Biological Soil Characteristics: Indicators for Assessing Soil Quality

Franco Widmer and Hans-Rudolf Oberholzer

Swiss Federal Research Station for Agroecology and Agriculture (FAL-Reckenholz)

Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

e-mail: franco.widmer@fal.admin.ch and hansrudolf.oberholzer@fal.admin.ch

Abstract

Soil indicators should allow to monitor changes in soil quality. General and technical criteria need to be met to make an indicator routinely applicable to soil quality monitoring. Microbiological soil characteristics (MSC) are attractive potential soil quality indicators (SQI). They are sensitive to changes in soil chemical and physical characteristics and may be considered sensors and integrators for a variety of stresses to soil. Soil microbial parameters like soil biomass and basal soil respiration are among the most important ones and have proven to be robust and powerful in soil quality monitoring. However, they do not reflect diversities and structures of soil microbial communities. More recently developed methods may serve this purpose. Analyses of community level substrate utilization, community level fatty acid profiles, or community level genetic profiles represent the most commonly used fingerprinting approaches for microbial community structures. However, these methods do not yet meet most of the criteria for robust and applicable indicators and have still limited power for determination of microbial diversity in soil. Thus, further development of fingerprinting indicators is required. We propose to currently apply the established and robust soil biological indicators. Focused research needs to be initiated to establish defined indicators of changes in soil microbial community structures. These indicators need to be included in the set of currently existing indicators in order to allow monitoring of MSCs at a higher resolution.

Keywords

soil quality indicator (SQI), microbiological soil characteristics (MSC), soil microbial diversity, soil microbial biomass

Introduction

A short and comprehensive definition of soil quality has been proposed by Doran and Parkin (1994): „The capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health“. The presently applied definition of soil fertility for legislation purposes in Switzerland is more detailed, but contains basically the same components (Anonymous, 1998). A more detailed definition of soil quality and of the factors that define it is very difficult to achieve and a large number of such definitions has been proposed (Patzel *et al.* 2000)

Characterization of soil quality may be approached by means of physical, chemical or biological methods depending on the particular functional aspect of the soil under consideration. All three approaches detect specific soil characteristics as well as possible interactions and thus can reflect changes in soil quality. Biomass, community structures, and specific functions of soil microorganisms appear to be of major importance for general soil functions and if detectable could serve as sensitive soil quality indicators (SQI). These MSCs of a soil reflect and integrate chemical, physical and biological soil properties over time, since microbial soil communities strongly depend on the conditions of the habitat they colonize. Thus, microbiological characteristics of a soil may provide indicators, which integrate short-, middle- and long-term changes in soil quality. As soils display a multitude of biological characteristics and many of them may not be accessible, specific indicators have to be chosen. These indicators should satisfy some general and technical requirements (Table 1).

Table 1: General and technical requirements for biological soil quality indicators (SQIs)

General requirements for biological SQIs:

- direct relation to the soil function under evaluation
- sensitivity to effects of general land use and farming practices
- sensitivity to potentially harmful changes in soil quality, e.g. contamination or changes in physical soil properties

Technical requirements for biological SQIs:

- reproducible results
- generally accepted and broadly applied method, e.g. ISO or DIN standards
- cost efficient
- availability of reference values for evaluation of results
- ecological significance of results

Soil microbial biomass and soil basal respiration meet these requirements. Two ISO-DIN methods have been established for the measurement of soil microbial biomass (ISO 14240-1; ISO 14240-2) and one DIN method for the measurement of soil basal respiration (DIN 19737). Additional information on the metabolic state of soil microbial communities and on carbon availability can be deduced from the metabolic quotient (qCO_2, quotient of basal respiration and microbial biomass carbon) as well as from the carbon quotient (qC, quotient of microbial carbon and organic carbon C_{mic}/C_{org}; Anderson and Domsch, 1985; Anderson and Domsch 1990).

