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Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type

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Abstract

Rhizosphere microbial communities are important for plant nutrition and plant health. Using the culture-independent method of PCR-DGGE of 16S rDNA for community analyses, we conducted several experiments to investigate the importance of pH, soil type, soil amendment, nutritional status of the plant, plant species and plant age on the structure of the bacterial community in the rhizosphere. At the same time, we assessed the spatial variability of bacterial communities in different root zone locations. Our results showed that the bacterial community structure is influenced by soil pH and type of P fertilization. In a short-term experiment (15–22 days) with cucumber and barley growing in a N deficient or a P deficient soil, the bacterial community structure in the rhizosphere was affected by soil type and fertilization but not by plant species. In a 7.5-week experiment with three plant species (chickpea, canola, Sudan grass) growing in three different soils (a sand, a loam and a clay), the complex interactions between soil and plant effects on the rhizosphere community were apparent. In the sand and the loam, the three plant species had distinct rhizosphere communities while in the clay soil the rhizosphere community structures of canola and Sudan grass were similar and differed from those of chickpea. In all soils, the rhizosphere community structures of the root tip were different from those in the mature root zone. In white lupin, the bacterial community structure of the non-cluster roots differed from those of the cluster roots. As plants matured, different cluster root age classes (young, mature, old) had distinct rhizosphere communities. We conclude that many different factors will contribute to shaping the species composition in the rhizosphere, but that the plant itself exerts a highly selective effect that is at least as great as that of the soil. Root exudate amount and composition are the key drivers for the differences in community structure observed in this study.

Introduction

Rhizosphere microbial communities carry out fundamental processes that contribute to nutrient cycling, plant growth, and root health. The extent to which these communities vary in relation to various environmental factors is thus of considerable interest to plant-microbial ecologists. Practical interests include the manipulation of microbial communities to promote plant-beneficial interactions involving hormone production, enhanced nutrient availability in nutrient-limited soils, and the natural suppression of root disease-causing microorganisms. Rhizosphere communities are influenced by soil and plant factors, but little is known about the relative importance of these factors. Moreover, the dynamic spatial and temporal nature of microbial communities in different root locations and over time is becoming more and more evident as methods requiring small sample volumes are used. A very important breakthrough for rhizosphere ecologists has been the advent of culture independent methods such as PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) of ribosomal DNA, which assess a much greater fraction of the microbial population than culture-dependent

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methods such as dilution plating. Culture-dependent techniques detect less than 10% of soil microorganisms (Bakken, 1985), and they are very labour intensive. With PCR-DGGE, microbial communities can be analysed for specific groups of microorganisms, and different root zones can be studied, since only small samples are required for the analyses. The variability between small samples is usually high, reflecting the high spatial variability in the soil, which emphasises the need for greater replication.

Soils have very distinct microbial communities (Gelsomino et al., 1999; Carelli et al., 2000), which are the result of many different selection factors. These include the physical and chemical characteristics of the soil (e.g., soil texture, nutrient and organic matter content and pH) and environmental factors such as climate and vegetation. Rhizosphere microbial communities can be regarded as a subset of the soil microbial community, therefore they too are influenced by soil chemical and physical properties. However, the species composition and the relative abundance of different species, which are collectively defined as the community structure, of rhizosphere communities differ from those in the bulk soil (Foster, 1986; Marilley and Aragno, 1999). This is a clear indication that plants have a strong influence on the microbial populations on their roots. Indeed, in many cases the rhizosphere communities of different plant species growing in the same soil are distinct (Ibekwe and Kennedy, 1998). Plants may even have very similar microbial community structures in different soils (Grayston et al., 1998; Miethling et al., 2000). However, the degree to which plants control the species composition of the rhizosphere microflora is not clear, as there are also studies in which plant species growing in the same soil had similar rhizosphere microbial communities, indicating that the influence of the soil may be greater than that of the plant (Buyer et al., 1999; Latour et al., 1999). In order to be able to manipulate microbial populations in the rhizosphere to the benefit of the plant, a better understanding of the relative importance of soil and plant factors for microbial rhizosphere communities is clearly needed.

Plant roots release 1–25% of the net photosynthesis as soluble and insoluble compounds into the rhizosphere (Merbach et al., 1999). Among rhizosphere microbial ecologists there is currently a consensus that differences in exudate amount and composition are likely to affect community structure because microbial species differ in their ability to metabolise and compete for different carbon sources. A wide range of factors have been shown to affect root exudation, including plant genotype (Rovira, 1959; Rengel et al., 1997; Grayston et al., 1998), plant age (Martin, 1971; Van Veen et al., 1991; Marschner et al., 2001b), nutritional status (Hoffland et al., 1989; Liljeroth et al., 1990; Marschner and Crowley, 1998; Fan et al., 2001) and colonisation by mycorrhizal fungi (Po and Cumming, 1997; Marschner et al., 1997).

