000), but no monitoring of human pathogenic bacteria in soil is carried out in Denmark.

Enumeration of pathogenic bacteria can be carried out either by cultivation or by molecular/immunological techniques. Methods relying on cultivation use growth media selective for specific groups of microorganisms, i.e. XLD agar for isolation of *Salmonella* and *Shigella* (Marsh *et al.* 1998) and MacConkey agar for isolation of coliforms (Atlas 1993). These methods are well-established, cheap, and easy to use. Molecular techniques may give a more accurate estimate of the population sizes, as they do not rely on growth of the bacteria. On the other hand they may detect dead bacteria as well as free DNA. Among the molecular methods, that would be suitable for a monitoring programme, are quantitative PCR (Lloyd-Jones *et al.* 1999) and specific fluorescent oligo-nucleotide probes (Szewzyk *et al.* 1993; Marsh *et al.* 1998). With immunological methods, specific antibodies are used instead of oligo-nucleotide probes (e.g. Hansen *et al.* 1997) and the detection limit can further be lowered when combined with immunomagnetic separation (Lund *et al.* 1988).

The drawback of using both the molecular and immunological techniques is that they technically are more demanding than the traditional culturing methods. Little is known about the occurrence of pathogenic bacteria in agricultural soil and investigations on the differences between fields receiving manure and/or sewage sludge and untreated fields are needed prior to implementation into a monitoring programme.

7 Indicators of bioavailability

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil microbial community health Leaching to groundwater Surface run-off	**Bioavailability**	Biosensor bacteria Plasmid-containing bacteria Antibiotic-resistant bacteria Catabolic genes

Chemical compounds may often be adsorbed to soil particles, such as clay minerals, and made unavailable to the biota. The bioavailable concentration will be equal to or lower than the total chemically extractable concentration. From an environmental viewpoint, the bioavailable fraction of a chemical compound may be a more relevant parameter than the chemically extractable fraction. Microorganisms can measure the bioavailability of a chemical compound in soil. Indicators of bioavailability represent measurements at the community and population levels.

7.1 Biosensor bacteria

Biosensor bacteria are designed to respond to certain stress situations (e.g. toxicity) through the use of reporter genes (Paton *et al.* 1997).

Environmental relevant bacteria can be selected and genetically modified by fusing reporter genes (e.g. bioluminescence) to the genes of interest and thereby give a certain signal to a specific response. Ultimately, fibre optic linked membrane bound biosensor probes may facilitate *in situ* ecotoxicity monitoring of soil (Paton *et al.* 1997).

Biosensor bacteria responding to mercury (Rasmussen *et al.* 2000) or chromate (Peitzsch *et al.* 1998) or zink (Paton *et al.* 1997) are presently available. The zink biosensor bacteria have been used for soil monitoring purposes, where it was the most discriminative method (C. Campbell, pers. comm. 2001). Commercial biosensor bacteria products for overall ecotoxicological analysis are available (Remedios™ (www.remedios.uk.com) and Microtox® (www.azurenv.com)).

7.2 Plasmid-containing bacteria

The frequency of plasmid-containing soil bacteria has been shown to be higher in polluted soils compared to agricultural soils, and to increase by addition of heavy metals to soil (Cambell *et al.* 1995; Drønen *et al.* 1998; Breen *et al.* 1992). Thus, measurement of numbers of plasmid-containing bacteria or numbers of plasmids in soil can be used as a general indicator of environmental contaminants. If numbers of plasmids increase at a site, an investigation to identify the stress factor (e.g. pollutants) can subsequently be initiated.

Two different approaches can be used to assess the occurrence of plasmids in soil, the endogenous and the exogenous approach. By the endogenous approach, plasmids are extracted from soil bacteria isolated on agar plates followed by a visualisation of the plasmids on agarose gels (Cambell *et al.* 1995). By the exogenous approach, suitable plasmid free recipient bacteria are used as "fishing rods". The plasmid free bacteria are mixed with a soil sample and allowed time to pick up (by conjugation) naturally occurring plasmids from the indigenous bacteria (Smalla *et al.* 2000; Drønen *et al.* 1998; Top *et al.* 1994). Plasmids are extracted and visualised as in the endogenous approach.

A major disadvantage of the endogenous plasmid extraction procedure is that it only analyses the fraction of soil bacteria that grow on cultivation media. This step is eliminated in the exogenous plasmid isolation procedure. However, only conjugative and mobilisable plasmids may be isolated by this method. The frequency and variability in plasmid numbers in different soil types should be estimated in order to standardise the method.

