



Indicators for evaluating soil quality

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Abstract

Interactions between the diversity of primary producers (plants) and of decomposers (microbes and mesofaunal communities), the two key functional groups that form the basis of all ecosystems have major consequences on the functioning of agricultural ecosystems. Soil microorganisms control the transformation and mineralization of natural compounds and xenobiotics. The soil microbiota, existing in extremely high density and diversity, rapidly modify the energetic performance and activity rates to changing environmental conditions. Thus, the microbial consortium possesses the ability to accommodate environmental constraints by adjusting (i) activity rates, (ii) biomass, and (iii) community structure. These parameters are particularly important to take into consideration when evaluating soil quality. The present paper gives an overview about the possibilities to use bacterial and fungal populations as an indicator for soil quality. Furthermore also the applicability of nematodes for the determination of soil health will be discussed.

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1. Selection of indicators

Soil quality is defined as the ‘continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, sustain biological productivity, to promote the quality of air and water environments, and to maintain plant, animal and human health’ (Doran and Safley, 1997). Since soil microorganisms can respond rapidly, they reflect a hazardous environment and are, therefore, considered when monitoring soil status. However, it is still unclear whether naturally occurring environmental factors can damage the genotypic ability of the soil microbiota to recover after harsh conditions and become healthy

again (Sparling, 1997). Research on the resilience of soil microbiota may be a significant task of molecular approaches.

The ideal soil microbiological and biochemical indicator to determine soil quality would be simple to measure, should work equally well in all environments and reliably reveal which problems existed where. It is unlikely that a sole ideal indicator can be defined with a single measure because of the multitude of microbiological components and biochemical pathways. Therefore, a minimum data set is frequently applied (Carter et al., 1997). Thus, the basic indicators and the number of estimated measures are still under discussion. However, national and international programs for monitoring soil quality presently include biomass and respiration measurements but extended also to nitrogen mineralization, microbial diversity and functional groups of soil fauna (Bloem et al., 2003).

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Irrespectively, it is essential to consider a set of abiotic and biotic properties and processes as soil indicators in ecosystems.

2. Soil microbial biomass

The soil microbial biomass can be defined as organisms living in soil that are generally smaller than approximately 10 μm . Most attention is given to fungi and bacteria, these two groups of microbes being the most important with reference to energy flow and nutrient transfer in terrestrial ecosystems (Richards, 1987). Fungi and bacteria are generally dominating within the biomass. However, most biomass estimates do not reliably exclude protozoa. The microbial biomass consists of dormant and metabolically active organisms. However, the presently widespread biomass estimates, either direct or indirect (biochemical) techniques, were not properly valid and checked for separating these fractions.

It has been suggested that the microbial biomass content is an integrative signal of the microbial significance in soils because it is one of the few fractions of soil organic matter that is biologically meaningful, sensitive to management or pollution and finally measurable (Powlson, 1994). With the development of the four now widespread indirect methods, fumigation-incubation (FI), substrate-induced respiration (SIR), fumigation-extraction (FE) and ATP content (Jenkinson and Powlson, 1976; Anderson and Domsch, 1978; Jenkinson and Ladd, 1981; Vance et al., 1987), a great deal of effort has gone into the measurement of the size of the microbial biomass and its associated nutrient pools. All these methods are designed to quantify the microbial biomass carbon in different soil samples, soil horizons, soil profiles and sites (Elliott, 1994). However, it must be realized that between different soil samples different biomass may occur without direct correlation to soil quality (Martens, 1995; Dilly and Munch, 1998).

Nevertheless the soil microbial biomass is the eye of the needle through which all organic matter needs to pass through (Jenkinson et al., 1987). As a susceptible soil component, the biomass may be therefore a useful indicator since pollution may reduce this pool as, e.g. demonstrated by Fritze et al. (1996) for heavy metals.