Differences in the distribution of these exudates along the root axes have been examined by many researchers using radiolabeled carbon pulses (e.g., Marschner et al., 1997) and other methods (Hoffland et al., 1989; Römheld, 1991; Dinkelaker et al., 1995), all of which show that root exudates are primarily released in the zone of elongation behind the root tips. A large proportion of the carbon is in the form of water-soluble substances such as sugars, organic acids and amino acids. The differences in type and quantity of carbon available in different root zones thereby select for distinct rhizosphere community structures (Yang and Crowley, 2000). As the root tip grows through the soil, microorganisms in its pathway will be the first colonisers. During rapid root growth, the zone of elongation behind the root tips is only sparsely colonised by soil microorganisms. Thereafter, microbial population densities increase rapidly a few centimetres behind the root tips where soluble, insoluble and volatile root exudates attract soil microorganisms and are used for microbial growth and metabolism. In contrast, along the older root parts, the primary substrates for microbial growth include cellulose and other recalcitrant cell wall materials from sloughed root cortex tissues. Certain components of root exudates can also have a selective influence on rhizosphere microorganisms by repelling some species and increasing the competitive ability of others (Geurts and Franssen, 1996). Variations in the distribution and quantities of these substances that include alkaloids, flavonoids, terpenes, and other secondary metabolites, and their relative importance in influencing rhizosphere community structure, are new research topics that are only just beginning to be investigated.

Sorting out the relative importance of plant and soil factors remains as a major task, in which experiments will need to be conducted to examine simultaneously multiple variables including plant species, soil type, and plant nutrition. In this paper, we present a series of case studies that have begun to investigate the interactions between plant roots and soils as well as the effect of nutrient status of the plant, plant age and soil nutrient content on rhizosphere community structure. Also,

the variability of bacterial rhizosphere communities along the root axes was assessed. Our hypothesis was that the plant would be one of the major factors influencing the rhizosphere community structure but that other factors, such as soil type or fertilization would also have an impact.

Materials and methods

Effect of pH on rhizosphere communities of sorghum

Sorghum (*Sorghum vulgare* L.) plants were grown in a Handford loamy sand with a baseline pH (H_2O) of 8.1 and in the same soil after adjustment of the pH to 6, 6.5, 7 and 7.5 for a total of 5 pH levels. The pH adjustments were made prior to the experiment by placing batches of the soil into beakers in a 1:1 water suspension that were then amended with 0.1 M HCl to the desired pH. The pH was adjusted daily by addition of dilute HCl, with mixing for a period of 10 days until the pH had stabilized in all soil samples. The soils were then drained, air-dried and 100 g aliquots were used to fill free-draining 95-ml plant growth container tubes. The soils were amended with minimal salts medium to provide nitrogen (10 mM NH_4SO_4), phosphorus (3 mM NaH_2PO_4), and trace elements, and were then planted with pre-germinated seeds of sorghum (3 replicates). After 18 days of growth, during which the plants were watered regularly until water started to leach from the pots, the plants were removed from the containers and root tips with adhering soil were sampled. At harvest the final pH values were 5.9, 6.8. 7.0, 7.5 and 8.1.

Effect of N and P fertilization on rhizosphere communities in barley and cucumber