Microbiological soil characteristics

For the evaluation of measurements of the above-mentioned microbial soil characteristics (MSC), reference systems are needed. A reference system is a database, which allows measured (indicator) values to be referred to and compared with. It consists of the definition of "normal" (reference) values and "normal" spatial and temporal variation of these values. Oberholzer and Höper (2000) have proposed a reference system for the evaluation of agricultural soil based on the most applied soil microbial parameter, i.e. soil microbial biomass, the metabolic quotient (qCO_2), and the carbon quotient (qC). For microbial biomass the reference system depends on multiple linear regression equations between microbial and abiotic soil properties. For two different regions, i.e. for the Lower Saxony in northern Germany and for Switzerland, similar multiple linear equations with similar soil properties and with similar coefficients of determination (higher than 0.7) have been found (Oberholzer *et al.*, 1999; Höper, 1999). In contrast to soil microbial biomass, soil respiration could not be estimated with acceptable precision by use of regression models. For assessment of soil quality respiration data could only be used indirectly for calculating the metabolic quotient. The metabolic quotient as well as the carbon quotient are suitable to define threshold values, which depend on soil texture. The reference system, is based on tilled arable soils under conventional agriculture, a situation which is common to Swiss and German agriculturally managed soils. This reference system is not applicable to grassland or forest soils, thus implying the need for development of additional reference systems. Precision of data from a reference system depends on the size and heterogeneity of the reference area included and on the number of data sets collected. Small and uniform reference areas result in a low standard error of the reference values. Similarly, a decrease in the number of reference data sets leads to an increase of uncertainty of the reference system. Thus, the reference system can be improved by selecting appropriate areas and by optimizing the quantity of data included. In addition to microbial information, the data set should include general soil information (e.g. soil texture, soil pH, organic carbon and total nitrogen contents), specific soil information (e.g. contamination and compaction),

site information (e.g. mean annual temperature and precipitation, and altitude), and land use information (broad land use, organic fertilizer input, actual and precedent crop) (Nowak *et al.*, 1999). Microbial parameters should be determined according to standardized methods (e.g. ISO or DIN). Inter-laboratory comparisons are of high value for the establishment of comparable data sets and may build the basis for a biological soil characteristics database (Beck *et al.*, 1997).

In addition to these approaches, more recently developed techniques such as analyses of total soil DNA (Pace, 1986; Widmer *et al.*, 1999; Amann, 2000), soil fatty acids (FA, e.g. phospholipid FA (PLFAs), FA methyl esters (FAMEs), and archaeal ether FAs (AEFAs)) (Zelles, 1999; Widmer *et al.*, 2001; Gattinger *et al.*, 2002) and community level substrate utilization (CLSU) (Bochner, 1989; Garland and Mills, 1991; Garland, J. L. 1996; Widmer *et al.*, 2001) allow for a more detailed investigation of soil microbiology. Each of these approaches offers a focus on specific aspects of MSCs and represents an independent analysis of differences or changes in soil microbial community structures or functions. For the DNA approach, total DNA is directly extracted from soil (Bürgmann *et al.* 2001). In several studies it has been shown, that information on soil DNA contents can provide additional information on MSCs. In addition, extracted soil DNA can be further analyzed for specific marker gene pools. Combinations of specific polymerase chain reaction (PCR) amplification of target gene pools and genetic fingerprinting techniques, such as restriction fragment length polymorphism (RFLP; Widmer *et al.*, 1998; Widmer *et al.*, 1999) denaturant gradient gel electrophoresis (DGGE; Muyzer and Smalla, 1998; Van Elsas *et al.*, 2001) or single strand conformation polymorphism (SSCP; Schwieger and Tebbe, 1998; Stach, *et al.*, 2001) are applied. These analyses yield DNA banding patterns (genetic fingerprints), which can be quantified and compared among different soil samples. The numbers, relative migration positions, and intensities of specific bands in the patterns can be quantified and interpreted in relation to microbial community structures in a soil. Specific genes can be isolated and their DNA sequences determined which allows identification of the organisms at the genus level and in certain cases even at the species or sub-species level. For the FA approach, the total soil FA-fraction is obtained and quantitatively analyzed by gas chromatography (GC). Identities of FAs can be determined by use of reference systems or mass spectrometry (Laczkó *et al.*, 1997; Zelles, 1999; Widmer *et al.*, 2001). The result of these analyses is a GC profile with identified and quantified peaks for specific FAs (e.g. a PLFA fingerprint). Differences in FA-fingerprints may be interpreted with respect to alterations of microbial community structures and microbial physiologies in a soil. For analyzing community level substrate utilization (CLSU) fingerprints, soil suspensions containing total soil microbial communities are inoculated to multi-well plates containing a selection of specific substrates (Garland & Mills, 1991; Di Giovanni *et al.*, 1999 Widmer *et al.* 2001). Growth of microorganisms in the wells is indicated by a specific dye, which can colorimetrically be quantified. The result of this analysis is a substrate utilization fingerprint of the different substrates (CLSU

