

# Functional diversity of soil organisms – a review of recent research activities in Germany

Christoph Emmerling<sup>1\*</sup>, Michael Schloter<sup>2</sup>, Anton Hartmann<sup>2</sup>, and Ellen Kandelers<sup>3</sup>

<sup>1</sup>Universität Trier, FB VI – Abt. Bodenkunde, Universitätsring 15, D-54286 Trier, Germany

<sup>2</sup>GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Bodenökologie, Ingolstädter Landstrasse 1, D-85764 Neuherberg/München, Germany

<sup>3</sup>Universität Hohenheim, Institut für Bodenkunde, D-70599 Stuttgart, Germany

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## Summary – Zusammenfassung

The aim of this review is to provide an overview of recent investigations on the functional diversity of soil organisms and to elucidate whether a combination of different phenotypic and genotypic assessment methods can give new insights into the relation of structural (phylogenetic) and functional diversity of soil microbial and faunal communities. The knowledge of functional gene sequences for the major microbial transformations enables studies of their presence and diversity in soils. The concomitant evaluation of phylogenetic identification and functional activity of even individual microbial cells *in situ* is now possible using such as fluorescence *in situ* hybridization and microautoradiography. Studies about microbial-faunal interactions clarifies the importance of soil organisms for soil processes.

**Key words:** functional diversity / ecosystem engineers / soil enzymes / catabolic profiles / functional gene analysis / genetic microdiversity

## Funktionelle Diversität von Bodenorganismen

Dieser Artikel gibt einen Überblick über neuere Untersuchungen zur funktionellen Diversität von Mikroorganismen, einigen Vertretern der Bodenmesofauna, sowie der Bodenmakrofauna. Es wird der Frage nachgegangen, inwieweit die Kombination von phänotypischen und genotypischen Untersuchungsmethoden die Erkenntnisse über die Beziehung von struktureller (phylogenetischer) und funktioneller Diversität von tierischen und mikrobiellen Lebensgemeinschaften erweitern kann. Die Detailkenntnis der Gensequenzen der zentralen mikrobiellen Transformationsprozesse erlaubt die Untersuchung der Anwesenheit und Diversität dieses Genpools in Böden. Eine gleichzeitige phylogenetische Identifizierung und Funktionsanalyse auf Einzelzellniveau ist durch den gleichzeitigen Einsatz von Fluoreszenz *in situ* Hybridisierung mit Gensonden und Mikroautoradiographie möglich. Studien zu Wechselwirkungen von Bodentieren und Bodenmikroorganismen haben die funktionelle Bedeutung von Bodenorganismen geklärt.

## 1 Introduction

One major function of a soil is its use as a habitat for soil organisms including maintaining of biodiversity, assemblage, and activity for both soil microflora and soil fauna. Soil microorganisms themselves are involved in major soil processes, such as humification, recycling, and mineralization of organic residues, leading to the plant availability of nutrients. The mechanical fragmentation of organic residues, stabilization of soil aggregates, or bioturbation and mixing of organic and mineral substances are governed primarily by soil animals. In turn, these activities positively influence the physico-chemical properties of soil and consequently soil fertility and quality.

In the case of cultivated soils, every single farming practice may influence soil fertility and quality status either in a positive or negative manner. As a consequence, the German Soil Protection Law (*BBSchG*, 1998) prescribes a range of farming practises which are favorable to conserve soil fertility and quality (i.e. conservation of soil organic matter and microbial activity) or which help to protect soils from negative farming influences like soil compaction and erosion.

Moreover, in the German Nature Protection Law (*BnatSchG*, 2002) paragraphs 2 (1.3, 1.8) and 5 (3.2) regulate the conservation of biodiversity in every ecosystem. The law outlines that the conservation and development of biodiversity is the key for the functioning of the ecosystem. Conservation of biodiversity includes conserving the diversity of habitats, communities, species, and genetic diversity within a species.

During the last decade a growing interest in biodiversity research has arisen among soil biologists worldwide. One of the reasons is that advanced techniques, such as molecular biological approaches, had opened new doors and perspectives in investigations on the structure and function, as well as stability/resilience, of soil ecosystems. Furthermore, research on biodiversity issues is a consequence of the crucial question if the loss of species will result in a loss of soil functions (*Reber and Wenderoth*, 1997; *Andrén and Balandreau*, 1999; *Griffiths et al.*, 2001). This aspect brings to question whether all soil organisms have the same importance for soil functioning or if a few species ('keystone species') are more relevant and, moreover, if the others are redundant (*Beare et al.*, 1995; *Pankhurst et al.*, 1996; *Bengtsson*, 1998; *Andrén and Balandreau*, 1999; *Wolters*, 2001). Soil micro-organisms relevancy is an issue which is controversial in the international research community. Even

\* Correspondence: PD Dr. C. Emmerling; E-mail: emmerling@uni-trier.de

though there is evidence supporting the opinion that functions of soil microorganisms are redundant (Andr en and Balandreau, 1999; Buckley and Schmidt, 2001). Naeem et al. (1994) and Griffiths et al. (2000), among others, found strong positive relationships between the structure and function of soil microorganisms.

