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Soil biodiversity monitoring in Europe: ongoing activities and challenges

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Summary

The increasing interest in soil biodiversity and its protection includes both the biodiversity conservation issues and the mostly unknown economic and ecological values of services provided by soil biodiversity. Inventory and monitoring are necessary tools for the achievement of an adequate level of knowledge regarding soil biodiversity status and for the detection of biodiversity hot spots as well as areas where current levels of biodiversity are under threat of decline. In this paper the main tools and methodological approaches for soil biodiversity measurement are presented. Technical aspects related to the inventory and monitoring activities at a large spatial scale are discussed. A short review of some current experiences of soil biodiversity monitoring at the European level is also presented.

Introduction

Humans have extensively altered the global environment and caused a reduction in the level of biodiversity. These changes in biodiversity alter ecosystem processes and change the resilience and resistance of ecosystems to environmental change. It is estimated that human activities have increased the rates of extinction by 100–1000 times (Lawton & May, 1995). In the absence of major change in policy and human behaviour these activities will continue to affect biodiversity.

The recent Conference of the Parties of the Convention on Biological Diversity (May 2008, Bonn) demonstrated that the need for action to protect biodiversity is unanimously acknowledged. Biodiversity conservation is essential both for ethical reasons and especially for the ecosystem services that the complex of living organisms provide for current and future generations. These ecosystem services are essential for the functioning of our planet.

Soil represents one of the most important reservoirs of biodiversity. The biological diversity in soils is several orders of magnitude higher than that found above ground (Heywood, 1995) and is seen as the last frontier for biodiversity on earth (Swift, 1999). Despite this, studies on soil biodiversity are often neglected and as such there is a paucity of knowledge on this subject. The majority of soil organisms are still unknown: it has been estimated that the currently described fauna of Nematoda, Acari and Protozoa represents less than 5% of the total number of species (Wall *et al*., 2001).

Relationships between ecosystem functioning and biodiversity are particularly evident in soil. Soils provide a high number of ecosystem services, thanks to the complex communities of organisms living there. The soil biota contribute, directly or indirectly, to nutrient cycling, waste (organic materials) decomposition, soil (structure) formation, and water regime control (Lavelle *et al*., 2006). The contribution of soil organisms to nutrient cycling in terrestrial ecosystems is well established, and quantified for a number of ecosystems (cf. Swift *et al*., 1998). Some of these processes, particularly within the N cycle, are performed only by very specific organisms, while others, such as soil organic matter decomposition, are carried out by a diverse group of bacteria, fungi, protozoans and invertebrates. Pimentel *et al*. (1997) estimated the global economic benefits of soil biodiversity at 1546 billion dollars, but the 'real' value of the services provided has still to be determined (Huguenin *et al*., 2006). Within the soil compartment, the most obvious service is waste recycling. Other services are less evident, such as plant pollination; many species of pollinators, in fact, have an edaphic phase in their early life cycle.

A necessary starting point to achieve the objective of preserving soil biodiversity is to reach an adequate level of knowledge on its

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extent and on its spatial and temporal distribution. If we consider rural areas, studies on soil microbiota are scarce in relation to those on other aspects of agricultural biodiversity (Weigel & Schrader, 2007). Soil biodiversity monitoring is essential for the early detection of possible decline in processing ability and to enable the adoption of measures to reverse such decline. Soil monitoring, in general, consists of the systematic determination of soil variables and their temporal and spatial variation. Adaptation of monitoring approaches to a living system, such as the complex of soil organisms, requires specific methodologies. The use of harmonized methodologies is essential to provide data that are comparable among sites (Morvan *et al*., 2008), but at the same time the specificity of soil organisms must be taken into account. These methodologies should enable representation of both the complexity and the high temporal and spatial variability that characterize soil biota.

The urgency to adopt soil biodiversity monitoring programmes is motivated by both the increasing pressures on soil biodiversity and the limited current knowledge. So, there is a clear need to summarize the status of soil biodiversity monitoring in Europe. After summarizing background information on the most important threats to soil biodiversity (including the legal tools, such as the EU official documents, in which they are listed) and general considerations on soil biodiversity inventories, monitoring and forecasting, this paper seeks to answer the following three questions:

1 How can soil biodiversity be measured?
2 Which monitoring activities are currently performed or planned in individual European countries and in the EU as a whole?
3 What recommendations can be given in order to monitor soil biodiversity efficiently?

During a recent workshop held in Brussels, oriented towards the identification of research needs for the implementation of the Soil Thematic Strategy, soil biodiversity status in Europe was identified as one of the main knowledge gaps. The conclusion was that there is a lack of data for a reliable assessment of threats to soil biodiversity at a regional scale, essential for the adoption of appropriate protection policies.

Background information

Threats to soil biodiversity

To some extent it is possible to base the evaluation of threats to soil biodiversity on the global evaluation of biodiversity pressure indicators proposed by Spangenberg (1999, 2007), bearing in mind the differences in the processes affecting above- and below-ground organisms. For Europe, the main anthropogenic disturbance factors or pressures have been identified at three levels of biodiversity: ecosystem, species and gene (EuroStat, 1999; EEA, 2004, 2005).

At the ecosystem level, the main pressures derive from over-exploitation, changes of climatic and hydrological regime, and change of geochemical framework. At the species level, the main pressures on soil biodiversity derive from changes in environmental conditions, change in geochemical framework, land-use change, competition from invasive species and effects of toxic compounds. At the gene level, the main pressures derive from changes in environmental conditions, effects of toxic compounds and the effects of genomics ('Genetic pollution').

Other pressure factors that are important for overall biodiversity are possibly less important for soil biodiversity. This is the case for habitat fragmentation and the consequent reduction of biotope size, which can theoretically be detrimental for soil biological diversity, but at scales that rarely occur in nature. For example, the scientific evidence for the effects of small-scale habitat fragmentation on soil organisms was in the order of a few square centimeters, far away from the 'real world processes' (Gonzalez & Chaneton, 2002; Rantalainen *et al*., 2006).

It is important to consider that in addition to the pressures listed above, any physical loss of soil, or other soil degradation processes, can lead to loss of biodiversity. Starting from the analyses carried out by Spangenberg (1999, 2007) regarding biodiversity in Europe, in Table 1 the main pressures on soil biodiversity, and the related driving forces, are listed.

Making inventories, monitoring, forecasting

The inventory of soil biodiversity (biodiversity in general) should consist of an estimation of taxonomic diversity at one (or several) site(s) at a given time. A second possible step, the monitoring activity, is achieved by estimating diversity at the same site, at more than one time, to allow inferences regarding change to be drawn. Inventories should be based on the adoption of standardized, quantitative and repeatable protocols of sampling and estimation of soil biodiversity. Any inventory protocol has to be designed to provide information on α and β-diversity. Di Castri *et al*. (1981) proposed the inclusion of a set of extensive sites and a subset of intensive sites, at which an in-depth and more accurate estimate of biodiversity should be carried out. Through the more complete inventory realized at the intensive sites, it would then be possible to calibrate the standardized quantitative sampling and estimation protocols. Replication in time of these protocols would then be the basis of monitoring activities. The selection of sites for inventory or monitoring programmes can be based on a hierarchical design, or a grid-based scheme (regular, irregular, stratified, etc.). In the hierarchical design, factors that mainly affect soil biodiversity are the first-level categories (i.e. land use/cover, soil type, etc.). The prediction of the possible distribution of living organisms in the environment can be achieved using the 'habitat suitability' approach. In a wider perspective habitat suitability models are based on the application of linear and non-linear multivariate statistical analysis on a spatial base. Habitat suitability models are often used to predict the likelihood of occurrence and abundance of species, using habitat attributes considered important for their survival, growth and reproduction (Laymon & Barrett, 1994; Guisan & Zimmermann, 2000); application of these models to soil organisms is scarce (Bonn & Schröder, 2001).

