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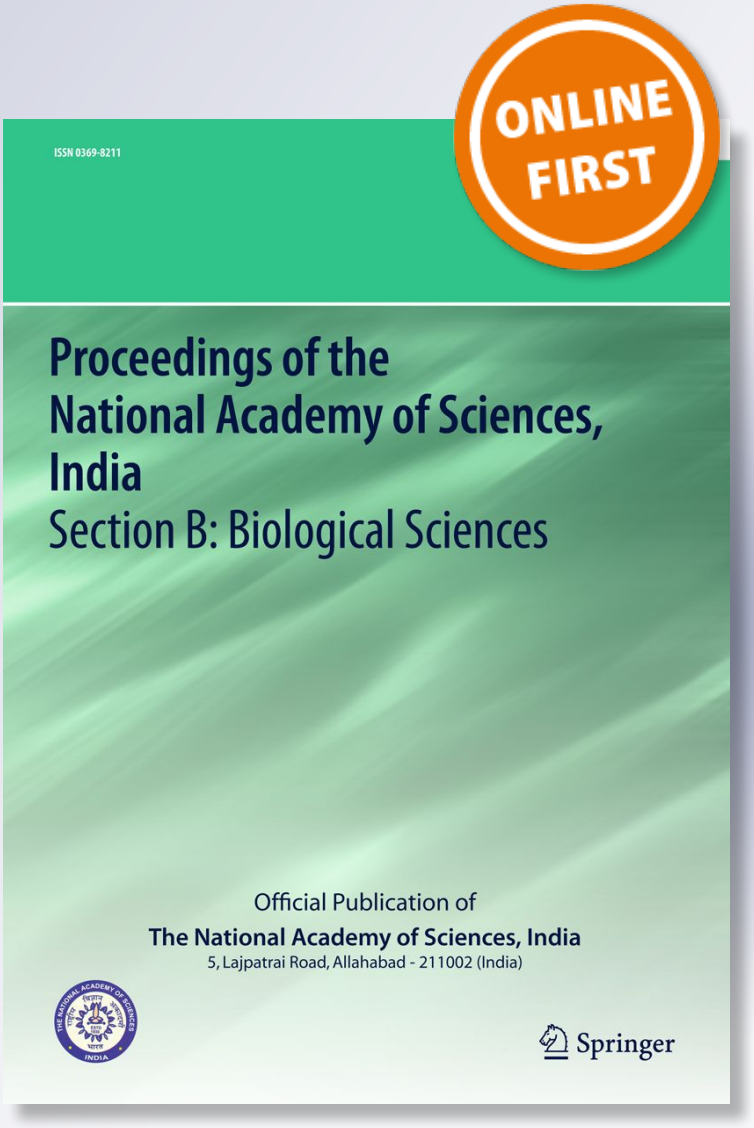

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REVIEW

Influence of Soil and Plant Types on Diversity of Rhizobacteria

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Abstract Biodiversity is an important ingredient of environmental conservation and is central to agriculture production. Most microbial diversity of the soil ecosystem is confined to the rhizosphere. Rhizodeposition through plant root exudates plays a major role in defining resident microflora, which differs from that in bulk soil. Rhizobacterial diversity is influenced by both plant and soil type. Soil factors, plant root exudates and agricultural management are the factors that determine the community composition within the rhizosphere.

Keywords Soil · Plant · Diversity · Rhizobacteria · Microbial communities

Introduction

Soil microorganisms play an important role in soil processes that determine plant productivity. The knowledge of soil microorganisms is limited in part by the inability to study soil microorganisms. Torsvik et al. [1] estimated that 1 g of soil contains 4,000 different bacterial "genomic units" as evident from DNA–DNA reassociation. The estimate suggests that about 5,000 bacterial species have been described. Approximately 1 % of the soil bacterial population can be cultured by standard laboratory practices. It is not certain that only this 1 % represent the entire bacterial population [2].

Microorganisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as formation of soil structure, decomposition of organic matter, toxin removal and the cycling of carbon, nitrogen, phosphorus, sulphur, etc. In addition, microorganisms play key roles in suppressing soil-borne plant diseases, in promoting plant growth and in bringing changes in vegetation. The diversity of microbial communities has effects on ecological function, soil suppressiveness to plant pathogens and resilience to disturbances in soil ecosystems [3, 4].

Application of pesticides [5], amendment with chitin [6], application of compost [7] or manure, and the introduction of genetically modified microorganisms [8] have all been shown to affect soil microbial community structures. The physicochemical properties of soil [9], distribution of soil particle size [10], the presence and age of specific plant species [11], and crop rotations [12] are the key determinative factors.

Soil microbial communities are often difficult to fully characterize mainly because of their immense phenotypic and genotypic diversity, heterogeneity and crypticity. Bacterial populations in soil top layers can go up to more than 10^9 cells per g soil and most of these cells are generally unculturable. The fraction of the cells making up the soil microbial biomass that have been cultured and studied is <5 % [13]. DNA-based methods offer the possibility to assess the total microbial diversity in soil [14].

Microbial Diversity Versus Community Structure

The term biodiversity has been defined in various ways. In microbial terms, it describes the number of different types

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(species) and their relative abundance in a given community in a given habitat. In molecular-ecological terms, it can be defined as the number and distribution of different sequence types present in the DNA extracted from the community in the habitat. However, the term "community structure" implies that information is included on the numbers of individuals of different recognizable taxa [15]. With respect to microbial diversity, the number of types present and the evenness of their distribution are important. A habitat with a larger number of species is more diverse, whereas an evenly distributed community is more diverse than an unevenly distributed community with the same number of species [3]. Plant type is a major determinant of the structure of microbial communities in soil, as plants are the main providers of specific carbon and energy sources.

Soil type is also a major determinant of the structure of microbial communities, as the combination of soil texture and structure, organic matter, microaggregate stability, pH, and the presence of key nutrients i.e., N, P, and Fe, determine the habitable niches in soil. Agricultural management regime, such as crop rotation, tillage, herbicide and fertilizer application and irrigation is the key determinant of microbial community structure in soil.

Conceptually, the extent to which plant and soil type influence the structure of microbial communities is dependent on their relative "strength". In addition, the nature of the microbial type affected also is significant, as some organisms may turn out to be virtually refractory to change (e.g., typical K-strategists or slow growers such as *Arthrobacter* types), whereas others are very prone to stimulatory or destimulatory effects e.g., typical r-strategists (bacteria characterized by high growth rates under conditions of enhanced nutrient supply) such as the pseudomonads [16].

Plant Type as the Determinant of the Structure of Microbial Communities in Soil

The rhizosphere effect is the most immediate influence of plants on soil systems [17]. However, since the microbial inoculations would mainly be performed in soils before the plant is grown up, the strains should also be able to survive in the soil and show a good saprophytic ability. To fulfill these requirements, progress must be made in the knowledge of which bacterial traits affect the soil and rhizosphere colonizing ability of microbes [18–20].

Plant roots release a wide variety of compounds into the rhizosphere soil, including ethylene, sugars, amino acids, organic acids, vitamins, polysaccharides, and enzymes. These materials create unique environments for the microorganisms living in association with plant roots in the rhizosphere. Different compositions of root exudates are expected to select different microbial communities in soil.