For soil animals, especially for those which are known as 'ecosystem engineers', it is hypothesized that it is much more easier to detect close relationships between species/life-strategies and soil functioning than for soil microflora. A decrease in the population of anecic earthworm species for example through soil tillage practices might result in a decrease in vertical macro pores and consequently to a decrease in water infiltration and preferential flow, and decomposition of organic residues (Edwards et al., 1992).

However, it should be clarified, that no species and no members of the same species are identical and therefore probably will not have the same function in soil (Lavelle et al., 1997; Bengtsson 1998; Wolters, 2001).

From a soil biological point of view the linkages between soil biodiversity and the functions and processes which are governed by the soil microflora and faunal community is not clearly and sufficiently identified up to now. Additionally, the determining factors for the spatial and temporal variability of soil biodiversity remain to be determined (Elliott and Lynch, 1994; Pankhurst et al., 1996; Brussaard et al., 1997; Lavelle et al., 1997; Bardgett and Shine, 1999; Tiedje et al., 1999; Wolters, 2001).

In this context, this review highlights some recent activities focused on studies from Germany. Since the invertebrates and the decomposer community in soil are among the most species rich in terrestrial ecosystems but still underrepresented in the research activities, the review does not claim to be complete and thus, special remarks are made on soil microorganisms, selected members of the soil mesofauna, and earthworm (*Lumbricidae*) communities.

## 2 Biodiversity/soil functioning relationship of faunal communities

Nor the diversity of soil invertebrates neither the link between diversity and soil function is still adequately researched (Lavelle et al., 1997; Wolters, 1991). Despite discrepancies in the use of terms, such as soil animal, biodiversity, soil function (Wolters, 2001), the heterogeneity and variability of soil conditions (Ekschmitt and Griffiths, 1998) are some major problems or aspects in this research field (Wolters, 1998).

However, the importance of soil faunal organisms on functions and processes in soil is generally accepted. In sum, soil micro-faunal organisms are involved especially in biochemical processes, like the decomposition of soil organic substance, the enhancement of microbial activity through their feeding activity, and thus potentially regulate microbial populations. Despite these functions, various members of soil meso-faunal organisms are to some extent important in regeneration and in mixing of soil organic matter in the soil and thus are also involved in physical

processes. In addition, large organisms, like earthworms and myriapods, are important members of the soil faunal assemblage due to their ability to change or create their habitat through their burial activity and thus modify the trophic resource base of organisms that are smaller and less mobile (Lavelle et al., 1997). This leads to the formation of voids, an increase in water infiltration and stabilization of soil aggregates, and the development of an humic top horizon in soil as an example. The biodiversity-soil functioning relationship at this hierarchical scale (the decomposer food web) is significant, for example, in natural forest ecosystems, since the decomposition of organic matter and the bioturbation and mixing of organic residues into the soil is related to the completeness of the existing decomposer food web and may result in the development of either mull-, moder- or raw humus profiles (Dunger, 1998; Graefe, 1995, 2000).

### 2.1 Functional diversity of soil mesofauna communities

The soil mesofauna comminute organic matter by feeding on detritus and by ingesting microbes and adhering detrital material, resulting in an increase in the surface area of the organic matter available for microbial attack (Swift et al., 1979; Didden et al., 1997). Feeding on microbes is one other important impact for microbial mediated degradation of soil organic matter. Moreover, predators, such as mites (Gamasina, Mesostigmata) influence population growth of other organisms and thus have an indirect effect on overall ecosystem performance (Koehler, 1997, 1999). The impact of soil mesofauna on the soil microbial community and nutrient supply was studied by Zechmeister-Boltenstern et al. (1998) and Kandeler et al. (1999a). However, Schlatte et al. (1998) and Sonnemann et al. (1999) concluded from microcosm experiments that microarthropods do not obligatory affect microorganisms probably due to the experimentally obtained conditions for microbes in the minicosms.

There is evidence for selective grazing of microarthropods on bacteria and fungi, for example by collembola (Larink, 1991). Conversely, little conclusive evidence exists concerning the phytopathological potential of soil mesofauna.

Studies concerning the functional diversity of the soil mesofauna or their activities deal with the problem that there are so many individuals in soil and that most of them are so small. Nonetheless, Schrader et al. (1997) reported beneficial effects from enchytraeid burrowing activities in compacted soils. Air permeability and hydraulic conductivity in soil were increased due to their activity (Didden et al., 1997). A significant increase in aggregate stability of casts from Collembola compared to soil aggregates was found by Heisler et al. (1996). According to results from Langmaack et al. (2001), the activity of Collembola and Enchytraeidae distinctly contribute to the rehabilitation of sealed soil surfaces and the development of a finely structured soil surface micro-relief. Results from Koehler and Weidemann (1995, 1998) showed microarthropods profoundly contributed to biogenic dune sand stabilization.

Nematodes are widely used in ecosystem studies (e.g. Ekschmitt et al., 2001a, b). A functional grouping of

nematodes is generally synonymous with allocation into feeding groups. Bongers and Bongers (1998) suggested the integration of the life strategy approach based on the life history of nematodes and the trophic group classification to obtain a better understanding of nematode biodiversity and soil functioning. Results from experimentally manipulated diversity of soil fauna revealed strong effects on nematode community (Alphei, 2001). There were markedly different patterns of nematode numbers and dominant groups related to increased complexity of the soil fauna, particularly to earthworm – nematode interactions (Alphei, 2001).