Table 1 Summary of the main pressures, sorces and driving forces on soil biodiversity

Pressure	Source	Driving force
Climate change	Increase in the greenhouse gas emissions to the atmosphere is recognized as the main cause of the climate change • CO_2 originates when organic materials are oxidized, mainly by burning fossil energy carriers, but also by natural processes such as soil and ocean respiration • N_2O release to the atmosphere originates from agriculture (N over-fertilization), industrial processes and vehicle engines. • CH_4 originates from rice paddies, wetlands, animal husbandry and waste site disposals	Energy consumption; Land use intensity; Agricultural intensity
Ecosystem/habitat disruption	Land-use change and the overexploitation of biodiversity can determine the disruption of ecosystems and habitats. Among the land-use change processes the conversion of agricultural land into urban areas (soil sealing), and the conversion of natural or seminatural habitats into agricultural land use are the most prominent threats to soil biodiversity	Land-use change; Land-use intensification
Soil erosion	Soil erosion is a natural process, but is usually exacerbated by human activities. The overexploitation of pasture or agricultural lands, can promote severe erosion	Land-use intensity; Energy consumption (via climate change)
Soil compaction	The use of heavy machinery in agriculture and the reduction in soil organic carbon content can determine soil compaction	Agricultural intensification
Chemical pollution	Long-range air pollutants Pesticides used in agriculture Persistent organic pollutants Heavy metals Trace elements from industrial processes and vehicle emissions	Agricultural intensification; Dissipative use of chemicals
Soil organic matter decline	Decline in soil organic matter is the result of a series of causes, among them: • Decoupling of animal husbandry and agricultural activities and consequent reduction of manuring practices • Intensification of agricultural practices (frequency and depth of tillage, continuous cropping, narrow crop rotations, reduction in return of crop residues, etc.) • Climate change	Agricultural intensification; Energy consumption (via climate change)
Human exploitation	Intensive agriculture Intensive animal husbandry and grazing Forest farming	Land-use change; Land-use intensification; Agriculture and animal husbandry intensity
GMO pollution	Accidental, deliberate or residual release of GMOs, with the subsequent establishment of modified organisms or of modified DNA in natural populations.	GMO production, trade and release
Invasive species	Accidental or deliberate introduction of foreign species as a result of globalization (global trade, tourism) The impact of invasive species may be exacerbated by climate change.	Globalization; Climate change
Habitat fragmentation	Land-use change processes, and the construction of linear transport infrastructure, generally lead to a reduction of natural and seminatural biotope size. This pressure for soil organisms, however, is not as dramatic as it is for other, above-ground, organisms.	Land-use change; Mobility infrastructures

How to measure soil biodiversity

A brief overview of methods for the determination of the diversity of soil microorganisms (Section 3.1), soil invertebrates (Section 3.2) and soil functions (Section 3.3) is given. In particular, the availability of standardized methods suitable for routine use in soil biodiversity monitoring programmes is highlighted. While some of these methods have been proposed by national agencies (e.g. in the United States by ASTM), the most suitable are those published by the International Organization for Standardization (ISO, Geneva, Switzerland) because they have been developed for monitoring purposes and because they are internationally widely accepted.

Microorganisms

Methodological overview. Soil microbial ecology is known to be an integrative science with strong interconnections between systematics, genetics, biochemistry, molecular biology, physiology, modelling, palaeobiology, soil science, parasitology, epidemiology and biotechnology, with important food, public health and environmental implications. Recently, much research has been undertaken in the development of molecular methods to enable the characterization of microbial information contained in the nucleic acids extracted from environmental samples. These developments have enabled the characterization of variations of the microbial community structure and diversity in multiple situations, allowing the identification of populations preferentially associated with environmental perturbations (for an extensive review see Ranjard *et al*., 2000). Altogether, these methodological developments have led to high-throughput screening and sequencing that are aiding the assessment of the metagenome (collective DNA from all microorganisms present in an ecosystem) and have provided the majority of the DNA sequences now found in databases. In the post-genomic era, a major challenge is to elucidate the functional role of the metagenome by linking the genetic structure and diversity of microbial communities with their functions. To fulfill this challenge, new approaches have been developed based on the coupling of molecular biology and isotopic techniques (DNA-/RNA-stable isotope probing) as well as the whole-scale characterization of the metatranscriptome (collective RNA from all microorganisms present in an ecosystem) and metaproteome (collective proteins from all microorganisms present in an ecosystem) (for review see Maron *et al*., 2007). In the context of soil microbial biosurvey, the use of these different approaches allows the assessment of various objectives such as:

1 estimating soil microbial diversity patrimony;
2 collecting soil microbial genetic resources;
3 ranking the contribution of pedo-climatic and land use factors by explaining the diversity and composition of bacterial communities; and
4 characterizing bacterial bio-descriptors of specific environments, land use and anthropogenic activities.

The diversity of soil microbial communities has been investigated for many years using methods based on isolation and culturing of microorganisms. Such techniques are known for their selectivity and are not representative of the entire bacterial community. The proportion of cells that can currently be cultured is estimated to be between 0.1% and 10% of the total population and very few data are available to indicate how closely this reflects the actual composition of these communities. Recent advances in the field of molecular biology (including extraction of nucleic acids, polymerase chain reaction (PCR) amplification, DNA cloning, and DNA sequencing) have made possible the development of techniques that no longer require the isolation and the culture of bacteria and thus reduce the bias associated with it. These methods involve a direct lysis of bacterial cells in soil followed by the extraction of the nucleic acids from the matrix and finally the analysis of targeted sequences of the whole genetic information.

These 'molecular ecology' methods have mostly been used to assess the composition of microbial communities (identification of genus, species or phylogenetic groups) and to monitor, over space and time, changes due to environmental disturbance, using as a target ribosomal genes and/or function-specific genes. More recently, the automation of these techniques makes it possible to work at a medium throughput, which is compatible with the need to characterize numerous samples from a structured programme. Altogether, such a strategy has enabled us to extend our understanding of microbial ecology and will continue to do so. However, due to the various biases or limitations of each technique (e.g. non-standardized DNA extraction procedures, and differential PCR amplification) they are not a substitute for more conventional methods (cultured population studies, measurement of activity, etc.) but must be viewed as complementary methods for use in investigating the ecology of bacteria in their natural habitats. In this respect, future prospects in microbial ecology must be of a polyphasic nature, combining selections of molecular biological and microbiological techniques to understand the relationships between microorganisms and their environment. Furthermore, data focusing on the activity of microbial populations and on gene expression and regulation *in situ* are still scarce, especially in soil environments. Development of tools addressing these points is a challenge for the coming years (for review see Maron *et al*., 2007).

An overview of the use of microorganisms in ecological soil classification and assessment, including monitoring programmes, was recently provided by Winding *et al*. (2005). Methods addressing the measurement of overall microbial parameters in soil have been standardized for some decades, mainly focusing on carbon and nitrogen cycles in soil (e.g. ISO, 1997a, b). More recently the need to standardize methods addressing the structure of microbial communities was identified as an important topic by standardization organizations. Currently, the standardization of two methods is under way: the extraction of DNA from soil as a prerequisite for several genetic methods as well as a method

for quantifying the functional diversity of microbial communities. As a minimum, measures of microbial biomass, respiration, N-mineralization and community profiling (e.g. DGGE, ARISA, PLFA, or CLPP) should be included in any battery for the monitoring of soil microorganisms (Winding *et al*., 2005).