fingerprint). This data can be interpreted in relation to metabolic activities of specific populations or communities in a sample.

Therefore, all three approaches yield fingerprint-type data sets that may be used for further qualitative or numeric analyses. It will be important to establish protocols which allow to compile these data for comparative analyses and which allow to perform an objective characterization and comparison of soil microbial communities (Widmer *et al.* 2001). In addition, these down stream analyses may be used to test new and possibly improved fingerprinting approaches for their value in soil quality analyses.

Assessing soil microbial diversity

One of the current challenges to soil microbial ecology is the assessment of actual soil microbial diversities, i.e. species distribution and species abundance (Hill *et al.*, 2000; Hughes *et al.*, 2001; Kennedy, 1999). This information is required for determination of diversity indices such as the Simpson's or the Shannon's diversity indices. The main problem encountered is that microorganisms in soil are difficult to visualize at species level resolution. Direct cell counting based on specific DNA staining or *in situ* hybridization techniques are of great value but lake the taxonomic resolution required. In addition, detection by *in situ* hybridization depends on active cells since target rRNA sequences need to be present in sufficient numbers in the cells for yielding detectable signals. Direct extraction of fatty acids derived from cellular membrane structures of soil microbial communities represents an additional approach to detect their *in situ* community composition. However, also with this approach the taxonomic resolution is limited since many microbial taxa share common fatty acids and resolution at the species level in community analyses is not possible. In addition, also here the physiological state of an organism may affect the fatty acid composition in its membranes. Although this may be an interesting aspect for ecological studies, it complicates analyses of community structures based on the fatty acids approach. Therefore many investigations focused on the amplification of specific marker genes from total soil DNA by using PCR. As for *in situ* hybridization, ribosomal RNAs and their genes offer a great marker system for this approach. High-resolution genetic fingerprinting techniques have been developed for analysis of gene pools present in soils. DNA sequence analysis of rRNA genes allows identification of respective organisms to the genus and in some cases even to the species or sub-species level. However, it has been shown that a specific genetic fingerprint or a single RFLP band does not necessarily derive from a monophyletic group of microorganisms and thus unequivocal identification of taxa is not possible. In addition, it is uncertain whether the gene pool composition present in the PCR amplification products is identical to the gene pool present in the soil. PCR amplification may introduce shifts in the composition of

the gene pool represented in the PCR product since not all target genes may be amplified at the same rate and some may not be amplified at all. This PCR bias is intrinsic to this method and represents a clear limit even though community level PCR has probably been the most powerful tool to shed light on the unseen world of soil microbial diversity. Cultivation dependent approaches such as community level substrate utilization are clearly limited for diversity studies due to be their selectivity for culturable microorganisms and the capability of multiple phylogenetic groups of organisms to use the same substrates. However, also this approach has successfully been applied for documenting changes in soil communities related to different soil treatments.