## 2.2 Earthworms as 'Ecosystem engineers' and soil functioning

Ecosystem engineers are organisms that may modify or create their habitat and thus may modulate the availability of resources to other species, and soil properties (Brussaard, 1998). Earthworms can be considered the most important 'ecosystem engineers' in arable soil, among others, for example myriapods, or enchytraeids in forest ecosystems (Huhta et al., 1998), due to their lasting effects on soil physical and bio-chemical properties. There is growing evidence that small soil fauna such as Enchytraeids and Collembola are also important engineers in arable soil (Schrader et al., 1997; Langmaack et al., 1999). However, the basic knowledge about the impact of earthworm communities on soil formation, development, and conditions has already been pointed out by Darwin over 120 years ago.

Nonetheless, much work has been done to clarify and quantify earthworms activity in soil. Earthworms can have important effects on the nutrient supply to plants (Makhschin, 1997). The amount of nutrients is higher in earthworms casts and burrows than in the surrounding soil (Heine and Larink, 1993; Buck et al., 1999). This is important since phosphorous and nitrogen are limiting growth factors of plants. Despite the accumulation of nutrients Scheu (1987) and van der Werff et al. (1995) found that the amount of water soluble phosphorus in earthworm casts was up to 6 times higher than in the surrounding soil indicating that earthworm activity does not only cause the accumulation of nutrients but also may increase the availability of phosphate from less available sources. The gut passage of litter components through earthworms may also severely affect litter decomposition and the mobilization of nitrogen in soil (Scheu, 1993a; Wolters and Ekschmitt, 1995; Helling and Larink 1998; Potthoff and Beese, 2000; Schmidt and Curry, 2001). After the application of organic residues the food consumption and the burrowing activity of earthworms generally increased (Emmerling and Paulsch, 2001). Scheu (1993b) concluded from laboratory experiments that the mineralization of two main structural components of plant litter, holocellulose and lignin, was increased due to earthworm processing by the endogeic species *Octolasion lacteum* (Oerley). For both C resources, holocellulose and lignin, earthworm processes caused a two-phase temporal alteration in mineralization. In soils experimentally treated with earthworms the soil microbial biomass was reduced and the metabolic activity and active microbial biomass was in

turn increased (Wolters and Joergensen, 1992; Scheu, 1992). The authors suggested that the earthworm casts were colonized by bacteria rather than by fungi which dominate the microbial biomass in soil.

Earthworms effects on soil physical properties are intensively documented. For example, Ziegler and Zech (1992) outlined that the activity of *Eisenia fetida* (S.) resulted in the formation of water-stable aggregates. This, in turn, may decrease the potential of soil erosion. In addition, Schrader and Zhang (1997) and Zhang and Schrader (1993) pointed out, that the efficiency of casting for the stabilization of the soil structure depended decisively on the sensitivity of a soil to physical disturbance. The experiments have been done with the two anecic species, *Lumbricus terrestris* (L.) and *Aporrectodea longa* (Ude), and the endogeic *A. caliginosa* (S.). The more sensitive the soil the more effective was the casting for water stable aggregation. As a result from Larink et al. (2001) earthworm casts showed 10–20 % higher values for porosity and about 50 % higher swelling values indicating a high potential of water uptake and increased pore space due to the microstructure. This pore space may function as an essential habitat for microflora and Protozoa (Bonkowski, 1995; Larink et al., 2001).

Despite the formation of casts, earthworms generally change the soil structure by the formation of macropores when penetrating the soil. Depending on the species and life-form characteristics most of the common species force radial pressures varying between 39–63 kPa (epigeic species), 72–93 kPa (endogeic), and 59–195 kPa (endogeic) (Keudel and Schrader, 1999). The burrowing activity leads to complex burrow systems and continuity of the burrows depending on earthworm species and life strategies (Langmaack et al., 1999b) and thus increases the hydraulic conductivity and percolation rates (Joschko et al., 1992). This function remains important especially in soils with compacted subsoil layers and surface water runoff.

## 3 Functional role of soil microbial communities and their metabolic potentials in nutrient cycling and energy transfer in soil

### 3.1 Enzyme activities as indicators of functional diversity

In terrestrial ecosystems, soil organisms play an essential role in the cycling of elements (mineralization and humification) and stabilization of soil structure. The mineralization of organic matter is carried out by a large community of organisms and involves a wide range of metabolic processes. Most of these processes are mediated by soil enzymes which are produced by soil microorganisms, roots and to some extent by soil animals. Therefore, the composition of soil biota determines the potential of the community for enzyme synthesis. The actual rate of enzyme production and the fate of produced and sorbed enzymes are modified by environmental effects and ecological interactions (Kandeler et al., 1996; Klose, 2001; Klose et al. 1999). For example, naturally occurring stresses, like water,

temperature, and substrate fluctuation regulate soil microbial populations and soil enzyme activities (Domsch, 1980; Domsch et al., 1983). Therefore, it is evident that the detection of human-induced changes of microbiological properties has to be measured against the background of natural variation (Brookes, 1993; von Steiger et al., 1996).

