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ORIGINAL PAPER

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Cereal/legume rotation effects on rhizosphere bacterial community structure in west african soils

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Abstract The increased use of cereal/legume crop rotation has been advocated as a strategy to increase cereal yields of subsistence farmers in West Africa, and is believed to promote changes in the rhizosphere that enhance early plant growth. In this study we investigated the microbial diversity of the rhizoplane from seedlings grown in two soils previously planted to cereal or legume from experimental plots in Gaya, Niger, and Kaboli, Togo. Soils from these legume rotation and continuous cereal plots were placed into containers and sown in a growth chamber with maize (*Zea mays* L.), millet (*Pennisetum glaucum* L.), sorghum (*Sorghum bicolor* L. Moench.), cowpea (*Vigna unguiculata* L.) or groundnut (*Arachis hypogaea* L.). At 7 and 14 days after sowing, 16S rDNA profiles of the eubacterial and ammonia-oxidizing communities from the rhizoplane and bulk soil were generated using denaturing gradient gel electrophoresis (DGGE). Community profiles were subjected to peak fitting analyses to quantify the DNA band position and intensities, after which these data were compared using correspondence and principal components analysis. The data showed that cropping system had a highly significant effect on community structure ($p < 0.005$), irrespective of plant species or sampling time. Continuous cereal-soil grown plants had highly similar rhizoplane communities across crop species and sites, whereas communities from the rotation soil showed greater variability and clustered with respect to plant species. Analyses of the ammonia-oxidizing communities provided no evidence of any effects of plant species or management history on ammonia oxidizers in soil from Kaboli, but there were large shifts with respect to this group of bacteria in soils from Gaya. The results of these analyses show that crop rotation can cause significant shifts in rhizosphere bacterial communities.

Keywords Crop rotation · DGGE · Plant nutrition · Rhizosphere · Soil microbiology

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Introduction

Cereal/legume rotations based on maize (*Zea mays* L.), pearl millet (*Pennisetum glaucum* L.), sorghum (*Sorghum bicolor* L. Moench.), cowpea *(Vigna unguiculata* L.) and groundnut (*Arachis hypogaea* L.) have been shown to allow significant, but site- and crop-specific increases in cereal yields within low input, subsistence-oriented agro-pastoral land use systems in the Sudano-Sahelian zone of West Africa (Bagayoko et al. 2000a, 2000b). Crop growth on acid sandy soils predominating in these systems is largely limited by the availability of phosphorus (Bationo et al. 1992). Research at multiple sites has suggested a complex interaction of chemical and biological factors, including increased mineral N, available P, elevated pH and arbuscular mycorrhizal infection, and a decrease in plant parasitic nematodes as causal mechanisms for rotation-induced increases in cereal yields (Alvey et al. 2000; Bagayoko et al. 2000a, 2000b). In principle, these chemical and biological changes should be accompanied by concomitant changes in the rhizosphere microflora. However, it is unknown how cropping systems affect the composition and structure of rhizosphere microbial communities. In subsistence agricultural systems, crop yields are directly dependent on the inherent soil fertility and on microbial processes that govern the mineralization and mobilization of nutrients required for plant growth. Furthermore, the impact of different crop species that

are used in various combinations is likely to be an important factor in determining the structure of plant-beneficial microbial communities that function in nutrient cycling, the production of plant growth hormones, and suppression of root diseases.