The N deficient soil was a sandy Cambisol from Ottendorf (Schleswig-Holstein, Germany). The loamy P deficient Phaeozem was collected from Wengelsdorf (Sachsen-Anhalt, Germany). Both soils were kept in large outdoor containers at the Institute for Plant Nutrition and Soil Science in Kiel, Germany, for several years and had been cropped with different plant species. The three soil treatments for the N deficient soil were: low N (50 mg kg^{-1} N), foliar N (with low N in soil (50 mg kg^{-1} N)) and high N (250 mg kg^{-1} N). N was added as NH_4NO_3. In the P deficient soil, the treatments with low P and foliar P received no additional P, while the high P treatment was fertilized with 150 mg kg^{-1} P as KH_2PO_4. The foliar treatment was used to increase the nutrient content of the plants without altering the nutrient content of the soil compared to the low soil treatment. The soils were filled in pots with 750 g pot^{-1} and planted with 6 imbibed seeds of barley (*Hordeum vulgare* cv. Scarlett) or 3 imbibed seeds of cucumber (*Cucumis sativus* cv. Delicatesse) per pot. The pots (3 replicates per treatment) were placed in a greenhouse with additional lighting (12 h, 500 μmol s^{-1} m^{-2}) and watered regularly. On d 6 the plants were thinned to 4 barley plants pot^{-1} and 2 cucumber plants pot^{-1}. From d 8 on, the plants with the foliar treatment received a 5% urea solution (foliar N) or a 10 mM NaH_2PO_4 solution (pH 6)(foliar P) every second day by applying the solutions on the leaves with a brush. For the first harvest on d 15, two barley plants or one cucumber plant were removed per pot. Care was taken not to disturb the remaining plants in the pot. At the second harvest on d 22, the remaining plants were removed. At each harvest, the roots were carefully removed from the soil. Loosely adhering soil was shaken off, the remaining tightly adhering soil ('rhizosphere soil') was then brushed from the entire root system. The results of shoot dry weight were subjected to a one-way ANOVA using SigmaStat (SPSS, Chicago, USA). Significance was tested with the Student-Newman-Keuls test or Tukey test ($P \leq 0.05$).

Effect of phosphorus nutrition on rhizosphere communities in chickpea, canola and Sudan grass

Chickpea, canola, and Sudan grass were grown in silica sand with no phosphorus amendment, or with phosphorus provided as 100 μg g^{-1} rock phosphate, or with 1% organic matter provided as composted biosolids. There were 2 replicates per plant species and treatment with one plant per pot. The plants were fertilized with a 0.1× Hoagland's nutrient solution without phosphorus, which was applied at each watering approximately every 3 days. After 4 weeks of growth, the plants were harvested and the root tips with adhering sand particles were sampled.

Effect of soil type, plant species and root zone location on rhizosphere communities

Three plant species (chickpea, canola and Sudan grass) were grown in open ended, 5 × 15 cm, brass cylinders containing intact cores of three California soils (a sandy soil, a sandy loam, and a clay) and were provided by watering the plants twice a week with a complete fertilizer solution with or without nitrogen

supplied as ammonium nitrate. After 7.5 weeks, the plants were harvested and DNA was extracted from soil adhering to the root tips and from mature root zones at the sites of lateral root emergence (for details see Marschner et al., 2001a).

Spatial variability of rhizosphere communities in cluster roots of white lupin

White lupin was grown in a quartz sand-soil mix with poorly available Ca phosphate and watered regularly. The plants were harvested after 21 and 35 days and DNA was extracted from the non-cluster roots, the young, mature and senescent cluster roots with adhering substrate (for details see Marschner et al., 2002).

PCR-DGGE

Bacterial community structure was examined by PCR-DGGE of 16S rDNA. DNA was extracted from the roots with adhering soil or from soil by bead beating. After removal of inhibiting substances such as humic acids, the DNA was bound to a silica matrix, washed with an ethanol-salt solution and eluted with water (for details see Marschner et al., 2001a, b). The bacterial 16S rDNA was amplified with universal bacterial primers (Heuer et al., 1997; Ovreas et al., 1997). DGGE was performed with 8% (wt/vol) acrylamide gels containing a linear chemical gradient ranging from 35% to 60% (7 M urea and 40% (vol/vol) formamide). Twenty μl of the PCR products were electrophoresed in 1X TAE buffer at 60 °C at a constant voltage of 150 V for 5 h using a Dcode® Universal Mutation Detection System (Bio-Rad) (for details see Marschner et al., 2001a, b).

In the present paper, band number was used to represent species number and band intensity the abundance of a given 'species'. Band positions in the different gels were compared by expressing them relative to a DNA standard mixture that was run as a reference lane in each gel. DNA band intensity was normalized by expressing the intensity of each band as a percentage of the mean band intensity of the gel (for details see Marschner et al., 2001a, b). Each peak represents individual groups of species having 16S rDNA sequences with similar melting behavior. The band intensity indicates the relative abundance of the group under these PCR conditions. Community structure based on relative band intensity and position were analysed by performing principal component analyses or canonical correspondence analyses. The significance of environmental variables such as fertilization or plant species was determined with Monte Carlo permutation tests (CANOCO 4.0, Microcomputer Power, Ithaca, USA)(for details see Marschner et al., 2001a, b).