3. Structural microbial diversity

However the measurement of the microbial biomass is a black box approach, without differentiating the heterogeneity of the microbial community. With the rise of molecular genetic tools in microbial ecology it became apparent that we know only a very small part of the diversity in the microbial world. Most of this unexplored microbial diversity seems to be hiding apparently in the high amount of yet uncultured bacteria. New direct methods, independent from cultivation, based on the genotype (Amann et al., 1995) and phenotype (Zelles, 1996) of the microbes allow a deeper understanding of the composition of microbial communities. Using, e.g. the rDNA directed approach of dissecting bacterial communities by amplifying the 16S rDNA (*rrs*) gene from environmental samples by polymerase chain reaction (PCR), and studying the diversity of the acquired *rrs* sequences, almost exclusively new sequences became apparent which are only to a certain degree related to the well studied bacteria in culture collections (Amann et al., 1995). Frequently occurring, yet uncultured bacteria became visible microscopically by using fluorescently labelled rRNA-directed oligonucleotide probes. Based on molecular studies it can be estimated that 1 g of soil consists of more than 10^9 bacteria belonging to about 10,000 different microbial species (Ovreas and Torsvik, 1998). This huge amount of diversity makes it often difficult to handle the microbial community structure as an indicator for soil quality.

However there are some studies which could demonstrate clear effects of changes in the farming management or contamination of a site on the total microbial community structure. Ovreas and Torsvik (1998) compared the influence of crop rotation and organic farming on microbial diversity and community structure. They found a higher diversity in soils which were under organic farming management. However almost nothing is known about sustainability of measured microbial parameters. Only Smit et al. (2001) investigated the seasonal fluctuation of bacterial soil community in a wheat field. Mainly for the monitoring of contaminations microbial diversity parameter are often used, for the assessment of soil quality. Muller et al. (2001), for example, investigated the long-term effects of long-term exposure to mercury on the soil microbial community along a gradient of

pollution. It could be shown that bacterial diversity was reduced in the contaminated soils, whereas there was no difference in fungal biomass. Most available information is about the effects of pesticides on microbes and their degradation by bacteria and fungi. Fantroussi et al. (1999) could demonstrate that due to the application of urea herbicides microbial diversity was decreased. Ibekwe et al. (2001) showed a clear impact of fumigants on the soil microbial community. Similar results were obtained for other pesticides (triadimefon) by Yang et al. (2000).

Due to the mentioned complicity of the whole microbial community it might be useful to look at indicator organisms only, which are correlated to soil quality, for example, beneficial microbes like *Rhizobium* or arbuscular mycorrhiza (AM).

AM are the most ancient and ubiquitous root symbioses, formed by fungi belonging to the order of Glomales (Zygomycetes) and 80% of terrestrial plants (Saif and Khan, 1975). AM fungi are obligatory biotrophic symbionts living in the roots of most terrestrial plants which positively affect on plant growth, and plant nutrition. The fungi involved act as biofertilizers, and are very important for agriculture (Gianinazzi and Schuepp, 1994). Furthermore, AM represent a direct interface between soil and roots, and a place of exchange not only of nutrient elements but also of toxic elements. Since Glomales and/or AM symbioses are sensitive to PAH (polycyclic aromatic hydrocarbons) and MTE (metallic trace elements), both could also be used as bioindicator of contaminated soils (Weissenhorn et al., 1995). The decline of AM occurrence and infectivity of AM in metal-polluted soils can be used as bioindicators of soil contamination.

Natural rhizobia populations are essential to increase the yield of leguminous crops. The importance of the interaction is based on the capacity of symbiotic *Rhizobium* strains to form nodules and fix atmospheric nitrogen. Some papers describe the influence of different farming systems on plant growth promoting *Rhizobium* (Miethling et al., 2000). The survival of *Rhizobium* on chickpea seeds, treated separately with one of the four commercial fungicides was improved by Kyei-Boahen et al. (2001) under laboratory conditions. Fungicide treatment in general decreased the viability of *Rhizobium* strains, forming capacity of nodulation, N₂ fixation, and plant growth. Also in

other studies effect of various pesticides (insecticides, fungicides and herbicides) on growth and efficiency of symbiotic properties were found (Madhavi et al., 1993). Although only very little is known about the evolution of natural bacterial populations through the years in relation to a host plant diversity and abundance of rhizobia might be a good indicator for soil quality.

4. Microbial activity

Soil microbial activity leads to the liberation of nutrients available for plants but also to the mineralization and mobilization of pollutants and xenobiotics. Thus microbial activity is of crucial importance in biogeochemical cycling. Microbial activities are regulated by nutritional conditions, temperature and water availability. Other important factors affecting microbial activities are proton concentrations and oxygen supply. The group of methods on soil microbial activities embraces biochemical procedures revealing information on metabolic processes of microbial communities. To estimate the soil microbial activity, two groups of microbiological approaches can be distinguished.