Root exudates are important nutritional sources for bacteria colonizing the roots. The composition of root exudates was shown to vary depending on the plant species and the stages of plant development [21]. Thus, the plant is supposed to profoundly influence the relative abundance of indigenous rhizobacteria as well as the population dynamics of introduced strains and other biotechnological interventions.

Cultivation-based and molecular methods, indicated that plant type is indeed a major factor influencing the structure of microbial communities. Using Community-Level Physiological Profiling (CLPP), Grayston et al. [11] compared the rhizospheres of wheat, ryegrass, bentgrass, and clover grown in two different soil types (dystic camnisol and eutic gleysol) at two sites in Scotland. A significant clustering of potential microbial activities along plant type (i.e., different plant species had different activities) was observed, but no differentiation was noted between microbial activities in the two soil types. Two studies by oilseed rape supported the hypothesis that crop type plays a major role in controlling the diversity of root-associated bacteria [22, 23]. Further Kaiser et al. [23] used cultivation-based and culture-independent (16S rRNA gene library) approaches. Although the soil types were different between the two studies, both strongly indicated that the plant plays a major role in determining the composition of the bacterial community in the rhizosphere, whereas soil type seemed to play only a minor role. Kowalchuk et al. [24], using PCR-DGGE, also demonstrated clear plant-induced effects on bacterial community structures in soil. The effects appeared to be limited to the direct rhizosphere and to be highly plant-specific and reproducible for a given plant species.

It is the loss of carbon compounds from the roots that influences the development of their multiplication and activity of microbial populations in the rhizosphere when compared with the bulk soil. This phenomenon is wide spread across all plant species as a general process, although the compounds lost from different plant species or even cultivars of particular species, can vary markedly in quality and quantity. In addition, plants can have specific mycorrhizal- and nodulation-based associations that fulfil unique functions. When considering the rhizosphere effect in general, the rhizosphere/bulk soil (r/s) ratios for bacteria, actinomycetes, and fungi are usually in the range of 2–20, 5–10, and 10–20, respectively. However, many of the bacteria in the rhizosphere and soil are unable to grow on laboratory media, which makes their study increasingly difficult. In young plant roots, however, it is thought that the rhizosphere bacterial communities are dominated by r-strategists, which are species with fast growth rates and capacities to utilize simple substrates [25]. As the roots mature, there is a shift in dominance to bacterial communities with relatively slow growth rates and the capacity to degrade more complex

substrates (k-strategists). As a rule, although a general increase in micro-organisms in the rhizosphere is always noted, the community structure and functional consequences of this increase are less well understood.

It is difficult to grow or enumerate microorganisms in ecological niches using traditional plate count methods. A variety of molecular methods have been developed to assay the presence or absence of these microorganisms in samples. The method of choice to determine what microorganisms are present in environmental samples is to amplify the conserved small subunit rRNA gene. In this process, DNA is isolated from the soil using bead beating, and polymerase chain reaction (PCR) with gene-specific primers is used to amplify the specific gene from the sample. To look at the diversity of small subunit rRNA genes (directly related to the diversity of microorganisms) present in the sample, the PCR products are either cloned and sequenced, or profiled by gel electrophoresis to allow the analysis of many samples. A variety of techniques are available for microbial community profiling, including denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis, single strand conformation polymorphism, and terminal restriction fragment length polymorphism. DGGE, in particular, has commonly been used to analyse microbial populations in a variety of samples [26]. The complexity of the banding pattern is used to assess the diversity of microorganisms present in the sample. Using such methods, the structures of rhizosphere microbial communities have been shown to be distinct from those of bulk soil, with lower density in the rhizosphere relative to bulk soil. The specific structure and diversity of the rhizosphere bacterial community varies between plant species and over time [27], and different root zones on the same plant can support distinct bacterial communities, reflecting qualitative and quantitative differences in root exudation [28]. In addition, soil type has a key role in determining the specific dominant bacteria colonizing the rhizosphere [29]. The structure of rhizosphere bacterial communities can also be influenced by root infection by pathogenic bacteria, which promote greater bacterial community variability compared with healthy roots [30]. Traditionally, pseudomonads have been considered to be important rhizosphere organisms. The term 'pseudomonads' has in the past been applied to bacteria now placed in different genera (e.g. *Burkholderia* and *Pseudomonas*), let alone of different species, and such conclusions need to be reconsidered. Many studies have shown elevated pseudomonad communities in the rhizosphere [30] and is not always the case, as in some circumstances *Bacillus* spp., may dominate [31]. While many rhizosphere pseudomonads are plant growth-promoting rhizobacteria, others inhibit plant growth and cause disease. However, it is not clear what makes some pseudomonads beneficial and others pathogenic, especially since they colonize the same ecological niches and possess similar mechanisms for plant colonization.

Little is known about plant specificity of root-associated bacteria, which are an important functional group of beneficial bacteria in the rhizosphere. A few studies have indicated a plant-dependent composition of culturable bacteria [22, 32]. Denaturing gradient gel electrophoresis (DGGE) fingerprints of PCR-amplified 16S ribosomal DNA (rDNA) genes from community DNA were used to study dominant bacterial populations in the rhizosphere of the three *Verticillium dahliae* Kleb. host plants-strawberry, potato, and oilseed rape-over two growing seasons [27]. Using this cultivation-independent approach, a plant-dependent abundance of dominant bacterial populations could be shown for most of the sampling times. The DGGE fingerprints showed plant-dependent shifts in the relative abundance of bacterial populations in the rhizosphere which became more pronounced in the second year. DGGE patterns of oilseed rape and potato rhizosphere communities were more similar to each other than to the strawberry patterns. In both years seasonal shifts in the abundance and composition of the bacterial rhizosphere populations were observed. Independent of the plant species, the patterns of the first sampling times for both years was characterized by the absence of some of the bands which became dominant at the following sampling times. *Bacillus megaterium* and *Arthrobacter* sp. were found as predominant populations in bulk soils. Sequencing of dominant bands excised from the rhizosphere patterns revealed that six out of ten bands resembled gram-positive bacteria. *Nocardia* populations were identified as strawberry-specific bands.

There is increasing evidence that gram-positive bacteria might be more dominant in the rhizosphere than previously supposed. *Bacillus* species were found as dominant populations in the rhizospheres of *Chrysanthemum* [33] and of Barley [34]. *Arthrobacter* spp. were also found as dominant populations in the molecular fingerprints of 16S rDNA fragments amplified from the rhizosphere DNA of maize grown in tropical soil [35], from the rhizosphere DNA of *Medicago sativa* and *Chenopodium album* [36], and from the rhizosphere DNA of *Chrysanthemum* [33]. One dominant DGGE band obtained at all locations of the barley rhizospheres grown in controlled pot experiments performed by Yang and Crowley [28] was identified as *Microbacterium*.