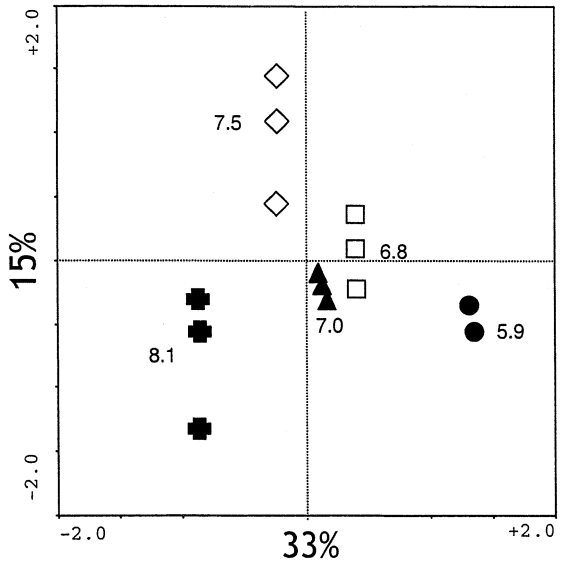

Figure 1. Ordination plot (canonical correspondence analysis) of bacterial rhizosphere communities of Sudan grass in a sandy loam adjusted to different pH values generated by canonical correspondence analysis of 16S rDNA DGGE profiles. The values on the axes refer to the % of the total variance explained by the axis. Communities are represented as symbols. Communities (symbols) close to each other have similar structures whereas communities far apart differ strongly in structure.

Results

Effect of pH on rhizosphere communities of sorghum

The bacterial rhizosphere community structure of sorghum was strongly affected by soil pH (Figure 1). Canonical correspondence analysis showed that the communities formed three distinct groups: the community structure at pH 5.9 was different from those at pH 6.8, 7.5 and 7.0, which again were different from that at pH 8.1.

Effect of N and P fertilization on rhizosphere community structure in barley and cucumber

The shoot dry weight of barley and cucumber grown in the N deficient soil increased significantly ($P < 0.05$) from d 15 to d 22 but was not affected by fertilization.

Table 1. Shoot dry weight of barley and cucumber after 22 days of growth in a N or a P deficient soil at high or low soil nutrient supply or foliar nutrient supply. Means of 3 replicates ± standard error

Nutrient supply	Cucumber		Barley	
	N soil	P soil	N soil	P soil
	mg shoot dw plant^{-1}			
Low	769 ± 63	330 ± 22	344 ± 48	96 ± 9
Foliar	1080 ± 178	312 ± 12	308 ± 88	97 ± 8
High	1206 ± 66	879 ± 68	463 ± 30	190 ± 44

In the P deficient soil, shoot dry weight of both plant species also increased significantly from d 15 to d 22. Shoot dry weight at high P supply was significantly higher than at low or foliar P supply (Table 1). There was some slight leaf scorching in the foliar P treatment in cucumber. The foliar N treatment did not cause any leaf damage.

The bacterial rhizosphere community structure was soil-specific, but did not differ between the two plant species (Figure 2). In both soils the rhizosphere community structure changed with plant age and was affected by fertilization. The community structure of the plants with low soil nutrient supply and plants with high soil nutrient supply formed two distinct groups, whereas the community structure of the foliar treated plants had similarities with both other groups. In the N deficient soil, fertilization had a significant effect on the bacterial rhizosphere community on d 22, but not on d 15. In the P deficient soil, fertilization had a significant effect at both harvests.

Effect of phosphorus nutrition on rhizosphere community structure in chickpea, canola and Sudan grass

After 4 weeks of growth, both plant species and phosphorus nutrition had a strong effect on the bacterial rhizosphere community structure in this experiment (Figure 3). The rhizosphere community structure of Sudan grass clearly differed from those of canola, while the rhizosphere community structure of chickpea showed similarities with both canola and Sudan grass, depending on type of fertilization. The rhizosphere community structure of Sudan grass was very similar in the three P treatments while those of chickpea and canola were influenced by fertilization. In chickpea, the community structures of the control and the treatment with rock P were different from those with organic matter. On the other hand, the rhizosphere community structure of canola with organic matter or rock P formed a group that was distinct from the community structure in the controls without fertilizer addition.