In the past, enzyme activities were measured and used as an index of microbiological functional diversity. Since microbial functional diversity includes many different metabolic processes, theoretically each enzyme activity of microbial cells should be measured (Nannipieri et al., 2002). A further possible approach is to measure the enzyme activities limi-

**Table 1:** The response of dehydrogenase and enzyme activities involved in carbon-, nitrogen-, phosphorus-, and sulphur-cycling to the type of vegetation and soil (n.d. not determined)(from Kandeler et al., 2001)

**Table 1:** Einfluss des Vegetation und des Bodentyps auf die Dehydrogenaseaktivität und Enzymaktivitäten des Kohlenstoff-, Stickstoff-, Phosphor- und Schwefelkreislaufes (n.d. not determined) (aus Kandeler et al., 2001)

soil enzyme activity	range of activities	vegetation / soil type	reference
xylanase activity mg glucose g <sup>-1</sup> (24 h) <sup>-1</sup>	13–24	spruce forest / n.d.	von Mersi et al. (1992)
	0.28–8.0	beech forest / n.d.	Zechmeister et al. (1991)
	3–17	agricultural land / n.d.	Tabatabai and Bremner (1969)
	1.8–3.0	grassland / Orthic Luvisol	Kandeler and Eder (1993)
	0.68–1.02	agricultural land / Eutric Cambisol	Kandeler and Murer (1993)
	0.75–2.00	agricultural land / Haplic Chernozem	Kandeler et al. (1999d)
	0.38–1.15	crop rotation / Phaeozem, Lithosol, Cambisol	Kandeler et al. (1996)
	0.24–1.83	agricultural land / Haplic Luvisol, Entisol	Stemmer et al. (1999)
β glucosidase μg p-nitrophenol g <sup>-1</sup> h <sup>-1</sup>	20– 55	grassland / Pachic Arguistoll	Ajwa et al. (1999)
	62– 98	agricultural land / Argixeroll	Burket and Dick (1998)
	36–160	forest / Haplohumult	Burket and Dick (1998)
	70–130	crop rotation / Fluvisol	Curci et al. (1997)
	130–310	crop rotation / Hapludalf	Deng and Tabatabai (1996)
	71– 86	crop rotation / Pachic Ultic Argixerolls	Miller and Dick (1995)
protease activity μg tyrosine g <sup>-1</sup> (2 h) <sup>-1</sup>	150–520	agricultural land / Haplic Chernozem	Kandeler et al. (1999d)
	224–514	pasture / Typic Dystrochrept	Haynes and Williams (1999)
	315–468	crop rotation / Phaeozem, Lithosol, Cambisol	Kandeler et al. (1996)
	120–430	wheat seeds / loamy sand	Badalucco et al. (1996)
	198–288	crop rotation / Haplic Luvisol	Friedel et al. (1996)
	304–624	agricultural land / Eutric Cambisol	Kandeler and Murer (1993)
arginine deaminase activity μg N g <sup>-1</sup> h <sup>-1</sup>	2.5– 5.0	grassland / Pachic Arguistoll	Ajwa et al. (1999)
	1.7– 2.0	crop rotation/Phaeocem, Lithosol, Cambisol	Kandeler et al. (1996)
	4.0–11.0	forest / sandy soils	Dilly and Munch (1995)
	0.1– 1.3	crop rotation / Fluventic Ustochrept	Franzluebbers et al. (1995)
arylsulfatase activity μg p-nitrophenol g <sup>-1</sup> h <sup>-1</sup>	30–50	grassland / Pachic Arguistoll	Ajwa et al. (1999)
	115–340	agricultural land / Hapludoll	Klose et al. (1999)
	6.9–213	pasture / Typic Dystrochrept	Haynes and Williams (1999)
	21–49	forest / Podzol	Staddon et al. (1998)
	1460–5912	forest / various soil types	Garcia and Hernandez (1997)
	50–350	crop rotation / Hapludalf	Deng and Tabatabai (1997)
	28–58	crop rotation/Phaeocem, Lithosol, Cambisol	Kandeler et al. (1996)
alkaline phosphatase μg p-nitrophenol g <sup>-1</sup> h <sup>-1</sup>	40– 80	grassland / Pachic Arguistoll	Ajwa et al. (1999)
	40–790	agricultural land / Aeric Vertic Epiaqualfs	Kim et al. (1998)
	100–500	crop rotation / Hapludalf	Deng and Tabatabai (1997)
	181–225	crop rotation / Ustochrept	Chander et al. (1997)
dehydrogenase μg TPF g <sup>-1</sup> (24 h) <sup>-1</sup>	114–155	crop rotation / Haplumbreps, Hapludalfs	Beyer et al. (1999)
	2–9	n.m. / Palixeralf	Marzadoni et al. (1996)
	0.6–0.9	crop rotation / Fluvisol	Curci et al. (1997)
	68–97	crop rotation / Ustochrept	Chander et al. (1997)
	148–207	crop rotation / Fluventic Xerochrept	Perucci et al. (1997)
	59–153	crop rotation /Phaeozem, Lithosol, Cambisol	Kandeler et al. (1996)

ting the overall rate of the metabolic pathway. These rate-limiting steps are very exergonic reactions and are the targets of metabolic regulation. For example, the determination of the phosphofructokinase-1 activity might give an idea of the potential rate of glycolysis in soil (Nannipieri et al., 2002).