Berg et al. [37] studied the effect of plant species on the abundance and diversity of bacterial antagonists by analyzing the abundance, the phenotypic diversity, and the genotypic diversity of rhizobacteria isolated from potato, oilseed rape and strawberry from bulk soil which showed antagonistic activity towards the soil-borne pathogen *V. dahliae* Kleb. A rather high proportion of antagonists from the strawberry rhizosphere was identified as

Pseudomonas putida B (69 %), while antagonists belonging to the Enterobacteriaceae (*Serratia* spp. and *Pantoea agglomerans*) were mainly isolated from the rhizosphere of oilseed rape. For *P. putida* A and B plant-specific genotypes were observed, suggesting that these bacteria were specifically enriched in each rhizosphere. Several recently published studies used repetitive extragenic palindromic (REP)-PCR fingerprints [38], such as those obtained by BOX-, REP-, or enterobacterial repetitive intergenic consensus (ERIC)-PCR, to explore the diversity of pseudomonads originating from rhizospheres and soils. Based on REP-PCR fingerprints all studies found an enormous genomic diversity of *Pseudomonas* spp. at the subspecies level [32]. Fromin et al. [32] reported that the genotypic structure of *Pseudomonas brassicacearum* populations analyzed by REP-PCR fingerprints are significantly influenced by the *Arabidopsis thaliana* genotype.

Studies show that plant species richness and plant functional diversity have a positive influence on overall catabolic activity and catabolic diversity of the culturable bacterial community in the bulk soil in an experimental grassland ecosystem [39]. Catabolic activity and catabolic diversity of culturable soil bacteria increased linearly with the logarithm of plant species number and with the number of plant functional groups. A reduction in plant biomass caused by a loss in plant diversity is expected to have strong effects on the decomposer community: microbial biomass is likely to decrease, because organic carbon sources limit soil microbial activity in most terrestrial ecosystems. Higher plant diversity may have influenced the soil bacteria by increasing the diversity of litter, the heterogeneity of soil microhabitats, or energy and material flows from the vegetation to the soil. The density of earthworms increased by 63 % across the range of diversities [40], suggesting that effects of plant diversity on bacterial activity were probably mediated to some extent by increased heterogeneity of soil microhabitats. Aboveground biomass increased with increasing plant species richness and enhanced flow to the soil may well also have contributed to the positive effect on bacteria.

Experimental results show a positive influence of plant diversity on C-source utilization patterns in soil samples and thus on the activity and functional diversity of culturable bacteria in the bulk soil [41]. The relationship may be a mutual one in that plants may also profit from diverse soil bacterial communities, e.g. mediated by better nutrient mineralization, growth stimulation, and enhanced antibiosis to pathogens [42]. In most agro-ecosystems, microbial growth is limited by input of fresh organic carbon and hence plant-biomass production. A sensitive measure for short-term responses to C inputs into soil is the microbial biomass supported per unit of total organic soil carbon C_{mic}/C_{org} ratio [43]. This ratio also significantly decreased with a decrease in plant species richness or plant biomass, which may be an early indicator for a longer-term decline in soil organic matter of low-diversity mixtures.

PCR-TGGE [44] was used to assess the degree of variation of dominant bacterial populations in respect of soil type (silty sand and loamy sand), plant type (clover, bean, and alfalfa) and developmental stage of the plant. Plant species had the greatest effect on the rhizosphere microflora, whereas the plant developmental stage had the lowest effect. The effect of soil type exceeded that of plant type in the soil habitat only, as the clustering of alfalfa plants in loamy sand was clearly distinct from the clustering of the same plants in the silty sand. Hence, the effect seen was dependent on the soil microhabitat sampled. Marschner et al. [29] examined bacterial community structures in the rhizosphere based on PCR-DGGE of soil rRNA genes as affected by three factors: plant species (chickpea, rape, and Sudan grass), soil type (a sandy soil, a sandy loam, and a clay), and root zone location. All three factors, as well as the interaction between them, had significant effects on the community structures in the respective rhizospheres. The bacterial community associated with chickpea was influenced mainly by soil type, whereas the communities of rape and Sudan grass were more affected by root zone than by soil type. Hence, the effects exerted by the different plants were, to varying extents, controlled by soil type, which makes the interactions complex. Finally, studies have addressed the rhizosphere communities of genetically modified plants related to those of conventional plants. Dunfield and Germida [45], using both PLFA and CLPP, found differences between the bacterial communities associated with genetically modified *Brassica napus* and conventional varieties. This difference may be linked to differences in the exudation by these plants [46].

In the context of sustained plant production systems, it is pertinent to appreciate that plants can alter the composition of soil microbial communities. Germida et al. [22] reported differences in rhizoplane communities of wheat and canola grown in the same field site, which indicates that plant species influence the composition of soil microbial and root-colonizing communities. The rhizosphere is a reservoir of genetically diverse populations of bacteria, the composition of which is determined by the selective influence of plant and soil type. The microbial communities in the soil milieu can undergo temporary variations in the population of a genotype as a function of root metabolites, which in turn changes with the metabolic processes of plants (plant age), soil species, cultivation techniques, distance from roots, i.e. rhizosphere effect and plant genotype [11]. Misko and Germida [47] claimed that different varieties of plant species could be selected for specific bacterial populations. These workers reported that

disomic substitution of a specific pair of chromosomes resulted in changed rhizosphere microflora of spring wheat (*Triticum aestivum*). Rhizodeposition also stimulates the germination of pathogen propagules and directed growth towards the root, which can lead to disease [48]. Soil-borne pathogens fall into two broad groupings. Pathogens like *Fusarium*, *Verticillium*, and *Pythium* rapidly kill all or a part of the host following their entry through plant roots. For some necrotrophic fungal pathogens with a broad host range including *Pythium* and *Fusarium*, plant exudate components including sugars and amino acids stimulate propagule germination and growth towards the root. For those pathogens with limited host ranges, propagule germination stimulants can be compounds specific to the host family, such as organic S compounds in the case of the interaction of *Sclerotium cepivorum* with *Allium* spp. Host cells are rapidly killed by cytolytic enzymes or toxins.

There are many examples of bacteria that can suppress the growth of pathogenic fungi in the rhizosphere [45]. Effective colonization of the root is a key factor determining the ability of these bacteria to exert biocontrol. A number of these bacteria produce anti-fungal metabolites, including antibiotics, extracellular enzymes, and HCN. Competition between rhizosphere bacteria and fungal pathogens for nutrients has also been identified as a biocontrol mechanism. For example, the sequestration of Fe^{3+} by bacterial siderophores and chelators can limit availability of the nutrient to pathogens, restricting their growth through the rhizosphere. Exposure of roots to non-pathogenic rhizosphere bacteria, including strains of *Bacillus* spp. and *Pseudomonas* spp., can induce resistance of host plants to some pathogenic fungi. Several mechanisms have been implicated in induced resistance, including enhanced production of phytoalexins, production of stress-related proteins and degradative enzymes, and the strengthening of epidermal cells [49].

The influence of plant type on the rhizosphere microbial community of *Triticum aestivum* and *Eleusine coracana* was studied [50]. Shannon diversity indices for wheat and *E. coracana* rhizospheric isolates based on genotypic fingerprinting were 0.6 and 0.2, respectively. Sequencing data showed predominance of genera, *Bacillus* and *Pseudomonas*. Using a model *Populus* system in a common garden with replicated clones of known genotypes, Schweitzer et al. [51] evaluated microbial biomass and community composition as quantitative traits. Plant genotype significantly influenced microbial community composition, explaining up to 70 % of the variation in community composition within *P. angustifolia* genotypes alone. These findings suggest that variation in above-and belowground traits of individual plant genotypes can alter soil microbial dynamics.