First, experiments in the field that often require long periods of incubation (i.e. Hatch et al., 1991; Alves et al., 1993) before significant changes of product concentrations are detected, i.e. 4–8 weeks for the estimation of net N mineralisation. In this case, variations of soil conditions during the experiment are inevitable, i.e. aeration, and may influence the results (Madsen, 1996). Furthermore field measurements are often difficult to interpret, for example, soil respiration determined in the field suffers in separating the activity of microorganisms and other organisms such as plants, which vary significantly in different systems and throughout the season (Dilly et al., 2000).

In contrast short-term laboratory procedures that are usually carried out with sieved samples at standardized temperature, water content and pH value. Short-term designs of 2–5 h minimize changes in biomass structure during the experiments (Brock and Madigan, 1991). Such microbial activity measurements include enzymatic assays that catalyze substrate-specific transformations and may be helpful to ascertain effects of soil management, land use and

specific environmental conditions (Burns, 1977). Laboratory methods have the advantage in standardizing environmental factors and, thus, allowing the comparison of soils from different geographical locations and environmental conditions and also results from different laboratories. They are frequently used to gain information on ‘functional groups’. However, laboratory results refer to microbial capabilities, as they are determined under optimized conditions of one or more factors, such as temperature, water availability and/or substrate.

When measuring soil enzyme activity, it is important to understand what type of information is being collected and how can it be used. Taylor et al. (2002) mentioned two main reasons for measuring soil enzymes. First, as indicators of process diversity, which informs about the biochemical potential, possible resilience and potential for manipulation of the soil system. Second, as indicators of soil quality, in the sense that changes in key functions and activities can provide information about the progress of remediation operations or the sustainability of particular types of land management. Despite the obvious benefits of having these types of information, Pettit et al. (1977) pointed out that it is important to realize the restrictions on enzyme assays and the limitations on the interpretation. Soil enzyme assays generally provide a measure of the potential activity, i.e. that encoded in the “soil genotype”, but this will rarely ever be expressed. However, it may represent the redundancy of the soil biochemical system and as such is an aspect of resilience. Some soil enzyme assays attempt to measure real activity, i.e. a phenotypic property, but are rarely successful. In considering soil enzymes as an indicator of soil quality, which enzymes are important? A case can be argued for at least 500 enzymes with critical roles in the cycling of C or N or both, but clearly this many cannot be measured routinely. If there is genuine redundancy in enzymatic functions in soil, the loss of activity of a specific “keystone” enzyme should not have a major effect. If, on the other hand, changes in the activity of some “benchmark” enzymes provide an early indication of changes in process diversity, soil enzymatic measurements have a clear role in the assessment of soil quality. The question that remains is which enzymes should be measured for this purpose?

5. Nitrogen turnover as an indicator for soil quality

Bioavailable nitrogen is one of the keys for plant growth in agriculture. At the same time nitrogen compounds like nitrate, nitrite or N_2O play an important role in environmental pollution. Therefore it is of great interest to understand the key processes in the nitrogen cycle in more detail, to define ways for a high productive agriculture which protects environment. On the one hand two main delivery processes (mineralisation and nitrogen fixation) are known. On the other hand nitrification and denitrification can cause significant losses of nitrogen from the bound pool.

The microbial mineralisation of proteins in terrestrial ecosystems fulfils the key function to mobilize organically bound nitrogen (Ladd and Butler, 1972). Nitrogen is transformed in the cycle as ammonium. Extra cellular proteases are produced by microbes and secreted to the environment to hydrolyze macromolecular polypeptides into smaller molecules, which can be removed by the cell (Kalisz, 1988). They have in general a very low substrate specificity. Results from Bach and Munch (2000) indicate that differences in protease activities are not caused by different microbial populations but by variable expression rates of the same community. As proteases are exoenzymes they are after secretion no longer under the regulation of the cell and can be stabilized by clay particles in the soil. Therefore the enzymatic activity for its own is no sensible indicator for the actual microbial proteolytic activity. Several attempts are made to identify eco-physiological conditions that cause an induction or repression of the peptidase expression in the habitat (Bach et al., 2001).