In many studies, a selection of the enzyme activities or microbial processes to be measured is done due to the importance of the process within element cycling (Fließbach et al., 2001; Landgraf et al., 2001; Rinklebe et al., 2001). Since the areas of high activity in soil are heterogeneously distributed within the soil matrix, enzyme activities and microbial processes were investigated at different scales. Micro-scale investigations over the past few years have improved our understanding of the mechanisms driving C- and N-turnover (Tiunov and Scheu, 1999). For example, the rhizo-, drilo-, und detritosphere were selected as microhabitats presenting "hot spots" of microbial activity and diversity (Beare et al., 1995). Until now, the up-scaling of data from the micro- to the plot- or regional scale remains difficult because spatial distribution patterns at these scales are still incompletely known.

During the last decades, investigations at the plot scale were the dominant sampling strategy for soil microbiological studies. Therefore, the response of microbial activities to land use (Friedel et al., 1996; Brake et al., 1999; Dilly and Nannipieri, 1998; Emmerling et al., 2001), fertilization (Kandeler et al. 1999d), organic farming (Fließbach and Mäder, 1997; Friedel and Gabel, 2001; Friedel et al., 2001), tillage (Ahl et al., 1998; Kandeler et al., 1999b, c), sewage sludge (Rost et al., 2001; Emmerling et al., 2000), compost (Niklasch and Joergensen, 2001), waste water irrigation (Friedel et al., 2000), and heavy metal pollution (Kandeler et al., 1996; Mayer et al., 2002; Chander et al., 2001) of various soil types in Western Europe is well known (see review of Kandeler et al. (2001) for ranges of enzyme activities in different ecosystems) (Tab. 1).

Much effort has also put into determining, whether soil microorganisms and their activities control the response of plant communities to a rising atmospheric carbon dioxide concentration and play an important role in the sequestration of extra carbon in soils (Jones et al., 1998; Kampichler et al., 1998; Niklaus et al., 2001) as well as to the effect of soil microorganisms to the elevation of temperature (Bardgett et al., 1999). Evidence from these studies has shown that changes in the activity of microbial communities due to environmental changes (e.g. soil management, soil pollution, climate change) can have lasting effects on ecosystem functioning (Alef and Kleiner, 1987; Perry et al., 1989; Kampichler et al., 1998). Since changes in microbial biomass and soil enzyme activities due to environmental changes may be manifested over a shorter time scale than changes in chemical soil properties (e.g. soil organic matter) (Beyer et al., 1999; Christensen, 1996), current concepts of soil monitorings include biological properties of soils (Brookes, 1993; Kandeler et al., 1994; von Steiger et al., 1996). On the basis of these data, it shall be possible to develop classification systems for soil enzyme activities (Tschierko and Kandeler, 1999; Stork and Dilly, 1998; Wirth, 1999).

## 3.2 Functional diversity of soil microbial communities

### 3.2.1 Polyphasic genotypic and phenotypic investigations of soil microbial diversity

It is well accepted that the taxonomic and genetic microbial diversity of soils and other environmental habitats cannot be studied using cultivation dependent techniques because only a small part of soil microbes responds to cultivation in laboratory media (Amann et al., 1995, Chatzinotas et al., 1998). Therefore, a set of cultivation-independent methods are necessary to get insights into complex microbial communities (for review see Hartmann et al., 1997).

These techniques allow a more comprehensive evaluation of the composition as well as the function of these communities. The molecular phylogenetic approach of 16S rDNA diversity assessment by specific PCR amplification and cloning / sequencing (Liesack et al., 1997) or denaturing / temperature gradient gel electrophoresis (D/TGGE) (Heuer et al., 2001) provides a powerful way to study the diversity of soil microbial communities (Tab. 2). Liesack and

**Table 2:** Phenotypic and genotypic methods for functional microbial diversity assessment

**Tabelle 2:** Phänotypische und genotypische Methoden zur Untersuchung der funktionellen mikrobiellen Diversität

Applied method:	Reference:
PCR-amplification of 16S rDNA and rRNA with subsequent T/DGGE-analysis or cloning	Felske et al. (1997), Liesack et al. (1997), Miethling et al. (2000), Smalla et al. (2001)
Evaluation of bacterial microdiversity using genetic fingerprinting techniques	Schlöter et al. (2000b)
PCR-amplification of functional genes: e.g. <i>amoA</i> , <i>nirK</i> , <i>nirS</i> , <i>narGH</i> , <i>nosGBn</i> , <i>nosZ</i> , <i>nifH</i> , <i>apr</i> , <i>npr</i> , <i>sub</i>	Bach et al. (2001), Bothe et al. (2000), Priemé et al. (2002), Mergel et al. (2001), Rotthauwe et al. (1997), Widmer et al. (1999)
Community-level catabolic profiles (CLCP) using the BIOLOG <sup>R</sup> -systems	Garland (1997), Heuer et al. (2002), Smalla et al. (1998)
Fluorescence in situ hybridization (FISH), combined with microautoradiography	Amann et al. (1995) Lee et al. (1999)
Stable isotope ( <sup>13</sup> C)-labeling	Radajewski et al. (2000)
Specific gene expression analysis (use of Green fluorescence protein, GFP, as reporter protein in translational fusions)	Egener et al. (1999) Steidle et al. (2001)
In situ fluorescence antibody labeling techniques of specific enzymes	Bothe et al. (2000), Metz et al. (2002)