Effect of soil type, plant species and root zone location on rhizosphere community structure

The study comparing three plant species (chickpea, canola and Sudan grass) grown in three California soils (a sandy soil, a sandy loam, and a clay soil) showed that plant species, root zone and soil type as well as the interactions between these variables had significant effects on the rhizosphere community structure, but that these varied for different combinations. Shoot dry weight of all three plant species was lower in the clay than in the other two soils (data not shown, see Marschner et al., 2001 for details). Additional N supply had little effect on plant growth. In all plant species and soils, the rhizosphere community structure of the root tips differed from those of the mature root zones but were not affected by N supply. The effect of soil type on the rhizosphere community structure was different for the three plant species (Figure 4). In chickpea, the rhizosphere community structure of the clay and the loam were similar and both differed from those in the sand. In canola, sand and loam formed one group with very similar rhizosphere community structure while those in the clay were different. Sudan grass had distinct rhizosphere community structures in all three soils. The rhizosphere community structure of all three plant species differed from each other in the sand and the loam. However, in the clay soil, canola and Sudan grass had very similar rhizosphere community structure that differed from those of chickpea (Figure 4).

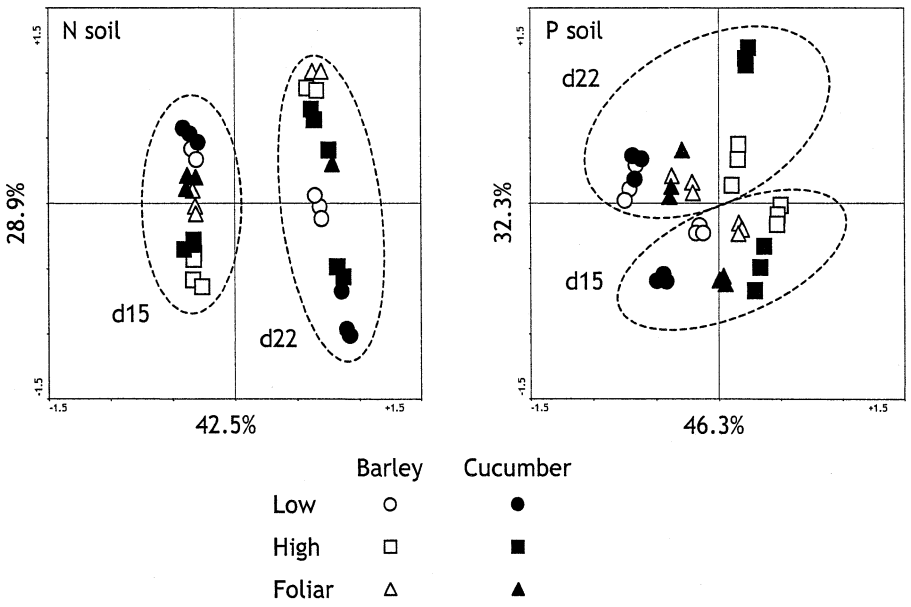

Figure 2. Ordination plot (canonical correspondence analysis) of bacterial rhizosphere communities of barley and cucumber on d 15 and d 22 for the N deficient and the P deficient soil with high or low soil nutrient supply or foliar nutrient supply generated by canonical correspondence analysis of 16S rDNA DGGE profiles. The dotted circles surround samples from the two harvest dates. For further information on the interpretation of the plots see Figure 1.

Spatial variability of rhizosphere community structure in cluster roots of white lupin

The bacterial community structure in the rhizosphere of white lupin was root zone-specific, and was further influenced by plant age (Figure 5). On d 21, the bacterial community structure formed two distinct groups. The first group was comprised of non-cluster roots while the second included all cluster roots. Cluster root age had no significant effect on the bacterial community structure in the rhizosphere. On d 35, however, all root zones had distinct community structures. The differences between the non-cluster and the cluster roots as well as between the cluster root age classes were significant.

Discussion

Our hypothesis was that the plant would be one of the major factors influencing the rhizosphere community structure but that other factors such as soil type or fertilization would also have an impact. The present studies support this hypothesis as they show that bacterial community structure in the rhizosphere is primarily affected by plant factors such as genotype, plant age and root zone location, but that it is also affected to varying degrees by soil factors such as soil type, nutrient availability and pH. Moreover, soil and plant factors interacted such that the selection pressures exerted by the plant and soil are modified with respect to secondary variables that arise with different plant-soil combinations.

The importance of soil pH for the rhizosphere community structure was evident in the experiment with Sudan grass grown in soil adjusted to different pHs (Figure 1). The rhizosphere community structure changed from pH 5.9 over pH 6.8–7.5 to pH 8.1. Thus, even pH changes of one unit may significantly affect the bacterial community structure as bacterial species differ in their pH optimum, i.e., the pH at which they are most competitive. However, soil pH can also affect the bacterial community structure indirectly by influencing nutrient availability and, importantly, root exudate amount and composition.