Outcomes and difficulties related to the characterization of soil microbial biodiversity at large spatial scales. Ecologists studying meso- and macro-organisms have long recognized that β-diversity (how community composition changes across a landscape gradient) is central for understanding the environmental factors driving the magnitude and variability of biodiversity. This conceptual vision is also relevant for microorganisms because patterns of β-diversity can offer valuable insights into the relative influence of dispersal limitations, environmental heterogeneity and environmental and evolutionary changes in shaping the structure of ecological communities (Green *et al*., 2004).

Although microorganisms are the most ubiquitous, diverse and abundant living organisms on Earth, and play a key role in a wide range of biogeochemical cycles, few studies have investigated the distribution of microbial diversity at scales broader than agricultural fields or forests. This situation may partly be explained by the characteristics of microorganisms such as: (i) their small size leading to a weak accessibility in a heterogeneous soil matrix, (ii) their high density (e.g. more than one billion per gram of soil), and (iii) their huge diversity (from 1000 to 1 000 000 species per gram of soil; Torsvik *et al*., 2002) combined with the difficulty in precisely defining their species. In spite of these limitations, the first study describing and investigating microbial biogeography was conducted by Beijerinck (1913), who defined the first postulate: '*everything is everywhere*, but, *the environment selects*'. Since this date, few authors have examined the full extent of microbial diversity and described its biogeographical patterns in order to verify this statement and to specify which environmental factors exert the strongest influence on indigenous microbial communities. Although recent advances in molecular biology have allowed the development of tools to assess bacterial diversity in environmental samples without culturing (see section 3.1.1), most studies have focused on cataloguing bacterial diversity at particular sites and describing how bacterial communities were affected by environmental perturbations (for review see Ranjard *et al*., 2000). As a result, data obtained from different studies are difficult to compare and the trends deduced are often inconsistent, illustrating our inability to generalize in microbial ecology.

Many host-associated microorganisms exhibit genetic and functional patterns that are related to the distribution of their hosts. As regards free-living microorganisms, most of the recent investigations are limited to the phylogeography of individual soil bacterial strains (Cho & Tiedje, 2000). These studies have generally demonstrated that the genetic distance between microorganisms was related to the geographic separation and highlighted correlations between the assemblage composition and environmental or geographic characteristics (for review see Martiny *et al*., 2006). To date, only a few publications have considered the whole soil microbial community and how it is structured across large spatial scales. Green *et al*. (2004), through DNA fingerprinting of the fungal community structure of 1536 Australian soils, demonstrated that despite a high local diversity, microorganisms might have only moderate regional diversity. On the other hand, Fierer & Jackson (2006) have performed a continent-scale description of soil bacterial diversity by considering about 100 different soils sampled from the north to the south of America. By applying a DNA fingerprinting method, they demonstrated that bacterial diversity was unrelated to site temperature, latitude and other variables that typically strongly influence plant and animal diversity, and that community composition was largely independent of the geographical distance. The environmental factor with the largest influence on bacterial diversity was soil pH, with the highest diversity in neutral soils and the lowest in acidic soils. These studies demonstrated the weak taxa-area relationships of soil microorganisms and consequently that microbial biogeography fundamentally differs from the biogeography of 'macro-organisms'. However, Johnson *et al*. (2003) demonstrated that variations in bacterial community DNA fingerprints from numerous agricultural soils were significantly correlated with soil texture and electrical conductivity but not with pH. The inconsistency between these results is probably due to the inadequacy of the sampling strategy, in terms of the soil sampled. This underlines the importance of increasing the number of studies of microbial biogeography to improve our understanding of microbial diversity, especially given the influence of microbial diversity on a wide range of environmental processes and consequently on the quality of our environment.

Soil invertebrates

Methodological overview. The soil biota is thought to harbour a large part of the world's biodiversity and to govern processes that are regarded as globally important components in the cycling of organic matter, energy and nutrients (e.g. Griffiths *et al*., 2000). Rough estimates of soil biodiversity indicate several thousands of invertebrate species (e.g. 1500–1800 invertebrate species were found in a German beech forest; Weidemann, 1986). The most important soil invertebrate groups in terms of numerical abundance and/or total biomass in temperate regions are: nematodes, micro-arthropods (mites and collembolans), enchytraeids and earthworms. These are found in the uppermost soil layers (i.e. the soil surface and the litter layer). It has been demonstrated that the use of higher taxonomic levels or trophic groups can provide relevant information on soil status. In this context the Maturity Index (Bongers, 1990), based on the composition of nematode communities, is probably the best known example of a tool based on soil organisms used in routine evaluations of agricultural sites. Most of these invertebrate groups have been proposed for monitoring purposes, either alone or in combination. However, the results are usually not comparable because sampling methods and study designs differ considerably (see Breure *et al*., 2005). Wall

et al. (2001, p. 115) stated in their 'Summary of Research Priorities for Soil and Sediment Ecosystems' that their highest priority was for the development of 'a strategy to increase the number of taxonomists working with below-surface invertebrates, as there are presently very few of these specialists globally'.

Besides the lack of taxonomy specialists, the use of invertebrates in soil monitoring programmes has been severely hampered by the lack of standardized methods. This demand became obvious when schemes for the biological classification and assessment of soils were proposed in Germany and in The Netherlands (Ruf *et al*., 2003; Breure *et al*., 2004). Because the biological characterization of a soil can lead to site-specific regulations (theoretically even a remediation of that site), any monitoring method must be standardized in order to be legally defensible. The methods applied for such purpose should be well-established and robust (Römbke *et al*., 2006). Based on the experience of the Tropical Soil Biology and Fertility (TSBF) Programme, Anderson & Ingram (1989) published a Handbook of Methods for Soil Biology and Fertility in Tropics, and 12 years later, Swift & Bignell (2001) published a Standard Method for the Assessment of Soil Biodiversity and Land Use Practices. Within the last few years, a working group of the ISO Technical Committee 190 Soil Quality reviewed appropriate candidates and proposed five methods for inclusion within the working programme, covering earthworms (ISO, 2006a), micro-arthropods (ISO, 2006b), enchytraeids (ISO, 2007a), nematodes (ISO, 2007b) and soil macrofauna (mainly arthropods living close to or on the soil surface) (ISO, 2008). As an example, the main features of the earthworm sampling method are presented in Table 2.

Discussion of the use of soil invertebrates in soil biodiversity assessment and soil quality indication. Soil invertebrates are a key component of soil biota. Below-ground diversity is essential for above-ground ecosystem function (Van Straalen, 2004). Some microfauna and mesofauna groups are highly abundant, their role in soil formation and transformation is well-recognized, the area covered during their life cycle is representative of the site under examination, their life histories permit insights into soil ecological conditions, and several species have already been recognized as useful biological indicators of soil quality.

Table 2 Hand-sorting and formalin extraction of earthworms

Guideline	International Standard ISO 23611-1
Species	Natural community (e.g. Lumbricidae, Glossocolecidae, etc.)
Principle	Combination of hand-sorting and formalin extraction
Method	Digging-out and hand-sorting of the soil within an area of 50 * 50 cm and a depth of *c*. 20 cm Application of 5–10 litres (several times) of a 0.5% aqueous formalin solution into the dug-out hole followed by a period of 30 minutes until the worms appear at the soil surface
Storage	Fixation in ethanol (70%) for 1–2 days, followed by 1–2 weeks in 4% formalin, then final storage in 70% ethanol
Parameters	Abundance, biomass, species composition
Remarks	In appendices: various modifications (e.g. sampling in the tropics [TSBF method] or fixation for genetic studies)

Soil fauna have a catalysing role in the cycling of elements but also an important function in vegetation diversity and succession. Although some soil animals are carnivorous, the most widespread ecosystematic activity of the soil meso- and macrofauna is the 'processing' and 'mixing' of organic detritus in the soil (Killham, 1994; Mulder, 2006). Moreover, ecosystem engineers, like termites, earthworms and large arthropods, directly or indirectly control the availability of resources to other organisms by causing physical state changes in biotic or abiotic materials (Verhoef, 2004). Due to their abundance the soil fauna can be seen as the soil's ecological insurance. Species diversity makes a community more stable and secure against catastrophic events (Van Straalen, 2004).