For these reasons, soil microbial ecologists are currently equipped with a large set of tools each of which has its specific advantages and limitations. All of them yield fingerprint-type data, which represent an image of the soil microbial community analyzed. An accepted definition of the taxonomic unit, which can be used for defining soil microbial diversity, is clearly lacking at this point. It appears obvious that detection of diversity at the species or even at the strain level is not feasible, since detailed knowledge on the microorganisms who live in soils is still missing and the available tools are not applicable for these analyses. For practical reasons soil microbial ecologist introduced ‘operational taxonomic units’ (OTUs), which can be recorded and used for diversity analyses. Typical examples of such OTUs are the fingerprint information obtained with the above-described methods, i.e. specific RFLP band intensities or RFLP pattern abundances, specific FA quantities, or extent of soil microbial growth on specific substrates. It is, however, very important to keep in mind that diversity of OTUs does not necessarily represent diversity of the soil microbial community analyzed. The difficulties encountered with this inaccessibility of soil microbial diversity has been described as ‘counting the uncountable’ in the review by Hughes and coworkers (Hughes *et al.* 2001). It is inevitable to define OTUs, which can be applied for assessing soil microbial diversities. Robust protocols, which meet the above-mentioned criteria (Table 1), need to be established and accepted for this purpose.

Conclusions

MSCs hold great potential to be used as indicators in soil quality evaluation. Some parameters, i.e. soil microbial biomass and soil basal respiration, have already gained broad acceptance and are being successfully applied in soil quality monitoring. In the recent past a variety of approaches has been developed for investigating soil microbial diversity. Much information on soil microbial community structures and their changes induced by various factors has been gathered. Due to is complexity, actual soil microbial diversity may not be accessible to soil quality assessment and therefore ‘Operational Taxonomic

Units' (OTUs) need to be defined. We propose to presently apply the established and robust soil biological parameters as biological SQIs in soil quality monitoring. Total soil DNA content may represent an additional MSC to be used as SQI. In addition, focused research activities need to be initiated to establish a set of OTUs for analysis of soil quality based on structural MSCs. Such indicators should subsequently be included in the set of existing indicators to allow monitoring of MSCs at a higher resolution.

References

Amann, R. (2000). Who is out there? Microbial aspects of biodiversity. Systematic and Applied Microbiology 23:1-8.

Anderson, T.H. and Domsch, K.H. (1990). Anwendung ökophysiologischer Parameter zur Charakterisierung mikrobieller Biomassen im Boden. Verhandlungen der Gesellsch. f. Ökologie (Osnabrück 1989). Band XIX/II: 324-329.

Anderson, T.-H. and K.H. Domsch (1985). Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biology and Fertility of Soils, 1, 81-89.

Anonymous (1998). Ordinance – Relating to Impacts on the soil (OIS) of 1 July 1998 (Verordnung über Belastungen des Bodens (VBBo) vom 1. Juli 1998), EDMZ, Bern.

Beck, T., Jörgensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R. and Scheu, S. (1997). An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol. Biochem. 29 (7), 1023-1032.

Bochner, B. (1989). "Breathprints" at the microbial level. ASM News 55:536-539.

Bürgmann, H., M. Pesaro, F. Widmer, and J. Zeyer (2001). A strategy for optimizing quality and quantity of DNA extracted from soil. Journal of Microbiological Methods 45:7-20.

Di Giovanni, G. D., L. S. Watrud, R. J. Seidler, and F. Widmer (1999). Comparison of parental and transgenic alfalfa rhizosphere bacterial communities using Biolog GN metabolic fingerprinting and enterobacterial repetitive intergenic consensus sequence- PCR (ERIC-PCR). Microbial Ecology 37:129-139.

DIN 19737: Bodenbeschaffenheit:Laborverfahren zur Bestimmung der mikrobiellen Bodenatmung. Beuth Verlag, Berlin.

Doran, J.W. and Parkin, T.B. (1994). Defining and Assessing Soil Quality. In: Doran et al (Eds): Defining Soil Quality for a Sustainable Environment. Soil Science Society of America Special Publication Number 35, 244 p.p

Garland, J. L. 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biology & Biochemistry 28:213-221.

Garland, J. L. and A. L. Mills (1991). Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-Level sole- carbon-source utilization. Applied and Environmental Microbiology 57:2351-2359.

Gattinger, A., M. Schloter, and J. C. Munch. 2002. Phospholipid, etherlipid and phospholipid fatty acid fingerprints in selected euryarchaeotal monocultures for taxonomic profiling. FEMS Microbiology Letters 213:133-139.

Hill, G. T., N. A. Mitkowski, L. Aldrich-Wolfe, L. R. Emele, D. D. Jurkonie, A. Ficke, S. Maldonado-Ramirez, S. T. Lynch, and E. B. Nelson. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15:25-36.