The strong effect of the soil type on the community structure in the rhizosphere was evident in the experiment with barley and cucumber (Figure 2) as well as in the study with the three Californian soils (Figure 4). In the latter case, the rhizosphere community structure of Sudan grass and canola were similar in the clay soil while they differed in the sand and the loam. This is in agreement with certain studies in which the experimental data have suggested that the soil was

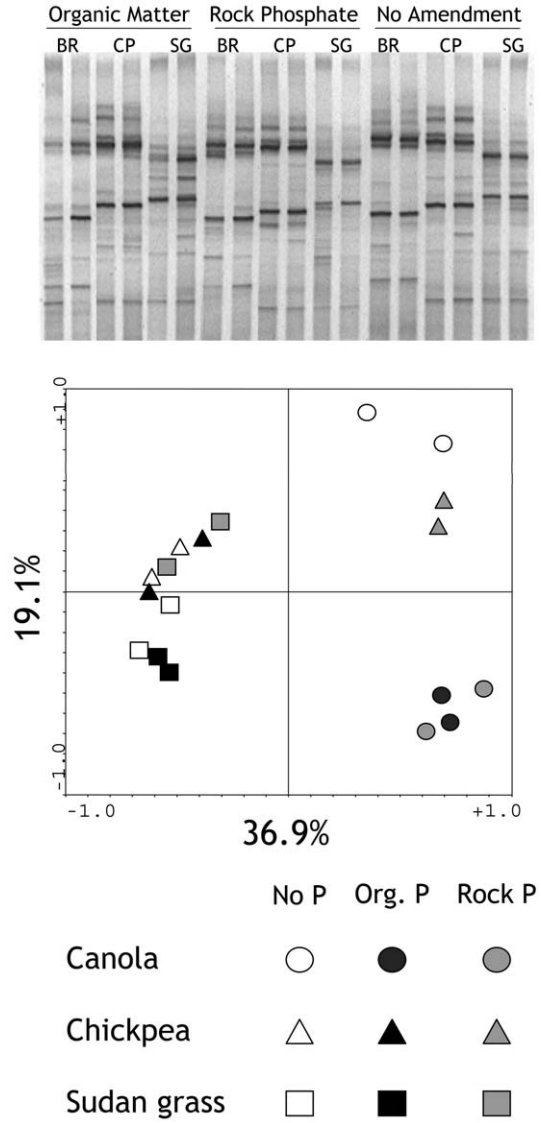

Figure 3. Ordination plot (principal correspondence analysis) of bacterial rhizosphere communities of Sudan grass, chickpea and canola under P deficiency (No P) or provided with phosphorus as rock phosphate (rock P) or organic P (Org P) generated from 16S rDNA DGGE profiles. For further information on the interpretation of the plot see Figure 1.

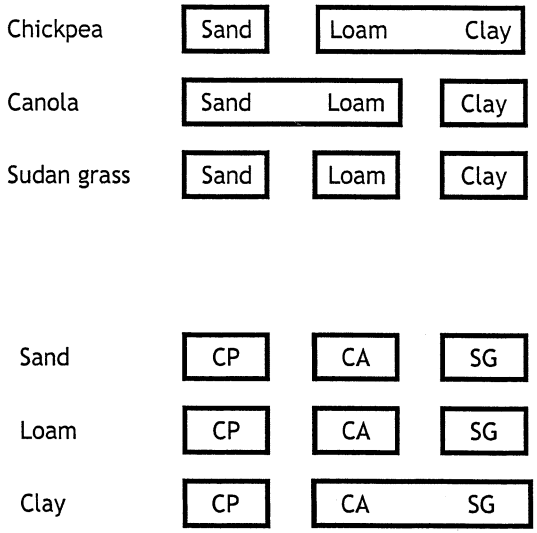

Figure 4. Rhizosphere bacterial communities of root tips of chickpea (CP), canola (CA) and Sudan grass (SG) growing in three different Californian soils (sand, loam or clay) generated by canonical correspondence analysis of 16S rDNA DGGE profiles. Significantly different communities are surrounded by separate rectangles ($P < 0.05$). (Based on Marschner et al., 2001a, reproduced with permission)