Plant Developmental Stage

The qualitative composition of root exudates is affected by plant developmental stages and in turn shows impact on microbial communities in rhizosphere [28, 52]. The effects of plant age were also observed by di Cello et al. [53] and Seldin et al. [54], who showed that populations of *Burkholderia cepacia* and *Paenibacillus azotofixans* in the maize rhizosphere changed during plant growth. Bacterial communities not only adapt to plant type, but also change over time with the same plant type [55]. Baudoin et al. [56] also reported clear differences between bacterial communities on maize in dependency of growth stage. It was also confirmed by Gyamfi et al. [57] that the plant growth stage had a strong impact on total bacterial as well as *Pseudomonas* communities. The fact that young plants contained bacterial communities that were distinct from those in other plant developmental stages was also observed by Duineveld et al. [33] with C*hrysanthemum*. When the root tips were compared with root base parts, the PCR-DGGE analyses revealed higher similarities between samples derived from root tips and between samples from young plants. The main sources of easily accessible substrates are sites at root tips and young roots. The young plants provide the highest amount of organic carbon available for microbial growth. Young roots and root tips might therefore represent excellent niches suitable for colonization by r-strategists.

A field experiment was conducted in dark brown clayey soil using three soybean genotypes and the results gleaned from both pot and field experiments indicated that bacterial communities in the soybean rhizosphere changed with growth stages, and higher number of DGGE bands observed in early reproductive growth stages, while surprisingly, a significant impact of genotypes on the bacterial communities was not observed in these experiments [58]. However, a plate culture experiment targeting the culturable bacterial communities detected a remarkable difference in the community structures of the rhizosphere between the two soybean genotypes, suggesting that a small portion of the total bacteria was influenced by genotype. Sequence analysis of DGGE bands indicated that bacterial phyla of Proteobacteria, Actinobacteria, Bacteroidetes, Nitrospirae, Firmicutes, Verrucomicrobia and Acidobacteria commonly inhabit the soybean rhizosphere.

Effects of Function

Plants, plant type and their growth stage can have strong effects on soil microbial communities viewed from the functional perspective [52]. To explore the effect of different plant species on the abundance and diversity of bacteria antagonistic to plant pathogens, isolates

originating from the rhizospheres of three host plants of *V. dahliae*-strawberry, potato, and oilseed rape-and from soil were analyzed for their antagonistic properties [37]. The abundance, taxonomic composition, and diversity of *V. dahliae* antagonists were shown to be plant-species dependent. The proportion of isolates with antagonistic activities was highest for the strawberry rhizosphere (9.5 %), followed by oilseed rape (6.3 %), potato (3.7 %), and bulk soil (3.3 %). Hence, plants affect their associated communities also in a functional way.

Microbial Communities Affected by Transgenic Plants

Several thousand field releases of transgenic crop plants have been performed during the last decade and several transgenic crop plants have been commercialized. However, there are actually very few studies [24] published which have tried to analyse the potential effects of transgenic crops on soil microbial communities. Two kinds of scenarios are recognized in which the large-scale use of transgenic crops could have an effect on microbial communities in rhizosphere and bulk soils:

(1) When the structural and functional composition of the soil microbial community in the vicinity of the roots is changed as a result of an altered root exudation or released transgene product with antifungal, antibacterial activity, or others.
(2) When bacterial rhizosphere populations would be able to capture and stably integrate transgenic plant DNA, in particular antibiotic resistance genes used as markers in transgenic crops.

To date only a few studies have been sought to analyse the potential effects of transgenic crop plants on the composition of bacterial communities in the rhizosphere under field conditions [59]. Heuer et al. [60] studied the structure and dynamics of bacterial communities in the rhizosphere of potato under field conditions, and to compare these to those of the transgenic T4-lysozyme-expressing potato plants. In contrast to many other transgenic plants, this genetic modification was targeted at bacteria, and it was shown that plant-associated bacteria were indeed affected, in so far as the susceptibility of the transgenic potato plants to infections by *Erwinia carotovora* was significantly reduced. It was demonstrated that a detectable amount of T4 lysozyme was released from the roots resulting in bactericidal activity at the root surface [61].

Influence of Soil Type on the Structure of Microbial Communities in Soil

Soil type along with the constellation of its physico-chemical factors represents another important factor influencing the structure of microbial communities. Gelsomino et al. [62] observed differences in the grouping of DGGE fingerprints obtained from 16 different soils from different geographical locations. These authors suggested that soil type largely determines the structure of bacterial communities seen by direct PCR-DGGE, and that similar soil types tend to select similar communities. In a study of microbial biomass and activity in four grasses in the US Northeast, soil texture was also shown to have a stronger effect than plant species [63]. Other studies have also indicated that soil type can have a marked influence on the microbial populations in the rhizosphere of maize. Chiarini et al. [64] compared the influence of soil type, cultivar, and growth stage of maize on the population size and structure of bacterial communities associated with the roots of field-grown maize. The greatest effect on density and community structure was exerted by soil type, whereas no significant difference between the effects of different maize cultivars was observed. In an analysis of the diversity of *Paenibacillus* populations in maize plants grown in two different soils, da Silva et al. [65] observed that soil type, rather than maize cultivar type, was the overriding determinative factor that influenced the community structures of *Paenibacilli* in the rhizosphere. Latour et al. [66] studied the effect of soil type and host plant type (flax and tomato) on the diversity of the populations of culturable fluorescent *Pseudomonas* spp. Although both soil type and host plant affected the diversity of fluorescent *Pseudomonas* species, soil type was clearly the dominant factor [67].

Soil texture can affect the rhizosphere microflora by limiting the availability of root exudates. For example, some amino acids and peptides are adsorbed and bound on clay minerals and used to a lesser extent than when present free in soluble state [67, 68]. The characteristics of fluorescent pseudomonad populations associated with the roots of two plant species (flax and tomato) were compared with those of fluorescent pseudomonads from an uncultivated soil [69]. The results from these studies, performed with the same soil, indicated that bacterial populations are not distributed at random. Several studies have clearly shown that the survival of different introduced strains of fluorescent pseudomonads varies in soils of different textures [70]. However, not much is known about the effect of the soil type on the selection achieved by the host plant toward the soilborne populations of fluorescent pseudomonads. Latour et al. [66] compared the diversities of fluorescent pseudomonads, from two uncultivated soils and from the roots of two plant species cultivated in these two soils. Numerical analysis of phenotypic characteristics allowed the clustering of isolates that showed high levels of similarity. This analysis indicated that both soil type and host plant had an effect on the diversity of fluorescent pseudomonads. However, of

the two factors studied, the soil was clearly the dominating one. The population associated with the roots of each plant species varied from one soil to the other which could possibly be ascribed to the differences recorded between the phenotypically diverse populations of fluorescent pseudomonads from the two uncultivated soils. The plant selection was, at least partly, plant specific. It was not related to bacterial species and biovars or to the presence of plasmid DNA. The phenotypic clustering of isolates was well correlated with genotypic characterization by repetitive extragenic palindrome-PCR fingerprinting.