Over the past 20 years the importance of soil faunal diversity for many ecosystem services has received increasing recognition. The increasing recognition of problems derived from soil degradation has contributed to identification of soil fauna research as a priority in soil quality assessments (Bongers, 1990, 1999; Van Straalen & Krivolutsky, 1996; Pankhurst *et al*., 1997; Van Straalen, 1997). Most soil animals have life cycles that are highly dependent on their immediate environment, interacting with soil in several different ways. To be able to evaluate their role and function, it is important to use methodologies that properly reflect either the number of species present, or the processes and roles that they play in the soil environment.

The growing interest in the employment of living organisms for the evaluation of soil conditions is justified by the great potential of these techniques, which are more sensitive than physico-chemical methods and give information that is more easily interpreted. The basic idea of bio-indicators is that the relationship between soil factors and soil communities can be reversed: when soil factors influence community structure, the structure of the community expresses information on the soil factors (Van Straalen, 1997, 2004). Studies on soil organisms often focus on one trophic level only (Brussaard *et al*., 2007), making an accurate assessment of soil fauna populations a challenge. To a certain extent, the focus on one trophic level is due to difficulties in extracting organisms efficiently from the soil matrix (Smith *et al*., 2008) and to uncertainties in taxonomic identification. Probably the best examples of accurate assessment of a taxonomic group of soil fauna are the Collembolan studies performed by Paulo Sousa and his colleagues (e.g. Ponge *et al*., 2006) in various European countries.

To retrieve information about soil quality, different properties of community structure, such as species richness and diversity, distribution of numbers over species, distribution of body-size over species, classification of species according to life-history attributes or to ecophysiological preferences and food-web structure, can be used (Van Straalen, 2004). The number of bio-indicator systems using soil and litter invertebrates is relatively high; some approaches use nematodes, enchytraeids, mites, collembolans, dipterans or coleopterans (e.g. Cortet *et al*., 1999; Van Straalen,

2004; Mulder, 2006). Recently, different authors have proposed new methods for soil quality assessment, based on soil fauna. Some of these methods are based on the general evaluation of microarthropods (Parisi *et al*., 2005), while others are based on the evaluation of a single taxon (Bernini *et al*., 1995; Iturrondobeitia *et al*., 1997; Paoletti, 1999; Paoletti & Hassal, 1999; Parisi & Menta, 2008).

The use of bioindicators highlights the interactions among the different pollutants and between them and the soil. Often, bio-monitoring techniques are not specific to the pollutant or environmental variable that functions as a stressor. For this reason, bio-monitoring cannot be considered a substitution for the physico-chemical analysis, but as a complementary methodology that allows a broader outlook on the study in question. Rutgers *et al*., (2000) proposed the 'Soil Quality Diamond', where soil assessment comprised three elements: chemical analysis, bioassays and ecological surveys. The last aspect involves three indicator groups of soil invertebrates: nematodes (Maturity Index), earthworms (biomass of epigeics, endogeics and anecics), and micro-arthropods (biodiversity of Collembola and Oribatida).

Soil functions

Soil organisms are involved in many soil functions, which are under increasing pressure from contamination, erosion, organic matter decline, compaction, salinization and landslides (EC, 2006). Most of these functions are performed through multitrophic interactions that ensure the resistance, resilience and recovery of these functions (FAO, 2003; Andren *et al*., 2004; Beck *et al*., 2005; Lavelle *et al*., 2006):

1 decomposition of organic matter, thus regulating the cycling of nutrients;
2 fixation of nitrogen from the atmosphere, making it available for plants;
3 degradation of anthropogenic compounds such as pesticides;
4 stabilization of soil aggregates, specifically by building clay-humus-complexes;
5 improvment of soil porosity due to burrowing activities;
6 influencing soil pH through nitrification and denitrification, resulting in mobility changes of heavy metals;
7 influencing heavy metal mobility under different redox conditions (e.g. in the sulphur cycle, especially important in areas with fluctuating water tables); and
8 last but not least, being prey for other organisms.

Because of this high number of functions, various methods have been developed to cover functional diversity, most of them referring to microbial activity. At short timescales, measures of microbial biomass, respiration, N-mineralization and a community profiling method (e.g. DGGE, PLFA, or CLPP) are recommended (Winding *et al*., 2005; Mulder *et al*., 2007).

Concerning soil invertebrates, probably the best example of functionally important species is anecic (i.e. deep-burrowing) earthworms such as *Lumbricus terrestris*. These organisms are considered to be ecosystem engineers (Jones *et al*., 1994), because they penetrate the soil by building burrows, thereby increasing pore space, and transport soil and organic matter by casting, functioning on organic material as a first step of organic matter breakdown, providing nutrients to plants, relocating seeds in the soil profile, changing the diversity and improving the activity of the microbial community by selective feeding and providing faeces rich in nutrients (Lavelle *et al*., 1997). Thus, any impact on these species, determined using either prospective tests (ISO, 1999) or retrospective monitoring methods (ISO, 2006a), will strongly affect soil structure and organic matter breakdown.

Finally, some integrative methods are used to measure functions performed by the soil organism community as whole. Here the litter-bag method, using mass loss of organic matter as an endpoint (OECD, 2006) or the bait-lamina test, focusing on the feeding rate of soil invertebrates (Kratz, 1998), could be used for this purpose, but in both cases the lack of an appropriate control has limited their use so far.

Ongoing activities and projects

In this section, examples of the use of soil biological parameters in European national monitoring programmes are presented. The aim of this compilation is to identify concepts, methods, assessments or other experiences that may be useful for an EU-wide monitoring programme of soil biodiversity. It should also be mentioned that other soil biodiversity monitoring schemes are now in progress in Europe, such as Ireland's soil nucleic archive (EPA, 2008) and the assessment of soil biodiversity within the UK's Countryside Survey (Black *et al*., 2003).

France

In France, a scientific project called 'ECOMIC-RMQS' coordinated by the Centre for 'Microbiologie du Sol et de l'Environnement' (CMSE, INRA Dijon, Burgundy, France) was started in 2006 with the aim of assessing for the first time, the microbial biogeography at the scale of the French territory. This integrated project will provide conceptual insights into the ecological theory on the community assembly by:

1 identifying the relative contribution of geographic isolation versus wide dispersal limit in bacterial diversification;
2 examining better the taxa-area relationship for bacteria; and
3 providing a better resolution of the hierarchy of the environmental parameters (plant cover, physico-chemical characteristics, climate factors, etc.) that contribute to the bacterial community diversity and composition.

In addition, this project could also give more applied outcomes such as:

1 the definition of the current level of bacterial diversity in French soils;

2 a better estimate of the impacts of land use and human activities on microbial diversity and distribution; and
3 the identification of bacterial bio-indicators specific to land use and human activities.

Soil sampling has been undertaken on the basis of the Réseau de Mesures de la Qualité des Sols (RMQS), on a 16 × 16 km systematic grid covering the whole of the French territory (Arrouays *et al*., 2002). The RMQS has a total of 2200 monitoring sites, including the 600 sites of the ICP forest level 1 European network.

The strategy for characterizing telluric bacterial communities is based on molecular tools such as quantitative PCR, DNA micro-array and DNA fingerprint directly on DNA extracted from soil. A robust method of direct extraction of DNA from soil was developed (Martin-Laurent *et al*., 2001; Ranjard *et al*., 2003).