the most important factor for determining rhizosphere community structure (Buyer et al., 1999; Latour et al., 1999). Apparently, some soils can override the plant effects on rhizosphere microorganisms; however, it is unclear which soil properties contribute to this. Clay soils generally support higher population densities of bacteria and are more aggregated than sand or loam soils. Results of this research suggests that clay soils may exert a strong influence on rhizosphere community structures, but this hypothesis needs further examination; for example, by incrementally increasing the clay content of a clay-sand mixture. However, the effect of soil structure on the bacterial community structure in the rhizosphere may also be indirect, via differences in plant growth and nutrition. It should be noted that in the experiment with barley and cucumber, where there were no differences in rhizosphere community structure between the plant species, the plants were harvested 2–3 weeks after germination and also that the study by Buyer et al. (1999) was conducted with very young plants. On the other hand, the plants in the experiment with the three Californian soils as well as with the different types of P amendment where clear plant species-specific differences in the bacterial rhizosphere community structure were found were 7.5 and 4 weeks old, respectively. This indicates that plant effects may become pronounced during plant development and emphasizes the strong effect plant age may have on the microbial community structure in the rhizosphere. Indeed, Carelli et al. (2000) showed that plant-specific effects on *Sinorhizobium meliloti* populations become more important than soil effects as plants mature.

The experiment with the three plant species grown in three different soils showed that plant and soil specific effects interact in a complex way (Figure 4). In

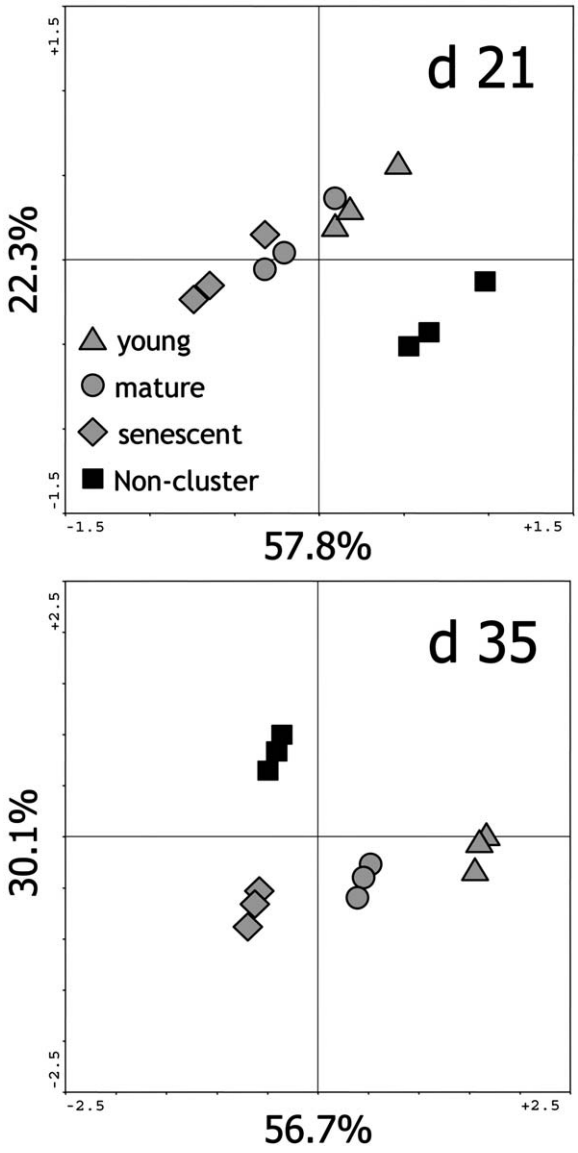

Figure 5. Ordination plot of rhizosphere bacterial communities of white lupin after 21 and 35 days in different root zones: non-cluster roots and young, mature or senescing cluster roots generated by canonical correspondence analysis of 16S rDNA DGGE profiles. For further information on the interpretation of the plots see Figure 1. From Marschner et al., 2002, reproduced with permission.

chickpea and canola the rhizosphere community structure of two soils were similar and differed from those of the third soil. However the groupings of the soils were different for the two plant species. Moreover, the rhizosphere community structure of Sudan grass was distinct in all three soils. The interactions between plant species and soil type are likely to involve the structure of the original soil microbial community as well as the nutritional status of the soil and the plant. In this context it should be noted that differences in management history between the soils could contribute to the observed differences between the soils.

While N fertilization had no effect on the community structure in the three California soils, both N and P fertilization strongly affected the rhizosphere community structure in the N or P deficient soils in the experiment with cucumber and barley (Figure 2). The stronger effect of fertilization in the German soils is probably due to their very low available N and P content, while the Californian soils were not N deficient. Alleviating N deficiency can increase the growth rate of bacteria (Christensen and Christensen, 1994) as well as the total number of bacteria (Liljeroth et al., 1990). In the two German soils the fertilization effect was at least partly plant-mediated because the bacterial rhizosphere community structure were also affected by foliar fertilization. This may be explained by the effect of the N and P status of the plant on root exudation (Hoffland et al., 1989; Liljeroth et al., 1990).