Microorganisms have also been shown to stimulate the amount and to affect the composition of the root exudates [17, 71–78]. Different studies were undertaken to compare the survival of introduced strains of fluorescent pseudomonads in soils with different textures [79]. Fine textures or soils amended with clay favor the survival of the introduced strains. Höper et al. [80] have also shown that an increase of the soil pH and amendment of a soil (pH 7) with clay (montmorillonite or illite) induces a significant increase of the density of the endogenous populations of fluorescent pseudomonads. Besides their survival, the levels of activity of fluorescent pseudomonads have also been related to soil characteristics. Suppression of fusarium wilts by siderophore-mediated iron competition has been associated with a low concentration of iron available for the pathogen; this low availability is related to a high pH and a high $CaCO_3$ content in the suppressive soils. The disease suppression related to phenazine production by *P. fluorescens* strain 2–79 has been positively correlated with the percentage of sand and pH and negatively correlated with iron and the percentages of silt, clay and organic matter [81]. The intensity of the carbon and iron competition has been demonstrated to be higher in the suppressive soil than in the conducive soil. This difference was related both to the higher levels of microbial activity and reactivity and to the low concentration of available iron [82].

Interactions among soil types, plant species (genotypes), and growth stages all affect rhizosphere microbial communities in an extremely complex manner. In some cases, the effect of soil type on the community is greater than the effect from the specific plant species [83], while in other cases the plant species may have a greater influence on community composition than soil type [37].

The observed dominant effect of soil type on microbial communities in the rhizosphere can thus be explained by the impact of the soil fabrics on the soil-inhabiting communities, which are the sources for rhizoplane and rhizosphere colonization. Also, soil texture may affect the rhizosphere microflora by limiting the availability of root exudates [17].

Agricultural Management Regime as the Determinant of the Structure of Microbial Communities in Soil

There is an enormous volume of literature on the application of bacteria for improvement of plant performance [84–92], but a few bacteria like *Azotobacter* and *Azospirillum* have been developed as commercial products. The organisms under most scrutiny for potential use in agriculture are bacteria belonging to the genera *Pseudomonas* and *Bacillus* species [93–97].

Soil management practices such as crop rotation, tillage, fertilizer, compost, manure or pesticide applications and irrigation greatly affect soil microbial parameters [16]. Tiedje et al. [98] compared the responses of microbial communities in three soils of different history to the application of 2,4-D (2,4-dichlorophenoxyacetate). They hypothesized that different land use practices will yield different microbial responses to the applied herbicide. However, the same population of 2,4-D degraders became dominant in three soils of different land use history, indicating that 2,4-D was a stronger selector. The impact of long-term grassland management regimes (N-fertilizer application and soil drainage) on microbial community structure was assessed by using PCR-DGGE and PLFA profiling. N fertilizer was found to exert a significant impact on the total bacterial and actinomycete community structures, whereas soil drainage had a significant impact on the actinomycete and pseudomonad communities [99]. This study strongly indicated that grassland management practice has an impact on the community structure of specific bacterial groups. Nusslein and Tiedje [100] showed that soil bacterial community shifts are correlated with a change from forest to pasture vegetation in a tropical soil. The G+C content of the pasture soil DNA was significantly higher than that of the forest soil DNA. Although α- and β-Proteobacteria dominated in the pasture soil, fibrobacter types were dominant in the forest soil. Four soils from eastern Washington State with contrasting soil management (no-till and conventional till) and environmental conditions were analyzed by PLFA and DGGE [101]. The results indicated that no-till soil practices improved the biological conditions. This conclusion was based on the fact that high microbial mass was determined by PLFA analysis and greater diversity of ammonia-oxidizing bacteria was associated with no-till soil.

In a long-term experiment performed by van Elsas et al. [102], permanent grassland was studied adjacent to farmland under rotation or under monoculture of maize. To assess the microbial community structure in these soils, several complementary methods were used, e.g., conventional enumeration on four different agar media for enumeration of culturable fungal, bacterial, *Bacillus*, and *Pseudomonas* populations, PCR-DGGE assessment of

microbial diversity using universal bacterial, fungal and group-specific primers (for *Bacillus* and *Pseudomonas*), and 16S and 18S rDNA clone libraries obtained from different treatments. The results obtained by all the methods showed clear differences in microbial community structures between different treatments. Higher microbial diversities and biomass were measured in the permanent grassland than in the arable land under monoculture or under rotation. Since root exudation is species-specific, it is a major factor that determines community composition within the rhizosphere [103].

Microbial Diversity and Community Structure in Polluted Soils

The use of diversity and changes in community structure as ecological indicators of perturbations and pollution have been investigated in soils subjected to different agricultural management and to heavy-metal contaminated sewage sludge [104]. Microbial diversity of an arable soil cropped to cereals, vegetables and potatoes was compared with that of an organic soil, used solely as a pasture field during the last decade; the two soils having similar texture and situated in western Norway 400 m apart. It was suggested that the total genetic diversity in the arable soil was approximately 24 times lower than in the relatively undisturbed pasture soil and that the overall genetic diversity provides a good indicator of disturbance caused by agricultural management. PCR combined DGGE indicated that the number of different bacterial types in both soils was high, meaning that there was a high "species" richness component of diversity in both soils. The bacterial populations in the arable soil were probably less evenly distributed than in the pasture soil, containing a few numerically dominant bacterial populations and many with low abundance.

The community structure of the low and high metal-contaminated soils was investigated by hybridization with group-specific phylogenetic probes [105]. The most abundant group of clones in the low metal-contaminated soil was the cytophaga-flexibacter-bacteroides group. This group was twice as abundant in the low as in the high metal-contaminated soil. In the high metal-contaminated soil, clones belonging to the α-Proteobacteria were numerically dominant. With respect to the isolates, 30–37 % of them belonged to Gram-positive bacteria with low mol% G+C. Accordingly, this was the largest group of isolates in both soils. In the high metal-contaminated soil the abundance of isolates and clones belonging to the α-Proteobacteria subclass differed markedly, as the percentage of clones was 38 % and that of isolates was only 14 %. These investigations revealed that the total microbial diversity in relatively undisturbed and unpolluted soil was high, and that upon perturbation and pollution the total soil microbial diversity was dramatically reduced.

Microbial Diversity in Relation to Suppressiveness of Soil to Plant Pathogens

Some soils are inhospitable to plant pathogens, by limiting either their survival or growth of the pathogens. Such soils are known as pathogen—or disease—suppressive and are found throughout the world.

Representatives of a range of bacterial (*Pseudomonas*, *Burkholderia*, *Bacillus*, *Serratia*, Actinomycetes) and fungal (*Trichoderma*, *Penicillium*, *Gliocladium*, *Sporidesmium*, nonpathogenic *Fusarium* spp.) groups have been identified as antagonists of soil-borne plant pathogens. The mechanisms by which these microorganisms make soil suppressive can be divided into several categories: nutrient competition, amensalism, microbial antagonism, parasitism and systemic induced resistance. Several antibiotic-producing *Pseudomonas* spp. were isolated from soil suppressive to diseases such as take-all of wheat, black rot of tobacco and fusarium wilt. Naturally occurring root-associated fluorescent *Pseudomonas* spp. producing the antibiotic 2,4-DAPG were highly enriched in take-all-suppressive soil and are key components of specific suppression of *Gaeumannomyces graminis* var. *tritici* [106]. This suppression was lost when 2,4-DAPG-producing *Pseudomonas* spp. were eliminated and, conversely, conducive soil gained suppressiveness to take-all when 2,4-DAPG-producing *Pseudomonas* strains were introduced.