Nitrogen fixation is performed by phylogenetically and physiologically diverse groups of prokaryotic organisms and poses a challenge to microbial ecologists in terms of diversity and activity assessment. The ecology of free-living diazotrophs has received little attention due to the low fixation rates that are usually attributed to non-symbiotic nitrogen fixation. Nevertheless, recurring accounts of unusually high N inputs into studied systems in addition to quickly activated N_2 -fixing activity in soil when energy sources are present indicate that this phenomenon plays an important role in natural systems and might have applications in land management. To provide better tools for

the study of this group of organisms, molecular approaches have been developed based on PCR amplification of the *nifH* gene and its mRNA transcripts for the group-specific detection of free-living diazotrophs in soil (Widmer et al., 1999).

Nitrification is the chemolithoautotrophic oxidation of ammonium via nitrite to nitrate. Nitrification can be measured directly using labeled nitrogen. Another possibility to determine potential nitrification is based on the addition of chlorate to inhibit the nitrite oxidation (Kandeler, 1989). The potential nitrification can be measured as accumulation of nitrite after addition of ammonium in short-term experiments. This method is well suited for measuring potential nitrification in high number of samples. A reduction in diversity for ammonia oxidizers for tilled soils was found by Bruns et al. (1999) in comparison to the native plots.

Denitrification is one of the key processes in the global nitrogen cycle as nitrate is turned into gaseous products (Flessa et al., 1995). During the process nitrate is stepwise reduced via nitrite, NO and N₂O to N₂. Due to the action of denitrifying microorganisms, the global dinitrogen content in the atmosphere is largely in balance due to the formation of the dinitrogen gas from terrestrial nitrate. On the other hand, nitrogenous oxides released from soils and waters have several impacts on the atmosphere. Nitrous oxide is next to CO₂ and CH₄ in its importance as a potent greenhouse gas. Nitric acid and its chemical oxidation product NO₂ are major constituents of acid rain, and NO and also N₂O interact with ozone in complex reactions and are major causes of the destruction of the protective ozone layer in the stratosphere. Nitrate is the main N-source for the growth of plants in agriculture but can simultaneously be used also by microorganisms in soils. Denitrification is generally regarded as an anaerobic process, but there are indications that it may take place also in well-aerated soils with high contents of bioavailable organic matter. The conditions which favor denitrification in soils have not yet been elucidated in much detail. It is, however, clear that any use of nitrate by bacteria means a loss of N for the growth of plants. Thus, denitrification has also severe impact on agriculture. In addition, products of denitrification (nitrate respiration) have manifold other, mainly adverse effects on soils but also on the atmosphere and waters. While the denitrification product N₂O can be easily measured using

gas chromatography, the determination of N₂ is not straight forward because comparatively small amounts of N₂ produced during denitrification have to be distinguished from a large background of 78% N₂ in the atmosphere. The methods available for measuring denitrification in the field are based on the use of the stable isotope ¹⁵N or on acetylene for blockage of the enzyme N₂O-reductase. In a preliminary study Cheneby et al. (2000) investigated denitrifying bacteria in three agricultural soils using classical cultivation techniques. They found a good correlation between number and diversity of denitrifiers and soil type.

6. Faunal indicators: nematodes

The use of faunal groups as indicators for soil quality needs a choice of organisms, that (a) form a dominant group and occurs in all soil types, (b) have a high abundance and high biodiversity and (c) play an important role in soil functioning, e.g. in food webs.

Nematodes fulfill these conditions and seem to be at present state of knowledge the most promising group, also because different tests in ecotoxicology, realized for single species as well as for communities, shows the suitability (Traunspruger and Drews, 1996; Freeman et al., 2000; Peredney and Williams, 2000; Haitzer et al., 1999). The group, composed of more than 11,000 species (Andrassy, 1992) includes species with different degree of tolerance against stress. Furthermore, the most important species used for ecotoxicologic assessments, *Caenorhabditis elegans*, is one of the few organisms with full genetic information. So, not only the classical toxicity parameter such as lethality, growth, reproduction and behavior can be realized, but also toxicity essays at molecular level, with the possibility to get information on the bioavailability of contaminants. Nematodes have life cycles over a broad range (a few days to over 2 years). This gives the possibility to integrate effects over different time scales.