One of the main aims of the project ECOMIC-RMQS is to build up and maintain a national soil DNA library (in the platform GenoSol, http://www.dijon.inra.fr/plateforme_genosol) that could be available to the whole scientific community in order to assess microbial diversity in the future with more powerful tools and/or other molecular analysis.

Germany

Germany is a federal republic, meaning that many governmental duties are covered at the level of the 16 'Länder' (Federal States). For example, despite the fact that there has been a federal Soil Protection Law (BBodSchG, 1998) in force for about 10 years now, monitoring activities at the several hundred permanent soil monitoring sites currently existing are not centrally co-ordinated. In the context of soil monitoring, and in particular soil biodiversity, this means that each state may have a different approach and the German government is focusing its activities mainly on initiating research projects. This also means that huge data sets on soil biodiversity generated in several states have not been assessed so far (although a compilation of these data is planned in the foreseeable future). However, due to activities of expert working groups, some recommendations for sampling methods (e.g. Barth *et al*., 2000) and assessment concepts (e.g. Beylich *et al*., 2005) are available. One proposal to address soil biodiversity, within planning processes in land use regulation, is given in Figure 1.

Soil biodiversity endpoints do not belong to the normal set of monitoring parameters but are performed on a case-by-case basis, often triggered by the interest of individuals responsible for soil protection, forestry or agriculture in State agencies – and on the available budget. Therefore, in some states almost nothing

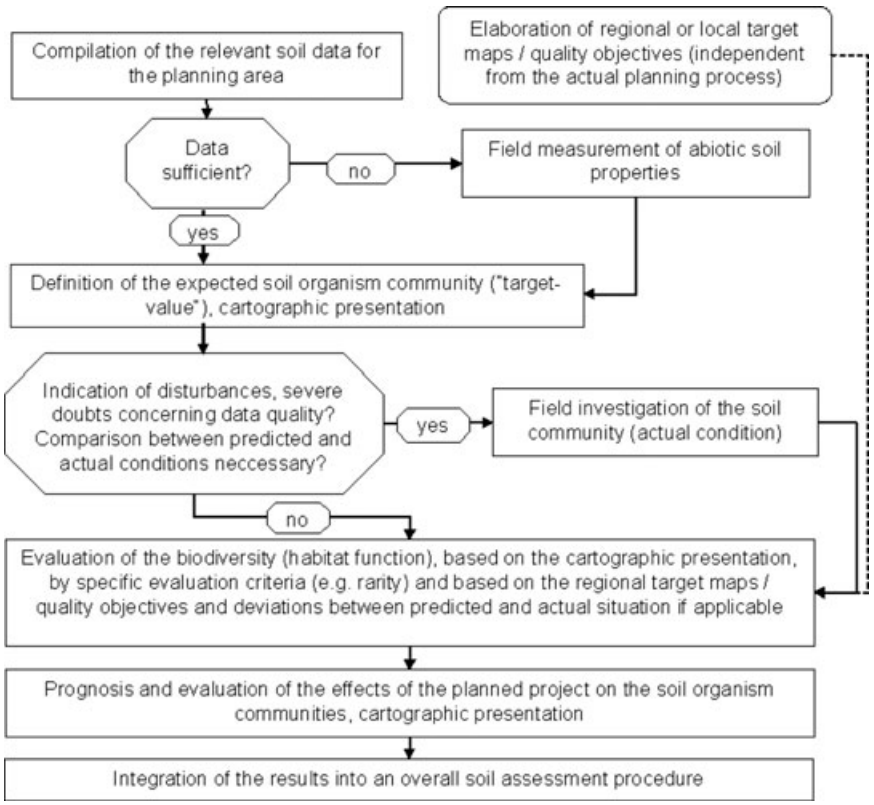

Figure 1 Decision tree for the assessment of the soil biodiversity (habitat for soil organisms) within planning processes (Figure taken from Beylich *et al*., 2005).

is known about soil biodiversity, while others, notably Baden-Württemberg, Brandenburg, Bayern Hamburg, Niedersachsen, Nordrhein-Westfalen, Schleswig-Holstein and Thüringen, have supported (irregularly) monitoring activities, mainly focusing on earthworms and microorganisms and less frequently on enchytraeids or collembolans. Recently, the knowledge on monitoring earthworm biodiversity has been compiled and discussed at a workshop organized by the German Federal Environmental Agency (UBA). There was an overall agreement that biological parameters should be included in soil quality assessment and that besides earthworms, other groups of organisms should also be used. However, it was also stated that further research is necessary in order to improve data interpretation and assessment criteria (Henneberg, 2007).

For about 10 years, based on activities starting in the state of Baden-Württemberg, the German Federal Environmental Agency (UBA) supported the development of soil biological classification and assessment concepts, more or less in parallel with Dutch activities (Römbke & Breure, 2005). So far, about 50 sites (mainly forests but also grassland and crop sites) have been sampled, for a wide range of soil organisms. Recently, both the UBA and individual states have supported research on the impact of global climate change on biodiversity in general, including soil biodiversity. An institute whose aim is to address these questions was founded in Frankfurt am Main in June 2008.

The Netherlands

The Dutch Soil Quality Network (DSQN) was established with the main aim of obtaining policy information regarding soil status and trends. Selected sites represent 70% of the soils and land uses of the Netherlands. A complete field sampling 'round' takes 5 years. This network was originally designed to collect data on abiotic characteristics of the soil (soil moisture, acidification, etc.) and it contains 200 locations on 10 soil type/land use combinations (20 replicates). Each year, 40 locations are sampled. The DSQN is concerned with 500 sites (200 locations sampled twice, plus 100 extra sites), including the Lheerbroekerzand monitoring area of the ICP forest European network. Apart from forests, the majority of sites in the Netherlands are rural and most sample locations are farms between 5 and 100 ha. Cultivation practices are recorded and the historical events are noted.

To investigate further the hierarchy of environmental parameters (plant cover, physico-chemical characteristics, climate factors, etc.) contributing to bacterial community diversity in relation to bacterial-grazing invertebrates (nematodes, collembolans, mites, enchytraeids and earthworms), all soil micro- and macro-organisms were monitored (Mulder *et al*., 2003; Schouten *et al*., 2004; Rutgers *et al*., 2008).

From the microbiological point of view, the Biological Indicators of Soil Quality project (BISQ) will give several applied outcomes such as:

1 the establishment of a state of bacterial diversity under different management regimes and soil textures in Dutch soils;
2 a better estimation of the impact of land use (livestock, pesticides) and human activities (liming, tillage) on microbial diversity; and
3 the identification of bacterial bio-indicators specific to land use and human activities, possibly in relation to transgenic crops.

Furthermore, the Netherlands Secretary for the Environment wishes to investigate the extent to which agriculture may affect ecosystem functioning below ground, or disturb fundamental microbial processes. From that point of view, the following questions will be addressed.

1 Is there microbial evidence of environmental stress in relation to some crops?
2 Does microbial competition for shared resources generate adaptive radiation?
3 Can a better ecological insight into shifts within the microbial community be obtained using the catabolic activity of bulk soil bacteria? And, if so, can we use it for better modelling?

Besides the aforementioned microbial methods, the community-level physiological profiles (CLPP) of the investigated soils have been measured in multiwell microplates (BIOLOG 'EcoPlates') specifically designed over a decade ago for micro-ecological studies by Insam (1997).

A European initiative ENVASSO (www.envasso.com)

The EU FP6 project ENVASSO (Environmental Assessment of Soil for Monitoring) addressed the eight threats to soil identified by the Commission (EC, 2002). The aim of the project was to design and test a single, integrated and operational set of EU-wide criteria and indicators that will provide a basis for a comprehensive harmonized soil and land information system for Europe. Indicators suitable for monitoring changes in soil biodiversity were selected from a literature review and an inventory of national monitoring programmes in the EU. Within this project decline in soil biodiversity was defined as the reduction of forms of life living in soils (both in terms of quantity and variety) and of related functions, causing a deterioration or loss of one or more soil functions. While the literature review allows the identification of about 100 possible indicators, the inventory of existing monitoring networks shows that few indicators are actually measured.