*Stackebrandt* (1992) were the first to demonstrate a hitherto unknown bacterial diversity in soil using the 16S rDNA-cloning approach. *Ludwig et al.* (1997) described a phylogenetically very diverse new bacterial phylum, *Holophaga / Acidobacterium*, which represents a considerable portion of active soil and sediment bacteria and which refused all cultivation attempts until now. Using the ribosome isolation technique from soil (*Felske et al.*, 1996) to extract bacterial rRNA, representing the metabolically active part of the community, the prominent activity of an uncultured member of the *Actinobacteria* was detected in grassland soil (*Felske et al.*, 1997). Nevertheless, by improving the isolation techniques, continuously new bacterial or fungal species are being cultivated from agricultural and forest soils as well as from the digestive track of soil animals and the rhizosphere of plants. This demonstrates that the search for functional microbial diversity of culturable microbes is continuously productive. Even within a given taxonomic unit (e.g. the soil bacterium *Ochrobactrum*) retrieved from soils of different locations and management, an abundant subspecies-microdiversity was found when highly resolving genomic fingerprinting techniques were applied (*Lebuhn et al.*, 2000; *Schlöter et al.*, 2000b). This probably reflects the very high diversity of rather isolated soil microhabitats enabling the selective development of different dominating ecotypes from an usually metabolically silent huge genetic pool of microbes at a rather small spatial scale. Yet, the degree of evolution and selection of new species or ecotypes and the mechanisms behind this phenomenon are not known in detail (*Schlöter et al.*, 2000b). In the case of *Ochrobactrum* sp. isolates enriched with a immunotrapping approach from differently managed soils (*Schlöter et al.*, 1996), it could be shown that these bacterial isolates had a high and unique diversity of enzymatic potential determined by catabolic fingerprinting on microplates (BIOLOG<sup>R</sup>) (*Schlöter et al.*, 1998, 2000a).

Community-level catabolic profiles (CLCP) as phenotypic fingerprinting technique using the commercially available BIOLOG<sup>R</sup>-system enable a very sensitive evaluation of the functional diversity of microbial communities (*Garland, 1997; Glimm et al.*, 1997). However, it has to be recognized, that under the test conditions, mostly the culturable subpart of the microbial community, employing fast growth rates of typically r-strategists, contribute to CLCP-analysis (*Smalla et al.*, 1998). Nevertheless, these CLCPs allow a rapid and sensitive insight into even minor shifts in functional community structures such as in the rhizosphere of different plant cultivars (*Heuer et al.*, 2002). Using metabolic and 16S rDNA fingerprinting, *Engelen et al.* (1998) monitored in detail the impact of a pesticide treatment on the structure and function of the soil bacterial community. *Di Giovanni et al.* (1999) used a combination of Biolog GN catabolic profiling and a genetic fingerprinting technique (enterobacterial repetitive intergenic consensus sequence-PCR, ERIC-PCR) to compare parental and transgenic alfalfa rhizosphere bacterial communities. The DGGE-analysis of PCR-amplified 16S rDNA also provided insight into the plant-dependent enrichment of rhizosphere bacterial communities and seasonal shifts (*Smalla et al.*, 2001) as well as the

influence of crop species, soil and the inoculation with *Sinorhizobium meliloti* (*Miethling et al.*, 2000). *Hengstmann et al.* (1999) compared the phylogenetic assignment of environmental 16S rDNA-genes with numerically abundant culturable bacteria from an anoxic rice paddy soil. *Wieland et al.* (2001) preferred to investigate the 16S rRNA diversity in soil and rhizosphere compartments, because rRNA-based investigations determine the abundance of ribosomes in the microbial community which should represent the active and dominant populations.

The huge 16S rDNA-data base which presently holds about 15.000 sequences allows for the design of phylogenetic oligonucleotide probes specific for certain taxonomic units with different resolution. The application of these probes carrying a fluorescent label were successfully used to identify single bacterial cells in their habitat by fluorescence *in situ* hybridization (FISH) combined with image analysis coupled confocal laser scanning microscopy (*Amann et al.*, 1995; *Hartmann et al.*, 1998). The FISH-technique could be combined with *in situ* labeling with fluorescence-tagged antibodies highly specific for certain bacterial strains or subpopulations (*Aßmus et al.*, 1997) or certain enzymes to monitor cellular functions, e.g. in denitrification (*Metz et al.*, 2002). A powerful approach to study functions and activities of single cells *in situ* is provided by the combination of the FISH-technique with microautoradiography (*Lee et al.*, 1999). Radioactive (e.g. <sup>14</sup>C)-labeled substrates are applied to natural microbial communities and the incorporation of this label into specific bacterial cells is monitored using microautoradiography. In a related approach with subsequent mass spectrometric analysis, the methanotrophic activity of microbial communities in the degradation and utilization of specific substrates can be traced using <sup>13</sup>C-stable isotope probing (*Radajewski et al.*, 2000).