Effect of Cropping System

Disease suppression can be influenced by cropping and management practice. Next to cover crops, compost application, tillage and crop rotation are important [1], as the densities of both soil-borne pathogens and the antagonistic microorganisms are affected. Effective crop rotation results in the lack of positive selection of the pathogen and provides time needed for the biological destruction of pathogen inoculum by antagonists residing in soil. The cultivation of plants influences the microbial activity of the soil and, therefore, the suppressiveness. For example, the cultivation of the leguminous cover plant *Pueraria javanica* significantly enhanced the suppressiveness to *Fusarium* wilt of a palm grove soil compared with soil that was kept uncultivated [107]. The cultivation of *P. javanica* induced changes in the microbial balance, increasing the population of non-pathogenic *Fusarium* spp. Root diseases are generally less severe in organic than in conventional farms, with reduction attributable to longer rotations, regular applications of organic amendments, and abstinence from, or reduction of, pesticide use [108]. Total populations of fungi and bacteria are generally higher in organically than in conventionally farmed areas. Populations of specific groups, i.e., fluorescent pseudomonads and

actinomycetes are also found to be higher in rhizosphere soil from organic farms than in those from conventional farms. A positive correlation was observed between microbial diversity and the disease-suppressive capacity of soil, as higher microbial diversities were measured in soil samples from the permanent grassland and grassland turned to arable land than in the long-term arable land under rotation [16].

Soil Amendment and Tillage

Organic amendments such as manure, compost and cover crops can positively affect the disease suppressiveness of soil. For instance, composts can suppress *Pythium* and *Phytophthora* root rot as well as *Ralstonia solanacearum* [7]. Organic amendments can be combined with the application of biocontrol agents to control diseases. During decomposition of organic matter in soil, the soil ecosystem is subjected to "oligotrophication," and the ratio of oligotrophic (K-strategist) to copiotrophic (r-strategist) organisms changes during microbial succession [108]. Knowledge on microbial communities and the major groups of microorganisms involved in the disease suppressiveness of soil is fundamental to a better understanding of the relevance of microbial diversity to disease suppression.

Conclusions

The two factors which have impact on microbial community structure are plant type and soil type which exert their effects in a complex manner. The fact that in some situations the soil and in others plant type is the determining factor affecting the soil microbial community may relate to the effects being either stronger or weaker in accordance with the relative strength of the selective forces exerted by soil or the plant. Also, this determining factor may be related to the complex microbial interactions in soil, including interactions between microorganisms and soil and microorganisms and plants. Plants clearly affect microbial communities but to what extent in time and space are not very clear. An improved understanding of how far the beneficial effects of plants extend in space and time will be an area for future work. The effects of abiotic conditions of soil (soil moisture content, temperature, etc.) on the relationships between the two main drivers of microbial community structure, plant and soil type, will enrich the understanding further. The availability of new and powerful technologies for studying cooperative microbial interactions in the rhizosphere guarantees a greater understanding of the processes which will facilitate their successful applications in biotechnology.

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References

1. Torsvik V, Salte K, Soerheim R, Goksoeyr J (1990) Comparison of phenotypic diversity and DNA heterogeneity in a population of soil bacteria. Appl Environ Microbiol 53:776–781
2. Kirk JL, Beaudette A, Hart M, Moutoglis P, Kironomos JN, Lee H, Trevors JT (2004) Methods of studying soil microbial diversity. J Microbiol Method 58:169–188
3. Abawi GS, Widmer TL (2000) Impact of soil health management practices on soilborne pathogens and root diseases of vegetables. Appl Soil Ecol 15:37–47
4. Manoharachary C, Tilak KVBR, Maheswari U, Singh D (2009) National conservation and characterization of microbes. Indian Farming 59:24–30
5. Heilmann B, Lebuhn M, Beese F (1995) Methods for the investigation of metabolic activities and shifts in the microbial community in a soil treated with a fungicide. Biol Fertil Soils 19:186–192
6. Hallmann J, Rodriguez-Kabana R, Kloepper JW (1999) Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. Soil Biol Biochem 31:551–560
7. Schönfeld J, Gelsomino A, van Overbeek LS, Gorissen A, Smalla K, van Elsas JD (2002) Effects of compost addition and simulated solarisation on the fate of *Ralstonia solanacearum* biovar 2 and indigenous bacteria in soil. FEMS Microbiol Ecol 43:63–74
8. De Leij FAAM, Sutton SJ, Whipps JM, Fenlon JS, Lynch JM (1995) Impact of field release of genetically modified *Pseudomonas fluorescens* on indigenous microbial populations of wheat. Appl Environ Microbiol 61:3443–3453
9. Kennedy AC, Smith KL (1995) Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170:75–86
10. Ranjard L, Richaume AS (2001) Quantitative and qualitative microscale distribution of bacteria in soil. Res Microbiol 152:707–716
11. Grayston SJ, Wang S, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30:369–378
12. Villich V (1997) Assessment of microbial diversity by fatty acid analysis. Dev Plant Pathol 11:71–74
13. Borneman J, Triplett EW (1997) Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. Appl Environ Microbiol 63:2647–2653
14. Ovreas L, Torsvik V (1998) Microbial diversity and community structure in two different agricultural soil communities. Microb Ecol 36:303–315
15. Liesack W, Janssen PH, Rainey FA, Ward-Rainey NL, Stackebrandt E (2000) Microbial diversity in soil: the need for a combined approach using molecular and cultivation techniques. In: Van Elsas JD, Trevors JT, Wellington EMH (eds) Modern soil microbiology. Marcel Dekker, New York
16. Garbeva P, van Veen JA, van Elsas JD (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Ann Rev Phytopathol 42:243–270
17. Manoharachary C, Kunwar IK, Sharat Babu K (2006) Rhizosphere: the hidden ecological niche and hot spot. Proc Nat Acad Sci India 76(B):321–342