The type of P amendment had a strong effect on the rhizosphere community structure of canola and chickpea but the amendment effect was plant species-specific (Figure 3). In canola the rhizosphere community structure of the controls differed from the other two treatments while in chickpea the community structure of the rock P amended soil differed from those of the controls or the organic matter amendment. On the other hand, the rhizosphere community structure of Sudan grass was not affected by the form of P nutrition. Canola is considered to be a P efficient plant species that exudes organic acids in response to P deficiency (Hoffland et al., 1989). It also excretes phosphatases (Rumberger, personal communication) that mobilise organic P. This could result in increased P availability in the rhizosphere from rock P and organic matter. Chickpea also exudes organic acids in response to P deficiency (Ohwaki and Hirata, 1992) and it is interesting to note that the two plant species that are known to exude organic acids (canola and chickpea) differ clearly in community structure from Sudan grass in presence of rock P. Altogether, the results of these experiments provide strong support for the hypothesis that root exudate quantity and composition are key factors for determining the bacterial community structure in the rhizosphere.

The spatial variability in bacterial rhizosphere community structure was shown in the experiment with the three plant species grown in the California soils (Marschner et al., 2001a) as well as in the experi-

ment with white lupin (Figure 5). In both experiments, the rhizosphere community structure were root zone specific, which may be attributed to differences in the type of carbon that is available for microbial growth along the root axes. Root exudation varies along roots (Merbach et al., 1999) and some substances such as organic acids and phytosiderophores are released mainly behind the root tip (Hoffland et al., 1989; Römheld, 1991). Exudation by cluster roots has been studied extensively and was shown to vary with cluster root age as well as with plant age (Neumann et al., 1999, 2000). Under strong P deficiency, young cluster roots exude mainly malate while mature cluster roots exude mainly citrate. Senescent cluster roots have lower organic acid exudation rates but higher acid phosphatase activity than the younger cluster roots. The differences in root exudation are likely to be the main reason for the specific rhizosphere community structure of the cluster root age classes. The lack of differentiation between the cluster root age classes on day 21 may be explained by a low organic acid exudation of the plants that were not yet strongly P deficient (Neumann et al., 1999).

Cluster roots differ not only physiologically, but also morphologically from non-cluster roots. The differences in rhizosphere community structure between cluster and non-cluster roots that were apparent at both harvests may therefore, in part, be due to the distinct morphology of the cluster roots with the high density of laterals and root hairs, which provide a completely different habitat than the non-cluster roots.

The rhizosphere community is a subset of the soil bacterial community, but not all soil bacteria are rhizosphere colonisers, therefore only a subset of the soil community will colonise the roots. These are often eutrophic species, such as pseudomonads, which have a high nutrient demand and can grow rapidly when the availability of nutrients is high (Marilley and Aragno, 1999). In the older root zones, the available carbon is much more recalcitrant and fast growing microorganisms will be replaced by slower growing microorganisms that are highly competitive in crowded, nutrient limited environments. In this manner, the rhizosphere is highly dynamic with respect to nutrient availability and chemical properties, and must be considered as a collection of distinct habitats that vary both spatially and temporally.

Conclusions

The present studies emphasize that many different factors will contribute to shaping the species composition and numerical predominance of specific bacteria, but the plant itself exerts a highly selective effect that is at least as great as that of the soil. Differences in amount and composition of root exudates appear to be the key drivers for the differences in community structure, but this idea needs to be confirmed by simultaneous analysis of microbial community structure and in situ collection of root exudates to determine the extent to which different plant substances including signal molecules influence rhizosphere community structures. The distinct rhizosphere community structure in different root zones emphasizes that for future studies of rhizosphere microbial ecology it is important that samples are taken from defined root zones. Although the structure of rhizosphere bacterial communities is highly dynamic, all studies to date suggest that they are consistent and reproducible for individual plant species at a given age that are subjected to the same conditions. This observation provides an opportunity for use of bacterial signatures for diagnostic analysis and possible manipulation of the rhizosphere in relation to disease, nutritional status, and the promotion of beneficial plant microbial interactions. It should be noted that the relationship between microbial community structure and function is, as yet, often not clear and will need to be investigated.

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