To study *in situ* activities of individual cells and populations, so called reporter constructs are successfully applied. For this reason, reporter genes coding for an fluorescent compound (e.g. the green fluorescent protein, Gfp) or for an enzyme (e.g. *gus* or *lac*, giving raise for a colorimetric or fluorimetric reaction) are fused to promoters of genes of interest. The activation of the promoter and the expression of particular genes can thus be studied in soil or rhizosphere microhabitats. *Egener et al.* (1999) demonstrated the activation of *nif*-genes of diazotrophic bacteria of the genus *Azoarcus* in the rhizosphere and *Steidle et al.* (2001) showed the *in situ* activation of bacterial communities by the action of signalling compounds of the N-acetylhomoserinlactone-type – a chemical language of certain bacterial communities.

### 3.2.2 The nitrogen cycle as example of molecular functional gene analysis

One of the most important functions of soil microorganisms in maintaining soil fertility is the cycling of nitrogen, as plants are dependent on the supply of nitrogen from the soil. Mineralization, nitrification, denitrification, and nitrogen fixation are the major microbial mediated processes of the global nitrogen cycle. However the relative contribution of different microbial populations to these N transformations in

soils and other habitats are scarcely known. The application of molecular biological techniques based on the knowledge of the corresponding functional genes and conserved regions within these genes, which are suitable for the development of specific primer systems, circumvent this problem. DNA-based studies enable to detect potentials of microbial communities for a specific function or process, while mRNA-based analyses allow the measurement of transcription rates. Finally, the immunological monitoring of enzymes of interest provide information about the actual expression. This new set of data need to be correlated with classic enzyme activity measurements and turnover and flux rates of particular metabolites.

### 3.2.2.1 Proteolysis

Hydrolysis of peptide compounds by extracellular microbial peptidases is the key step of N mineralization, the release of ammonium and the subsequent nitrogen cycling processes in ecosystems. Only limited information is available about the microbial sources and the nature of soil peptidases. Due to their ecology (degradation of different proteins), peptidases show a high heterogeneity with only short stretches of structurally conserved regions. To investigate peptidases in more detail, Bach et al. (2001) designed functional probes for the genes encoding alkaline metallopeptidases (*apr*), neutral metallopeptidases (*npr*), and serine peptidases (*sub*) based on the known functional gene sequences. Reference bacteria with known peptidase genes and proteolytic bacteria from a grassland rhizosphere soil, a garden soil, and an arable field were investigated for their genotypic proteolytic potential. Using this approach it was possible to quantify the particular genes from different soil samples using Real time PCR (Bach et al., 2002). In a long-term experiment, where conventional farming and precision farming are compared, the influence of the differentiated application of fertilizer on the microbial mineralization potential in the plots under precision farming could be demonstrated by increased copy numbers of peptidase genes. As most of the work in field of functional genomics is based on the mRNA, Bach et al. (1999) developed a magnetic capture-hybridization method for the isolation of prokaryotic mRNA for the neutral proteases.

### 3.2.2.2 Nitrification

Ammonia-oxidizing bacteria (AOB) are the key microbes in the process of regeneration of nitrate, because they are responsible for the conversion of ammonia to nitrite. Rothauwe et al. (1997) developed a primer pair, which amplify a 491 base pair fragment of the ammonia monooxygenase subunit A gene (*amoA*). As AOB are related to two distinct phylogenetic lineages, the primers are not able to detect all *amoA*-containing bacteria. However the *amoA*-containing bacteria of the  $\beta$ -proteobacteria, which includes the genera *Nitrosomonas* and *Nitrosospira*, can be amplified. Kowalchuk et al. (2000) demonstrated that the community structure of ammonia-oxidizing bacteria belonging to the  $\beta$ -proteobacteria is highly dynamic in semi-natural chalk grassland soils at different stages of secondary

succession. A predominance of *Nitrosospira* sequence cluster 3 in early successional fields was replaced by *Nitrosospira* sequence cluster 4 in late successional fields. Pink et al. (2001) used a subunit of ammonia monooxygenase (*AmoB*) to raise polyclonal antibodies against the enzyme. These antibodies were highly specific for the detection of the four genera of ammonia oxidizers of the beta-subclass of Proteobacteria. These antibodies are suitable tools to study the regulation of expression of the *amoB* gene in soil samples.