18. Nautiyal CS (2000) Biocontrol of plant diseases for agricultural sustainability. In: Upadhyay RK, Mukherji KG, Chamola C (eds) Biocontrol potential and its exploration in sustainable agriculture. Kluwer Academic, Dordrecht
19. Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK (2005) Diversity of plant growth and soil supporting bacteria. Curr Sci 89(1):136–150
20. Nautiyal CS (2006) Biological control of plant diseases by natural and genetically engineered fluorescent *Pseudomonas* spp. In: Ray RC, Owen PW (eds) Microbial biotechnology in horticulture. Science Publishers, Enfield
21. Jaeger CH, Lindow SE, Miller W, Clark E, Firestone MK (1999) Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. Appl Environ Microbiol 65:2685–2690
22. Germida JJ, Siciliano SD, de Renato FJ, Seib AM (1998) Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. FEMS Microbiol Ecol 26:43–50
23. Kaiser O, Puhler A, Selbitschka W (2001) Phylogenetic analysis of microbial diversity in the rhizoplane of oilseed rape (*Brassica napus* cv. Westar) employing cultivation-dependent and cultivation-independent approaches. Microb Ecol 42:136–149
24. Kowalchuk GA, Buma DS, De Boer W, Klinkhamer PGL, van Veen JA (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Van Leeuwenhoek 81:509–520
25. Brimecombe MJ, De Leij FA, Lynch JM (2001) The effect of root exudates on rhizosphere microbial populations. In: Pinto R, Varanini Z, Nannipierei P (eds) The rhizosphere. Marcel Dekker, New York
26. Singh BK, Walker A, Morgan JAW, Wright DJ (2003) Effect of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos degrading bacterium. Appl Environ Microbiol 69:5198–5206
27. Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67:4742–4751
28. Yang CH, Crowley DE (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Appl Environ Microbiol 66:345–351
29. Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1451
30. Yang CH, Crowley DE, Menge JA (2001) 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. FEMS Microbiol Ecol 35:129–136
31. Macrae A, Lucon CMM, Rimmer DL, O'Donnell AG (2001) Sampling DNA from the rhizosphere of *Brassica napus* to investigate rhizobacterial community structure. Plant Soil 233:223–230
32. Fromin N, Achouak W, Thiery JM, Heulin T (2001) The genotypic diversity of *Pseudomonas brassicacearum* populations isolated from roots of *Arabidopsis thaliana*: influence of plant genotype. FEMS Microbiol Ecol 37:21–29
33. Duineveld BM, Kowalchuk GA, Keijzer A, van Elsas JD, van Veen JA (2001) Analysis of bacterial communities in the rhizosphere of *Chrysanthemum* via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Appl Environ Microbiol 67:172–178
34. Normander B, Prosser JI (2000) Bacterial origin and community composition in the barley phytosphere as a function of habitat and presowing conditions. Appl Environ Microbiol 66:4372–4377
35. Gomes NCM, Heuer H, Schonfeld J, Costa R, Hagler-Mendonca L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant Soil 232:167–180
36. Schwieger F, Tebbe CC (2000) Effect of field inoculation with *Sinorhizobium meliloti* L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*). Linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. Appl Environ Microbiol 66:3356–3365
37. Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K (2002) Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. Appl Environ Microbiol 68:3328–3338
38. Rademaker JLW, Louws FJ, Rossbach U, de Bruijn FJ (1999) Computer-assisted pattern analysis of molecular fingerprints and database construction. In: Akkermans A, van Elsas JD, DeBruijn FJ (eds) Molecular microbial ecology Manual. Kluwer Academic Publisher, Dodrecht
39. Stephan A, Meyer AH, Schmid B (2000) Plant diversity affects culturable soil bacteria in experimental grassland communities. J Ecol 22:988–998
40. Spehn EM, Joshi J, Schmid B (2000) Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant Soil 224:217–230
41. Haack SK, Garchow H, Klug MJ (1995) Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl Environ Microbiol 61:1458–1468
42. Kim KY, Jordan D, McDonald GA (1998) *Enterobacter agglomerans*, phosphate solubilizing bacteria, and microbial activity in soil: effect of carbon sources. Soil Biol Biochem 30:995–1003
43. Insam H, Domsch KH (1988) Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. Microbial Ecol 15:177–188
44. Wieland G, Neumann R, Backhaus H (2001) Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Appl Environ Microbiol 67:5849–5854
45. Dunfield KE, Germida JJ (2001) Diversity of bacterial communities in the rhizosphere and root interior of field grown genetically modified *Brassica napus*. FEMS Microbiol Ecol 38:1–9
46. Di Giovanni GD, Wastrud LS, Seidler RJ, Widmar F (1999) Comparison of parental and transgenic alfalfa rhizosphere bacterial communities using Biolog GN metabolic fingerprinting and entero-bacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR). Microb Ecol 37:129–139
47. Misko AL, Germida J (2002) Taxonomic and functional diversity of pseudomonads isolated from the roots of field-grown canola. FEMS Microbiol Ecol 42:399–407
48. Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511
49. van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Ann Rev Phytopathol 36:453–459
50. Mittal S, Johri BN (2007) Assessment of rhizobacterial diversity of *Triticum aestivum* and *Eleusine coracana* from northern region of India. Curr Sci 93:1530–1537
51. Schweitzer JA, Bailey JK, Fischer DG, LeRoy CJ, Lonsdorf EV, Whitham TG, Hart SC (2008) Plant–soil–microorganism interactions: heritable relationship between plant genotype and associated soil microorganisms. Ecology 89:773–781
52. Picard C, di Cello F, Ventura M, Fani R, Guckert A (2000) Frequency and biodiversity of 2,4-diacetylphloroglucinol-