Use of soils fauna as indicators offers different possibilities. Single species bioassays are important to assess effects of single stressors and bioconcentration studies. However, these tests are often realized in laboratory experiments, with soil samples transferred in experimental systems and spiked with contaminants. Experiments on community level are ecologically

more relevant. They integrate interactions of all soil factors including management and pollutants effects. Effects recorded by nematode bioassays reflect, e.g. also the environmental conditions of the community, so effects of the physical habitat or the food availability. Community assays offer analysis of different features: abundance of individuals or species, biomass of species, species composition, feeding strategies, presence and abundance of key species. The data obtained are to be analyzed by different techniques, univariate or multivariate, depending on the required quality of response of the experimental device. Different measures are used. Shannon index gives the distribution of species abundance and also reveals rare species (higher index = higher diversity). Simpson index shows the distribution of species abundance, with more weight to common species (higher index = higher dominance). The evenness (value between 0 and 1) gives information on the distribution of species abundance (higher index = higher diversity). Feeding types are reflected by the index of trophic diversity. The maturity index (scales from 1 to 5) is an indicator for the persistence of colonizers or for the life strategies of nematodes (disturbance indicated by a low index).

Multivariate methods consider species or groups in combination with data an abundance or biomass. Similarities or dissimilarities between such assemblages are visualized by cluster analysis. Multivariate statistics tests the differences in community structure.

7. Aggregation of indicators

The aggregation of indicators for evaluating soil quality should consider the complexity of microbial life in soil. Multiple indicators can be regarded to refer to the 'driving forces' for C and N cycling in soils. As a minimum data set microbial biomass content and microbial activity rates including enzyme activities were often estimated together with measures on some basic soil components, i.e. organic C content (Carter et al., 1997). Data sets can be compared by designing sun ray plots (Dilly and Blume, 1998; Kutsch et al., 1998; Dilly and Kutsch, 2000). They show the pattern of the considered features and prospectively may evaluate with reference to both real and acceptable values of properties and processes and thus characteristics with respect to thresholds, limits or the window of viability.

The lower the serration of the star as in case of the A horizon of a wet grassland in contrary to maize monoculture, the higher is the association between the microbial features and link between microbial processes (Dilly and Blume, 1998).

In contrast to this star approach, canonical component analyses that represent state-space orientation are frequently lacking in a clear explanation of ecological interrelations between dependent and controlling, e.g. biotic and abiotic factors. Furthermore, the rationale with respect to 'emergent' properties of soils and ecosystems (Müller, 1996; Dilly and Kutsch, 2000) will not be achieved.

In addition, microbial activities related to microbial biomass are used for evaluating environmental conditions calculating, i.e. the metabolic quotient, which is the ratio between CO₂ production under standardized conditions and microbial C content (Anderson and Domsch, 1993). Finally, soil microbial activities of C and N cycles should be related to soil C and N stocks providing information concerning transformation intensity in labile pools by looking at substrate transformation and product formation.

To evaluate soil quality, spatial heterogeneity of microbiological characteristics in ecosystems is important to take into account since microbiological features may vary scale-dependently (Stork and Dilly, 1998).

For holistic approaches, indicators may be displayed in hierarchical schemes for analyzing interactions and signal transfers in different subsystems (Dilly and Kutsch, 2000; Dilly et al., 2001). The abundance of specific populations and active components are probably more variable in contrast to the biomass. Particularly the activity of the whole biomass may change considerably with reference to environmental impact in contrast to biomass itself. These alterations may only slightly or slowly be affected in more stable ecosystem components such as the soil organic C content.

8. Conclusions

The great abundance and diversity of microorganisms in soil have high metabolic potentials. Since microorganisms are generally growth-limited in soils, they may poorly exploit their capabilities. In contrast, soil microorganisms respond rapidly to stressors by

adjusting (i) activity rates, (ii) biomass, and (iii) community structure. Combining soil microbiological estimates, e.g. in sun rays or quotients, seems to be of great relevance for evaluating soil quality. This is shown in four papers presented by: (1) Ruf et al. 'A biological classification concept for the assessment of soil quality'; (2) Anderson 'Microbial eco-physiological indicators to assess soil quality'; (3) Eckschmitt et al. 'On the quality of soil biodiversity indicators—three case studies at different spatial scales'; (4) Schloter et al. 'Influence of precision farming on the microbial community structure and selected functions in nitrogen turnover with indicator value for soil quality.

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