### 3.2.2.3 Denitrification

Denitrification is the respiratory reduction of nitrate or nitrite to dinitrogen or nitrous oxide by aerobic bacteria under anoxic conditions. During denitrification,  $\text{NO}_3^-$  is sequentially reduced to  $\text{N}_2$  via a set of four enzymes: nitrate reductase (NaR), nitrite reductase (NiR), nitric oxide reductase (NOR), and nitrous oxide reductase ( $\text{N}_2\text{OR}$ ). These enzymes convert nitrate to nitrite (NaR), nitrite to NO (NiR), NO to  $\text{N}_2\text{O}$  (NOR), and  $\text{N}_2\text{O}$  to  $\text{N}_2$  ( $\text{N}_2\text{OR}$ ), respectively (Zumft, 1997). As the gene sequences for *narGH*, *nirS/K*, *norCB*, and *nosZ* are known, primers have been constructed for all of the corresponding genes. Priemé et al. (2002) demonstrated the high richness of different *nir*-genes in denitrifying communities of two different soil types (forest- and marsh soil). However, whereas *nirK*-gene fragments were amplified from both soils, *nirS*-gene fragments could only be amplified from the marsh soil. As with the monooxygenase subunit A (*AmoA*), antibodies were raised against the nitrite reductase (NiRK) to monitor the enzyme and the corresponding organisms in the environment (Metz et al., 2002). These antibodies were highly specific for the detection of the NiRK and could be applied in complex environmental samples. Molecular aspects of tracking nitrifying and denitrifying populations in soils are summarized by Bothe et al. (2000). Aspects of the current knowledge about the ecology of ammonia-oxidizing and denitrifying bacteria including topics about the use of proteomic tools and antibodies are reviewed. Since the substrate and products of the denitrification process ( $\text{N}_2\text{O}$  emissions,  $\text{NO}_3^-$  leaching etc.) have a high impact on environmental quality, the European Community has recently granted a new COST-action in the field of denitrification.

### 3.2.2.4 Nitrogen fixation

Nitrogen fixation is the key process of the global conversion of the atmospheric dinitrogen molecule to ammonia, which is catalyzed by the nitrogenase complex of prokaryotic microorganisms (Triplett, 2001). For  $\text{N}_2$ -fixing bacteria, *nifH* (the gene coding for dinitrogenase reductase, the small subunit of the nitrogenase complex) is well conserved in all diazotrophs and is therefore suitable for developing molecular tools to screen for the occurrence of nitrogenase in different habitats. Widmer et al. (1999) were one of the first who demonstrated surprisingly high microdiversity of the *nifH* gene pool in soil samples. RFLP analyses of cloned *nifH* PCR products revealed characteristic patterns for each soil type. Among 42 *nifH* clones

obtained from a forest litter library nine different RFLP patterns were found; among 64 *nifH* clones obtained from forest soil libraries 13 different patterns were detected. Only two of the patterns were found in both the litter and the soil, indicating that there were major differences between these nitrogen-fixing microbial populations. These differences may be related to special habitat conditions for plant litter degradation and mineralization processes. Mergel et al. (2001) investigated populations of N<sub>2</sub>-fixing and denitrifying bacteria in an acid forest soil near Cologne by gene probing. Southern hybridizations indicated that the highest number of total bacteria, of denitrifying and N<sub>2</sub>-fixing microorganisms occurred in the upper soil layer.

### 3.2.3 Functional genomics – new tools for old questions

Completed and ongoing microbial genome projects have produced rapidly increasing amounts of information about genes involved in specific pathways. Most of the sequence information, presently available, have been based on human pathogen microbes. However, the sequencing of the genome of some soil bacteria has been completed. The symbiotic bacteria *Sinorhizobium meliloti* and *Mesorhizobium loti*, the plant pathogenic organism *Agrobacterium tumefaciens* but also *Bacillus subtilis*, *Pseudomonas aeruginosa* or *P. putida* have all been completed. All available information has been collected in a database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). This help to acquire improved knowledge of genes and their regulatory network which should lead to the development of improved tools for the investigation of the relationship between environmental parameters and ecosystem functions of complex microbial communities in natural environments.

## 4 Conclusions

Research on microorganisms, mesofauna, and 'ecosystem-engineers' over the last decade has revealed that conservation efforts on the biodiversity of soil organisms through various farming practices or legal guidelines will be positively linked to the processes and functions in soil which are governed by them. However, questions about the functional importance of a single species and the value or level of soil biodiversity which is needed for soil functioning have not yet been answered until now. In the future, new methods and techniques such as the analysis of variations in the abundance of <sup>13</sup>C and <sup>15</sup>N will provide information on the role of soil fauna in ecosystem functioning and soil food web interactions (Wolters, 2000; Albers et al., 2001; Scheu, 2002).

Soil microbiological research over the past ten years has given evidence about the responses of the functional diversity of soil microbial communities to environmental change in different soil conditions, but the relationships between genetic diversity and taxonomic diversity is poorly known and even less is known about the manner in which genetic diversity and taxonomic diversity of microorganisms affect microbial functional diversity (Insam and Rangger, 1997; Nannipieri et al., 2002).

Using a polyphasic approach, including different phylogenetic and functional gene diversity assessments as well as immunological studies of the expression of enzymes and catabolic fingerprinting, more insight into the functional microbial diversity of soils is possible. In addition, spatially highly resolving *in situ* measurements of the population structure and function of microbial communities using fluorescently labeled probes and confocal laser scanning microscopy adds detailed information about the identification of microbes with concomitant expression analysis of enzymes or microautoradiographic labeling with single cell resolution. Together with classic enzyme measurements and data about transformation rates, the knowledge about functional diversity of soil microbial communities is going to reach a new quality.

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