7.3 Antibiotic resistant bacteria

Restricted use of antibiotics (e.g. growth promoters) in agriculture has reduced but not eliminated antibiotic resistant bacteria in livestock and food (Anonymous 1998). Urban effluents, which also contain antibiotics, have been demonstrated to result in an increase in the number of antibiotic resistant bacteria in riverine environments (Goni-Urriza *et al.* 2000). Antibiotic substances have been detected in

outlets of sewage treatment plants (Witte 2000), manure and agricultural fields (Halling-Sørensen *et al.* 1998). Although the measured concentrations of antibiotic substances are generally below the minimum inhibitory concentration (MIC) to microorganisms, they may nevertheless select for the outgrowth of resistant bacteria in the soil ecosystem. Very little, however, is known about the occurrence of resistant microorganisms in agricultural soil. Heavy metal pollution may also indirectly select for antibiotic resistant bacteria, since a correlation between bacterial antibiotic resistance and mercury concentration in riverine sediments has been observed (McArthur *et al.* 2000). Thus, monitoring antibiotic resistant bacteria in soil will not only allow an assessment of the potential risk of antibiotic resistant bacteria to humans (human health), but can also be used as an indicator of industrial and urban pollution (potential leaching or surface run-off).

Enumeration of antibiotic resistant bacteria can be carried out either by cultivation and/or molecular techniques. Methods relying on cultivation on selective growth media containing antibiotics (tetracycline, kanamycin, etc.) are well established, cheap, and can easily be implemented in a monitoring programme. By use of these methods, not only can numbers of resistant bacteria be estimated, but the MIC and the breakpoint value may also be determined. This is necessary because an antibiotic concentration appropriate to distinguish between resistant and sensitive bacteria of one species, may not be applicable to another (Petersen *et al.* 1997). A well-known drawback of the cultivation methods is non-culturability of some bacteria. This can be overcome by molecular techniques, which estimate the population sizes of the resistance genes. PCR and molecular gene probe analysis (Aminov *et al.* 2001; Chee-Sanford *et al.* 2001; Schnabel *et al.* 1999) can possibly be used to detect a specific resistance gene in a soil sample and to develop quantitative PCR methods (Lloyd-Jones *et al.* 1999).

Since little is known about the occurrence of antibiotic resistant bacteria in agricultural soil, some baseline testing is required to investigate the possible differences between treated (i.e. with manure/sludge) and untreated fields. Monitoring of antibiotic resistant bacteria may be complemented with measurements of bioavailable concentrations of antibiotics by use of biosensor bacteria (Hansen *et al.* 2001) or plasmid-containing bacteria (see above).

7.4 Incidence and expression of catabolic genes

When the degradation pathway of a chemical compounds (e.g. pesticides) is known, key enzymes and catabolic genes can be identified and quantified. The presence of degradable chemical compounds in a soil is presumed to provoke a higher incidence and expression of corresponding catabolic genes due to either growth of bacteria or the spreading of the catabolic genes to the microbial community. Catabolic genes may, however, also be present due to their involvement in the degradation of naturally occurring and related organic compounds. The incidence of specific catabolic genes thus gives information on the ability of a soil to modify or degrade xenobiotic compounds. An elevated expression of the catabolic genes will, on the

other hand, indicate a partial or complete degradation of the corresponding organic compound.

Several methods have been proposed for determination of the incidence and expression of specific catabolic genes. These include conventional culturing of degradative microorganisms, activity measurements of specific degradative key enzymes, and molecular methods for detection of catabolic genes (e.g. PCR, qPCR) and measurements of their expression (e.g. mRNA, rRNA, biosensor bacteria). The molecular methods are described elsewhere (see chap. 1.1, 5.3, 7.1 and 7.3) and only the culturing technique will be dealt with here.

The potential for degradation of a xenobiotic compound in soil can be estimated by incubation of a soil slurry spiked with the compound (radiolabelled or unlabelled) of interest and subsequent determinations of either radiolabelled CO_2-production, the respiration rate (see chap. 2.1) or cell growth. The incubation approach is also used for isolation of consortia or pure cultures able to grow on and degrade specific xenobiotic compounds (Shuttleworth *et al.* 1997). The assay, though, is entirely dependent on the activity of the microorganisms and their culturability at the incubation conditions provided.

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