For monitoring it was considered that only three key indicators per soil stress were practical. However, this was considered a difficult task for indicating biodiversity decline due to the complexity of soil biota and multi-functionality in soils. Therefore stringent criteria were applied to the selection process to evaluate: (i) methodology standardization, (ii) complementarity to other indicators, and (iii) interpretation at both scientific and policy levels.

The key indicators selected were chosen as representative of three functional levels in soil: (i) abundance, biomass and species diversity of earthworms-macrofauna, (ii) abundance and species diversity of Collembola-mesofauna, and (iii) microbial respiration.

Table 3 Priority level of indicators for decline in soil biodiversity (ENVASSO)

Key issue	Groups of species	Level I (all core points of the monitoring network)	Level II (all core points or selected points depending on relevance to specific issues and availability of resources)	Level III (optional)
Species diversity	Macrofauna	Earthworm species	All macrofauna	
	Mesofauna	Collembola species Enchytraeidae (if no earthworms)	Acarina sub-orders	Activity based on litter bags or on bait lamina
	Microfauna		Nematode (functional) diversity based on feeding habits	Protista
	Microflora		Bacterial and fungal diversity based on DNA/PLFA extraction	
	Vascular plants			For grassland and pastures
Biological functions	Macrofauna			Macrofauna activity (e.g. biogenic structures, feeding activity)
	Mesofauna			Mesofauna activity
	Microflora	Soil respiration	Bacterial and fungal activity	

Of course, in principle when considering soil biodiversity, all soil organisms and the biological functions that they provide are important and should be assessed. However, for reasons of practicability it was decided to select this minimum set of three representative ecological groups (priority level I, Table 3) to act as surrogate measures for overall changes in biodiversity. Depending on the availability of resources and any specific requirements, this minimum set of indicators could be extended in some regions (priority levels II and III, Table 3). Procedures and protocols, based upon current ISO standards and adapted for assessment at a European scale, were tested in pilot sites established in four countries (France, Ireland, Portugal and Hungary) in order to assess the ease of measurement of the selected indicators and their efficiency in indicating the decline in soil biodiversity. The results obtained proved the effectiveness of each indicator and its sensitivity to detect change across a range of land-use categories at a European scale.

Conclusions

The understanding of the relationship between soil biodiversity and soil or ecosystem functions is still not complete, but increasing pressures are being imposed on the living organisms of the soil. The evaluation of the regional distribution of soil biodiversity, as a function of climate, soil type, land use and management is scarce. However, there is every reason to believe that declines in soil biodiversity are following the general trend observed above ground. For these reasons, monitoring activities are necessary in order to protect soil biodiversity. Monitoring soil biodiversity will enable the detection of biodiversity hot spots as well as areas subject to change, and the implementation of ecosystem management successfully.

The experiences presented in this paper focus on the monitoring of soil microbial diversity, but also on bacterial-grazing invertebrates and earthworms. From the microbial perspective, we might conclude that ECOMIC-RMQS is a powerful network to detect biogeographical trends and taxonomical patterns thanks to novel molecular techniques, whereas DSQN focuses on the ecophysiological response of soil microbial communities under environmental stress. The possible combination of these two approaches is a challenge for the future of applied microbiology in Europe and elsewhere.

Concerning soil invertebrates, the standardization of protocols for sampling, extraction and determination represents an important advancement for the adoption of these organisms in large-scale soil biodiversity monitoring programmes. The difficulties related to the taxonomic classification remain a bottleneck and as such the research into the use of biodiversity indexes, based on higher taxonomic level, or key species is highly pertinent and could aid the overcoming of these difficulties.

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References

Anderson, S.E. & Ingram, J.S.I. 1989. *Tropical Soil Biology and Fertility: A Handbook of Methods*. CABI, Aberystwyth.

Andren, O., Baritz, R., Brandao, C., Breure, A.M., Feix, I., Franko, U. *et al*. 2004. Organic matter and biodiversity. Task Group 3 on soil biodiversity. In: *Reports of the Technical Working Groups Established Under the Thematic Strategy for Soil Protection, 21319 EN/1* (eds L. VanCamp, B. Bujarrabal, A.R. Gentile, R.J.A. Jones, L. Montanarella, C. Olazabal & S.-K. Selvaradjou), pp. 357-397. EU Commission, Luxembourg.

Arrouays, D., Jolivet, C., Boulonne, L., Bodineau, G., Saby, N. & Grolleau, E. 2002. Une initiative nouvelle en France: La mise en place d'un Réseau multi-institutionnel de Mesures de la Qualité des Sols (RMQS). *Comptes rendus des Seances de l'Academie d'Agriculture de France*, **88**, 93–103.

Barth, N., Brandtner, W., Cordsen, E., Dann, T., Emmerich, K.-H., Feldhaus, D. *et al*. 2000. Boden-Dauerbeobachtung. Einrichtung und Betrieb von Boden-Dauerbeobachtungsflächen. In: *Bodenschutz, XI/00* (eds D. Rosenkranz, G. Bachmann, W. König & G. Einsele), Erich Schmidt Verlag, Berlin.

BBodSchG 1998. Gesetz zum Schutz vor schädlichen Bodenveränderungen und zur Sanierung von Altlasten (Bundes-Bodenschutzgesetz). *Bundesgesetzblatt I*, **502**.

Beck, L., Römbke, J., Breure, A.M. & Mulder, C. 2005. Consideration for the use of soil ecological classification and assessment concepts in soil protection. *Ecotoxicology & Environmental Safety*, **62**, 211–229.

Beijerinck, M.W. 1913. De infusies en de ontdekking der bakterien. In: *Jaarboek van de Koninklijke Akademie van Wetenschappen*, pp. 1–28. Mueller, Amsterdam.

Bernini, F., Avanzati, A.M., Baratti, M. & Migliorini, M. 1995. Oribatid mites (Acari Oribatida) of the Farma valley (Southern Tuscany). *Notulae Oribatologicae LXV/ Redia*, **78**, 45–129.

Beylich, A., Broll, G., Graefe, U., Höper, H., Römbke, J., Ruf, A. *et al*. 2005. Biologische Charakterisierung von Böden. Ansatz zur Bewertung des Bodens als Lebensraum für Bodenorganismen im Rahmen von Planungsprozessen. *BVB-Materialien*, **13**, 1–78.

Black, H.I.J., Parekh, N.R., Chaplow, J.S., Monson, F., Watkins, J., Creamer, R. *et al*. 2003. Assessing soil biodiversity across Great Britain: national trends in the occurrence of heterotrophic bacteria and invertebrates in soil. *Journal of Environmental Management*, **67**, 255–266.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance on nematode species composition. *Oecologia*, **83**, 14–19.

Bongers, T. 1999. The maturity index, the evolution of nematode life history traits, adaptive radiation and c-p scaling. *Plant & Soil*, **212**, 13–22.

Bonn, A. & Schröder, B. 2001. Habitat models and their transfer for single and multi species groups: a case study of carabids in an alluvial forest. *Ecography*, **24**, 483–496.

Breure, A.M., Mulder, C., Rutgers, M., Schouten, T., De Zwart, D. & Bloem, J. 2004. A biological indicator for soil quality. In: *Agricultural Impacts on Soil Erosion and Soil Biodiversity: Developing Indicators for Policy Analysis* Proceedings from an OECD Expert Meeting. March 25–28, 2003, pp. 485–494. Rome.

Breure, A.M., Mulder, C., Römbke, J. & Ruf, A. 2005. Ecological classification and assessment concepts in soil protection. *Ecotoxicology & Environmental Safety*, **62**, 211–229.