Hughes, J. B., J. J. Hellmann, T. H. Ricketts, and B. J. M. Bohannan. 2001. Counting the uncountable: statistical approaches to estimating microbial diversity. Applied and Environmental Microbiology 67:4399-4406.

Höper, H. (1999). Die Bedeutung abiotischer Bodeneigenschaften für bodenmikrobiologische Kennwerte. - Ergebnisse aus der Bodendauerbeobachtung in Niedersachsen. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 89, 253-256.

ISO 14240-1 (1997): Soil quality: determination of soil microbial biomass. Part 1: Substrate induced respiration. Beuth Verlag, Berlin.

ISO 14240-2 (1997): Soil quality: Determination of soil microbial biomass. Part 2: Fumigation-extraction method. Beuth Verlag, Berlin.

Kennedy, A. C. 1999. Bacterial diversity in agroecosystems. Agriculture Ecosystems & Environment 74:65-76.

Laczkó, E., A. Rudaz, and M. Aragno (1997) Diversity of antropogenically influenced or disturbed soil microbial communities In: Microbial Communities – Functional Versus Structural Approaches. Eds, Insam H. and Rangger A., Springer Verlag, Heidelberg, p. 57-67

Muyzer, G. and K. Smalla. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 73:127-141.

Nowak, K., Oberholzer, H.-R., Mäder, P. and Laczkó, E. (1999). Datenbank für bodenmikrobiologi-sche Kennwerte - Konzept. VBB-BSA der Arbeitsgruppe "Vollzug Bodenbiologie". Bodenschutz-fachstelle Kanton Bern, Zollikofen.

Oberholzer H.-R., Rek J., Weisskopf P. and Walther U., (1999). Evaluation of soil quality by means of microbiological parameters related to the characteristics of individual arable sites. Agribiological Research 52 (2), 113-125.

Oberholzer H.-R. and Höper H. (2000). Reference systems for the microbiological evaluation of soils. VDLUFA Kongressband 2000 Stuttgart-Hohenheim. Generalthema «Nachhaltige Landwirtschaft» Teil II Beiträge zum Thema «Biologische Bewertung von Böden». VDLUFA Schriftenreihe 55/II - 2000. pp19-34

Pace, N. R., D. A. Stahl, D. J. Lane, and G. J. Olsen. (1986). The analysis of natural microbial-populations by ribosomal-RNA sequences. Advances in Microbial Ecology 9: 1-55.

Patzel N., Sticher H. und Karlen D.L. (2000). Soil fertility - phenomen and concept. Journal of Plant Nutrition and Soil Science 163: 129-142

Schwieger, F. and C. C. Tebbe. 1998. A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. Applied and Environmental Microbiology 64:4870-4876.

Stach, J. E. M., S. Bathe, J. P. Clapp, and R. G. Burns. 2001. PCR-SSCP Comparison of 16S rDNA sequence diversity in soil DNA obtained using different isolation and purification methods. FEMS Microbiology Ecology 36 :139-151.

Van Elsas, J. D., P. Garbeva, and J. Salles. 2002. Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. Biodegradation 13:29-40.

Widmer, F., R. J. Seidler, P. M. Gillevet, L. S. Watrud, and G. D. Di Giovanni (1998). A highly selective PCR protocol for detecting 16S rRNA genes of the genus *Pseudomonas* (sensu stricto) in environmental samples. Applied and Environmental Microbiology 64:2545-2553.

Widmer, F., B. T. Shaffer, L. A. Porteous, and R. J. Seidler (1999). Analysis of *nifH* gene pool complexity in soil and litter at a Douglas Fir forest site in the Oregon Cascade mountain range. Applied and Environmental Microbiology 65: 374-380.

Widmer F., A. Fließbach, E. Laczkó, J. Schulze-Aurich, and J. Zeyer (2001). Assessing soil biological characteristics: a comparison of bulk soil DNA-, PLFA-, and community Biolog™-analyses. Soil Biology & Biochemistry 33: 1029-1036

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in Soil: a review. Biology and Fertility of Soils 29:111-129.