producing bacteria isolated from the maize rhizosphere at different stages of plant growth. Appl Environ Microbiol 66: 948–955
53. di Cello F, Bevivino L, Chiarini R, Fani R, Paffetti D (1997) Biodiversity of a *Burkholderia cepacia* population isolated from the maize rhizosphere at different plant growth stages. Appl Environ Microbiol 63:4485–4493
54. Seldin L, Rosado AS, da Cruz DW, Nobrega A, van Elsas JD (1998) Comparison of *Paenibacillus azotofixans* strains isolated from rhizoplane, rhizosphere, and non-root-associated soil from maize planted in two different Brazilian soils. Appl Environ Microbiol 64:3860–3868
55. Nacamulli CB, Dalmastri C, Tabacchioni S, Chiarini L (1997) Perturbation of maize rhizosphere microflora following seed bacterization with *Burkholderia cepacia* MCI 7. FEMS Microbiol Ecol 23:183–193
56. Baudoin E, Benizri E, Guckert A (2002) Impact of growth stages on bacterial community structure along maize roots by metabolic and genetic fingerprinting. Appl Soil Ecol 19:135–145
57. Gyamfi S, Pfeifer U, Stierschneider M, Sessitch A (2002) Effects of transgenic glufosinate-tolerant oilseed rape (*Brassica napus*) and the associated herbicide application on eubacterial and *Pseudomonas* communities in the rhizosphere. FEMS Microbiol Ecol 41:181–190
58. Xu Y, Wang G, Jin J, Liu J, Zhang Q, Liu X (2009) Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. Soil Biol Biochem 41:919–925
59. Kowalchuk G, Bruinsma M, van Veen JA (2003) Assessing responses of soil microorganisms to GM plants. Trends Ecol Evol 18:403–410
60. Heuer H, Kroppenstedt RM, Lottmann J, Berg G, Smalla K (2002) Effects of T4 lysozyme release from transgenic potato roots on bacterial rhizosphere communities are negligible relative to natural factors. Appl Environ Microbiol 68:1325–1335
61. Ahrenholtz I, Harms K, de Vries J, Wackernagel W (2000) Increased killing of *Bacillus subtilis* on the hair roots of transgenic T4 lysozyme-producing potatoes. Appl Environ Microbiol 2000(66):1862–1865
62. Gelsomino A, Keijzer-Wolters A, Cacco G, van Elsas JD (1999) Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. J Microbiol Methods 38:1–15
63. Groffman PM, Hanson CC, Kiviat E, Stevens G (1996) Variation in microbial biomass and activity in four different wetland types. Soil Sci Soc Am J 60:622–629
64. Chiarini L, Bevivino A, Dalmastri C, Nacamulli C, Tabacchioni S (1998) Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Appl Soil Ecol 8:11–18
65. da Silva KRA, Salles JF, Seldin L, van Elsas JD (2003) Application of a novel *Paenibacillus*-specific PCR-DGGE method and sequence analysis to assess the diversity of *Paenibacillus* spp. in the maize rhizosphere. J Microbiol Methods 54:213–231
66. Latour X, Corberand T, Laguerre G, Allard F, Lemanceau P (1996) The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. Appl Environ Microbiol 62:2449–2456
67. Buyer JS, Roberts DP, Russek-Cohen E (1999) Microbial community structure and function in the spermosphere as affected by soil and seed type. Can J Microbiol 45:138–144
68. Kuske CR, Ticknor LO (2002) Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Appl Environ Microbiol 68:1854–1863
69. Miethling R, Wieland G, Backhaus H, Tebbe CC (2000) Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. Microb Ecol 40:43–56
70. Lemanceau P, Corberand T, Gardan L, Latour X, Laguerre G (1995) Effect of two plant species, flax (*Linum usitatissinum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations of fluorescent Pseudomonads. Appl Environ Microbiol 61:1004–1012
71. Araujo MAV, Mendonca-Hagler LC, Hagler AN, van Elsas JD (1994) Survival of genetically modified *Pseudomonas fluorescens* introduced into subtropical soil microcosm. FEMS Microbiol Ecol 13:205–216
72. Boeuf-Tremblay V, Plantureux S, Guckert A (1995) Influence of mechanical impedance on root exudation of maize seedlings at two development stages. Plant Soil 172:279–287
73. Dashman T, Stotzky G (1984) Adsorption and binding of peptides on homoionic montmorillonite and kaolinite. Soil Biol Biochem 16:51–55
74. Meharg AA, Killham K (1995) Loss of exudates from the roots of perennial ryegrass inoculated with a range of micro-organisms. Plant Soil 170(345):349
75. Rovira AD (1969) Plant root exudates. Bot Rev 35:35–60
76. Hale MG, Moore LD (1979) Factors affecting root exudation. Adv Agron 31:93–98
77. McCully NM (1999) Roots in soil: unearthing the complexities of roots and their rhizospheres. Ann Rev Plant Physiol Mol Biol 50:695–702
78. Uren NC (2007) Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere biochemistry and organic substances at the soil-plant interface. FLCRC Press, Boca Raton
79. Heijnen CE, Hok-A-Hin CH, Van Elsas JD (1993) Root colonization by *Pseudomonas fluorescens* introduced into soil amended with bentonite. Soil Biol Biochem 25:239–246
80. Höper H, Steinberg C, Alabouvette C (1995) Involvement of clay type and pH in the mechanisms of soil suppressiveness to fusarium wilt of flax. Soil Biol Biochem 27:955–967
81. Ownley BH, Weller DM, Alldredge JR (1992) Relation of soil chemical and physical factors with suppression of take-all by *Pseudomonas fluorescens* 2–79. In: Keel C, Koller B, Défago G (eds) Plant growth-promoting rhizobacteria: progress and prospects. IOBC/WPRS bulletin, Zurich
82. Lemanceau P (1989) Role of competition for carbon and iron in mechanisms of soil suppressiveness to *Fusarium* wilts. In: Tjamos EC, Beckman CH (eds) Vascular wilt diseases of plants: basic studies and control. Springer, Berlin
83. Singh BK, Munro S, Potts JM, Millard P (2007) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36:147–155
84. Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth promoting rhizobacteria. Curr Microbiol 4:317–330
85. Whipps JM (1997) Developments in the biological control of soil-borne plant pathogens. Adv Bot Res 26:1–133
86. Okon Y, Labandera-Gonzalez CA (1994) Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. Soil Biol Biochem 26:159–170
87. Saxena AK, Tilak KVBR (1994) Interaction among beneficial soil microorganisms. Indian J Microbiol 34:91–106
88. Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770
89. Okon Y, Bloemberg GV, Lugtenberg BJJ (1998) Biotechnology of biofertilization and phytostimulation. In: Altman A (ed) Agricultural biotechnology. Marcel Dekker, New York
90. Malik RDS, Kavimandan SK, Tilak KVBR (1996) Soil microorganisms increase agricultural production. Indian FMG 2:15–16

91. Gupta A, Gopal M, Saxena AK, Tilak KVBR (2003) Effects of co-inoculation of plant growth promoting rhizobacteria and *Bradyrhizobium* sp. (*Vigna*) on growth and yield of green gram (*Vigna radiata* (L.) Wilczek). Trop Agric 80:28–35
92. Tilak KVBR (2007) Biofertilizers: prospects and potentials. J Ecofriendly Agric 2(1):1–19
93. Pal KK, Tilak KVBR, Saxena AK, Dey R, Singh CS (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. Microbiol Res 156:209–223
94. Nautiyal CS, Mehta S, Singh HB (2006) Biological control and plant growth-promoting *Bacillus* strains from milk. J Microbiol Biotechnol 16:184–192
95. Tilak KVBR, Ranganayaki N, Manoharachari C (2006) Synergistic effects of plant growth promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan* (L) Milsp.). Eur J Soil Sci 57:67–71
96. Venkateswalu B, Desai S, Prasad YG (2009) Agriculturally important microorganisms for selected ecosystems: challenges in technology development and application. In: Khachatourians GG, Arora DK, Rajendran TP, Srivastava AK (eds) Agriculturally important microorganisms, vol 1. Academic World International, New Delhi, p 2009
97. Nautiyal CS, Tilak KVBR (2009) Agriculturally important rhizobacteria as bioinoculants for enhancing plant growth and soil health. In: Khachatourians GG, Arora DK, Rajendran TP, Srivastava AK (eds) Agriculturally important microorganisms, vol 11. Academic World International, New Delhi
98. Tiedje JM, Asuming-Brempong S, Nusslein K, Marsch TL, Flynn SJ (1999) Opening the black box of soil microbial diversity. Appl Soil Ecol 13:109–120
99. Clegg CD, Lovell RDL, Hobbus PJ (2003) The impact of grassland management regime on the community structure of selected bacterial groups in soil. FEMS Microbiol Ecol 43:263–270
100. Nusslein K, Tiedje JM (1999) Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Appl Environ Microbiol 65:3622–3626
101. Ibekwe AM, Kennedy AC, Frohne PS, Papriernik SK, Yang CH (2002) Microbial diversity along a transect of agronomic zones. FEMS Microbiol Ecol 39:183–191
102. van Elsas JD, Garbeva P, Salles JF (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
103. Griffiths BS, Ritz K, Ebblewhite N, Dobson G (1998) Soil microbial community structure: effects of substrate loading rates. Soil Biol Biochem 31:145–153
104. Nannipieri P, Ascher J, Ceccherini MT, Loretta L, Giacomo P, Giancarlo R (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670
105. Sandaa RA, Torsvik V, Enger Ø (2001) Influence of long-term heavy-metal contamination on microbial communities in soil. Soil Biol Biochem 33:287–295
106. Raaijmakers JM, Weller DM (1998) Natural plant protection by 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. Mol Plant Microbe Interact 11:144–152
107. Abadieu C, Edel V, Alabouvette C (1998) Soil suppressiveness to *Fusarium* wilt: influence of a cover-plant on density and diversity of *Fusarium* populations. Soil Biol Biochem 30:643
108. van Bruggen AHC, Semenov AM (1999) In search of biological indicators for soil health and disease suppression. Appl Soil Ecol 15:13–24