Brussaard, L., De Ruiter, P.C. & Brown, G.G. 2007. Soil biodiversity for agricultural sustainability. *Agriculture, Ecosystems & Environment*, **121**, 233–244.

Cho, J.-C. & Tiedje, J.M. 2000. Biogeography and degree of endemicity of fluorescent *Pseudomonas* strains in soil. *Applied & Environmental Microbiology*, **66**, 5448–5456.

Cortet, J., Gomot de Vauflery, A., Poinsot-Balaguer, N., Gomot, L., Texier, C. & Cluzeau, D. 1999. The use of invertebrate soil fauna in monitoring pollutant effects. *European Journal of Soil Biology*, **35**, 115–134.

Di Castri, F., Hadley, M. & Damlamian, J. 1981. MAB: the man and the biosphere program as an evolving system. *Ambio*, **10**, 52–57.

EC 2002. Communication from the Commission to the Council, the European Parliament, the Economic and Social Committee and the Committee of the Regions, Towards a Thematic Strategy for Soil Protection, Brussels. COM (2002) 179, 16/4/2002.

EC 2006. Communication "Thematic Strategy for soil protection", COM (2006) 231, 22/9/2006.

EEA 2004. The state of biological diversity in the European Union. *Malahide Information Paper, Malahide/Inf2*. European Environment Agency, Copenhagen.

EEA 2005. *European Environment Outlook*. European Environmental Agency Report, Vol. 4/2005, Luxembourg.

EPA 2008. *Overview of the NSDB Archive* [WWW document]. URL http://erc.epa.ie/nsdb/index2.jsp [accessed on 6 November 2008].

EuroStat 1999. *The Environmental Pressure Index Programme*. EUROSTAT, Luxembourg.

FAO (Food and Agriculture Organization) 2003. *Biological Management of Soil Ecosystems for Sustainable Agriculture*. World Soil Resources Reports. Rome.

Fierer, N. & Jackson, R.B. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 626–631.

Gonzalez, A. & Chaneton, E.J. 2002. Heterotroph species extinction, abundance and biomass dynamics in an experimentally fragmented microecosystem. *Journal of Animal Ecology*, **71**, 594–602.

Green, J.L., Holmes, A.J., Westoby, M., Oliver, I., Briscoe, D., Dangerfiedl, M. *et al*. 2004. Spatial scaling of microbial eukaryote diversity. *Nature*, **432**, 747–750.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F. *et al*. 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. *Oikos*, **90**, 279–294.

Guisan, A. & Zimmermann, N.E. 2000. Predictive habitat distribution models in ecology. *Ecological Modelling*, **135**, 147–186.

Henneberg, P. (ed.) 2007. *Bodenbiologische Bewertung von Boden-Dauerbeobachtungsflächen (BDF) anhand von Lumbriciden*. UBA-Texte 34/07. Umweltbundesamt, Dessau-Roßlau.

Heywood, V.H. 1995. *Global Biodiversity Assessment*. Cambridge University Press, Cambridge.

Huguenin, M.T., Leggett, C.G. & Paterson, R.W. 2006. Economic valuation of soil fauna. *European Journal of Soil Biology*, **42**, S3–S15.

Insam, H. 1997. A new set of substrates proposed for community characterisation in environmental samples. In: *Microbial Communities: Functional Versus Structural Approaches* (eds H. Insam & A. Rangger), pp. 259–260. Springer, Berlin.

ISO 1997a. *Soil Quality–Biological Methods–Determination of Nitrogen Mineralization and Nitrification in Soils and the Influence of Chemicals on These Processes*. ISO 14238, Geneva.

ISO 1997b. *Soil Quality–Determination of Soil Microbial Biomass–Part 1: Substrate Induced Respiration Method*. ISO 14240-1, Geneva.

ISO 1999. *Soil Quality–Effects of Pollutants on Earthworms–Part 3: Guidance on the Determination of Effects in Field Situations*. ISO 11268-3, Geneva.

ISO 2006a. *Soil Quality–Sampling of Soil Invertebrates Part 1: Hand-Sorting and Formalin Extraction of Earthworms*. ISO 23611-1, Geneva.

ISO 2006b. *Soil Quality–Sampling of Soil Invertebrates Part 2: Sampling and Extraction of Microarthropods (Collembola and Acarina)*. ISO 23611-2, Geneva.

ISO 2007a. *Soil Quality–Sampling of Soil Invertebrates Part 3: Sampling and Soil Extraction of Enchytraeids*. ISO 23611-3, Geneva.

ISO 2007b. *Soil Quality–Sampling of Soil Invertebrates Part 4: Sampling, Extraction and Identification of Free-Living Stages of Nematodes*. ISO 23611-4, Geneva.

ISO 2008. *Soil Quality–Sampling of Soil Invertebrates Part 5: Sampling and Extraction of Soil Macrofauna*. Draft ISO 23611-5, Geneva.

Iturrondobeitia, J.C., Saloña, M.I., Pereda, J., Caballero, A.I. & Andrés, M.C. 1997. Oribatid mites as an applied tool in studies on bioindication: a particular case. *Abhandlungen und Berichte des Natuurkundemuseums Görlitz*, **69**, 85–96.

Johnson, M.J., Lee, K.Y., Scow, K.M. 2003. DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. *Geoderma*, **114**, 279–303.

Jones, C.G., Lawton, J.H. & Shachak, M. 1994. Organisms as ecosystem engineers. *Oikos*, **69**, 373–386.

Killham, K. 1994. *Soil Ecology*. Cambridge University Press, Cambridge.

Kratz, W. 1998. The bait lamina test – general aspects, applications and perspectives. *Environmental Science & Pollution Research*, **5**, 94–96.

Lavelle, P., Bignell, D. & Lepage, M. 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. *European Journal of Soil Biology*, **33**, 159–193.

Lavelle, P., Decaens, T., Aubert, M., Barot, S., Blouin, M., Bureau, F. *et al*. 2006. Soil invertebrates and ecosystem services. *European Journal of Soil Biology*, **42**, S3–S15.

Lawton, J.H. & May, R.M. 1995. *Extinction Rates*. Oxford University Press, Oxford.

Laymon, S.A. & Barrett, R.H. 1994. Developing and testing habitat-capability models: pitfalls and recommendations. In: *Wildlife 2000: Modelling Habitat Relationships of Terrestrial Vertebrates* (eds J. Verner, M.L. Morrison & C.J. Ralph), pp. 87–91. University of Wisconsin Press, Madison, WI.

Maron, P.A., Ranjard, L., Mougel, C. & Lemanceau, P. 2007. Metaproteomics: a new approach for studying functional microbial ecology. *Microbial Ecology*, **53**, 486–493.

Martin-Laurent, F., Philippot, L., Hallet, S., Chaussod, R., Germon, J.C., Soulas, G. *et al*. 2001. DNA extraction from soils: old bias for new microbial diversity analysis methods. *Applied & Environmental Microbiology*, **67**, 2354–2359.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L. *et al*. 2006. Microbial biogeography: putting microorganisms on the map. *Nature Review Microbiology*, **4**, 102–112.

Morvan, X., Saby, N.P.A., Arrouays, D., Le Bas, C., Jones, R.J.A., Verheijen, F.G.A. *et al*. 2008. Soil monitoring in Europe: a review of existing systems and requirements for harmonisation. *Science of the Total Environment*, **391**, 1–12.

Mulder, C. 2006. Driving forces from soil invertebrates to ecosystem functioning: the allometric perspective. *Naturwissenschaften*, **93**, 467–479.

Mulder, C., De Zwart, D., Van Wijnen, H.J., Schouten, A.J. & Breure, A.M. 2003. Observational and simulated evidence of ecological shifts within the soil nematode community of agroecosystems under conventional and organic farming. *Functional Ecology*, **17**, 516–525.

Mulder, C., Wouterse, M., Rutgers, M. & Posthuma, L. 2007. Transgenic maize containing the Cry1Ab protein ephemerally enhances soil bacterial communities. *Ambio*, **36**, 359–361.

OECD (Organisation for Economic Co-operation and Development) 2006. In: *OECD Series on Testing and Assessment No. 56: Guidance Document on the Breakdown of Organic Matter in Litter Bags*. ENV/JM/MONO, pp. 23–36. OECD, Paris.

Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. (eds.) 1997. *Biological Indicators of Soil Health*. CABI, Aberystwyth.

Paoletti, M.G. 1999. The role of earthworms for assessment of sustainability and as bioindicators. *Agriculture, Ecosystems & Environment*, **74**, 137–155.

Paoletti, M.G. & Hassal, M. 1999. Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. *Agriculture, Ecosystems & Environment*, **74**, 157–165.

Parisi, V. & Menta, C. 2008. Microarthropods of the soil: convergence phenomena and evaluation of soil quality using QBS-ar and QBS-c. *Fresenius Environmental Bulletin*, **17**, 1170–1174.

Parisi, V., Menta, C., Gardi, C., Jacomini, C. & Mozzanica, E. 2005. Microarthropod community as a tool to assess soil quality and biodiversity: a new approach in Italy. *Agriculture, Ecosystems & Environment*, **105**, 323–333.

Pimentel, D., Wilson, C., McCullum, C., Huang, R., Dwen, P., Flack, J., Tran, Q., Saltman, T. & Cliff, B. 1997.. Economic and environmental benefits of biodiversity. Bioscience **47**, 747–758.

Ponge, J.F., Dubs, F., Gillet, S., Sousa, J.P. & Lavelle, P. 2006. Decreased biodiversity in soil springtail communities: the importance of dispersal and landuse history in heterogeneous landscapes. *Soil Biology & Biochemistry*, **38**, 1158–1161.

Ranjard, L., Poly, F. & Nazaret, S. 2000. Monitoring complex bacterial communities using culture-independent molecular techniques: application to soil environment. *Research in Microbiology*, **151**, 167–177.

Ranjard, L., Lejon, D.P.H., Mougel, C., Schehrer, L., Merdinoglu, D. & Chaussod, R. 2003. Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. *Environmental Microbiology*, **5**, 1111–1120.

Rantalainen, M.L., Haimi, J., Fritze, H. & Setälä, H. 2006. Effects of small-scale habitat fragmentation, habitat corridors and mainland dispersal on soil decomposer organisms. *Applied Soil Ecology*, **34**, 152–159.

Römbke, J. & Breure, A.M. 2005. Ecological soil quality – classification and assessment. *Ecotoxicology & Environmental Safety*, **62**, 185–308.

Römbke, J., Sousa, J.P., Schouten, T. & Riepert, F. 2006. Monitoring of soil organisms: a set of standardised field methods proposed by ISO. *European Journal of Soil Biology*, **42**, S61–S64.

Ruf, A., Beck, L., Dreher, P., Hund-Rinke, K., Römbke, J. & Spelda, J. 2003. A biological classification concept for the assessment of soil quality: "Biological Soil Classification Scheme" (BBSK). *Agriculture, Ecosystems & Environment*, **98**, 263–271.

Rutgers, M., Faber, J.H., Postma, J.F. & Eijsackers, H. 2000. *Site-Specific Ecological Risks: A Basic Approach to the Function-Specific Assessment of Soil Pollution*. The Netherlands Integrated Soil Research Programme, Wageningen.

Rutgers, M., Mulder, C., Bloem, J., Schouten, T. & Brussaard, L. 2008. Biodiversiteit van de bodem in beeld: 10 jaar meten aan biologische bodemkwaliteit. *Bodem*, **4**, 29–32.

Schouten, T., Breure, A.M., Mulder, C. & Rutgers, M. 2004. Nematode diversity in Dutch soils, from Rio to a biological indicator for soil quality. *Nematology – Monographs and Perspectives*, **2**, 1–14.

Smith, J., Potts, S. & Eggleton, P. 2008. Evaluating the efficiency of sampling methods in assessing soil macrofauna communities in arable systems. *European Journal of Soil Biology*, **44**, 271–276.

Spangenberg, J.H. 1999. Indikatoren für biologische Vielfalt. In: *Zugänge zur Biodiversität-Disziplinäre Thematisierungen und Möglichkeiten integrierender Ansätze* (eds C. Görg, C. Hertler, E. Schramm & M. Weingarten), Metropolis, Marburg.

Spangenberg, J.H. 2007. Biodiversity pressure and the driving forces behind. *Ecological Economics*, **61**, 146–158.

Swift, M.J. 1999. Towards the second paradigm: integrated biological management of soil. In: *Interrelação Fertilidade, Biologia do Solo e Nutrição de Plantas*, pp. 11–24. UFLA, Lavras, Brasil.

Swift, M. & Bignell, D. (eds.) 2001. *Standard Methods for Assessment of Soil Biodiversity and Land Use Practice*, pp. 37. International Centre for Research in Agroforestry, Bogor, Indonesia.

Swift, M.J., Andren, O., Brussaard, L., Briones, M., Couteaux, M.M., Ekschmitt, K. *et al*. 1998. Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. *Global Change Biology*, **4**, 729–743.

Torsvik, V., Øvreås, L. & Thingstad, T.F. 2002. Prokaryotic diversity – magnitude, dynamics, and controlling factors. *Science*, **296**, 1064–1066.

Van Straalen, N.M. 1997. Community structure of soil arthropods. In: *Biological Indicators of Soil Health* (eds C.E. Pankhurst, B.M. Doube & V.V.S.R. Gupta), pp. 235–264. CABI, Aberystwyth.

Van Straalen, N.M. 2004. The use of soil invertebrates in ecological survey of contaminated soils. In: *Vital Soil: Function, Value and Properties* (eds P. Doelman & H.J.P. Eijsackers), pp. 159–194. Elsevier, Amsterdam.

Van Straalen, N.M. & Krivolutsky, D.A. (eds.) 1996. Bioindicator systems for soil pollution. *Proceedings of the NATO Advanced Research Workshop on New Approaches to the Development of Bioindicator*

Systems for Soil Pollution Moscow, Russia, April, 1995, pp. 24–28. Kluwer, Dordrecht.

Verhoef, H. 2004. Soil biota and activity. In: *Vital Soil Function, Value and Properties* (eds P. Doelman & H.J.P. Eijsackers), pp. 99–125. Elsevier, Amsterdam.

Wall, D.H., Snelgrove, P.V.R. & Covich, A.P. 2001. Conservation priorities for soil and sediment invertebrates. In: *Conservation Biology. Research Priorities for the Next Decade* (eds M.E. Soulé & G.H. Orians), pp. 99–123. Island Press, Society for Conservation Biology, Washington, DC.

Weidemann, G. 1986. Artenzahlen in den Ökosystemen des Sollings. In: *Ökosystemforschung – Ergebnisse des Solling-Projekts 1966–1986* (eds H. Ellenberg, R. Mayer & J. Scheuermann), pp. 219–225. Verlag Eugen Ulmer, Stuttgart.

Weigel, H.-J. & Schrader, S. (eds.) 2007. Forschungsarbeiten zum Thema Biodiversität aus den Forschungseinrichtungen des BMELV, pp. 206. *Landbauforschung Völkenrode*,.

Winding, A., Hund-Rinke, K. & Rutgers, M. 2005. The use of microorganisms in ecological soil classification and assessment concepts. *Ecotoxicology & Environmental Safety*, **62**, 230–248.