During the colonization of plant roots by soil bacteria, microorganisms from the bulk soil undergo selective enrichment in the plant rhizosphere in response to different root exudate components. Because different plant species release different types and quantities of exudates, plants exert species-specific effects on the soil microbial community that result in broad shifts in the microflora (Lynch 1990). Although not well investigated, it can be presumed that, as a sequence of plant species are grown on a soil, the predominant bacteria associated with the previous crop species will exert at least some temporary influence on the rhizosphere bacterial communities in the rhizosphere of the subsequent species, particularly during early growth. In practice, crop rotations have been explicitly used to disrupt disease cycles (Curl 1963), or in the case of legumes to fix atmospheric N_2 for the subsequent non-leguminous crop (Baldock et al.1981; Pierce and Rice 1988). When examined at the community level, crop rotations can cause changes in substrate utilization patterns, which suggest that soil bacterial communities under crop rotation have greater species diversity than under continuous cultivation with the same crop (Lupwayi et al. 1998). However, the consequences of such shifts in the bacterial community structure for nutrient uptake and plant growth remain unclear. New methods for community fingerprinting based on 16S rDNA profiles offer particular promise for monitoring such changes in the diversity of bacterial communities in the rhizosphere. In addition, it is also of interest to examine specific functional groups, such as ammonia-oxidizing bacteria, which can affect the chemistry and nutrient availability in the rhizosphere. The link between ammonia-oxidizing bacteria, soil pH, and N availability is of particular importance for plant nutrient uptake. The conversion of ammonia to nitrate is an acidifying process while the uptake of nitrate in excess of cation uptake by plant roots leads to an alkalinization of the rhizosphere (Armstrong and Helyar 1992; Gahoonia et al. 1992), which will influence the bioavailability of phosphorus (Lyndsay 1979), and affect the population size and activity of bacteria that produce plant growth hormones and antibiotics (Naseby and Lynch 1999). On the other hand, conversion of ammonia to nitrate allows N to be lost from the root zone by leaching or by denitrification. Thus, ammonia-oxidizing bacteria represent a key functional group that may significantly alter biological and nutritional parameters in the rhizosphere.

Using cultivation-based techniques, it has been estimated that more than 70% of the culturable bacteria isolated from the rhizosphere may be common to different plant species (Sturz et al. 1998). However, cultivation-based methods are very selective as only a small proportion of soil microorganisms can be cultured (Bakken 1985; Faegri et al. 1977; Torsvik et al. 1990). Therefore culture-independent methods such as fatty acid extraction (Ibekwe and Kennedy 1998; Cavigelli et al. 1995), T-RFLP (terminal-restriction fragment length polymorphism; Liu et al. 1997) and DGGE (denaturing gradient gel electrophoresis; Muyzer et al. 1993; Muyzer and Smalla 1998) are increasingly used for microbial community analysis. Previously, 16S rDNA profiles have been used to study the effect of the plant iron nutritional status on bacterial communities in different root zones (Yang and Crowley 2000) and plant species-soil interactions in soils with different textures (Marschner et al. 2000). Community analyses based on DGGE provide relatively low resolution of total species diversity (Muyzer et al. 1993; Ovreas and Torsvik 1998), but have been shown to be particularly useful for producing unique community fingerprints that can be used for the analysis of changes or shifts in populations of the predominant community members (Ferris et al. 1996; Massana et al. 1997; Ovreas et al. 1997; Ovreas and Torsvik 1998; Yang and Crowley 2000). Changes in the relative predominance of community members between identically treated samples also can be examined statistically by subjecting the DGGE profiles to image analysis followed by statistical analysis of the DNA band peak data using procedures such as factor analysis, correspondence analysis, and other multivariate statistical methods (Eichner et al. 1999; Yang and Crowley 2000).

In this experiment, we examined the eubacterial community structures of the rhizoplane of four crop seedlings, maize, millet, groundnut, and cowpea, which were grown under identical conditions in a growth chamber with soils from continuous cereal or legume/cereal rotation plots.

Materials and methods

Soil sampling

In December 1998 soil samples were taken from millet/cowpea field experiments at Gaya, Niger (11°59'N, 3°32'E) and maize/groundnut experiments at Kaboli, Togo (8°45'N, 1°35'E). Some basic soil properties are described in Table 1 (Buerkert et al. 2000). These field experiments contained one crop per year and were established in 1995 (Gaya) and 1996 (Kaboli) leading to a 3- and 4-year cropping history before sampling. Continuous cereal plots and plots from cereal/legume rotations that were last sown to legumes were sampled at either site by taking eight cores (5×15 cm) from each of two duplicate plots of 10×10 m for both cropping treatments. All 16 cores were mixed together into one aggregate sample. This aggregate sample was dried in the shade, sieved to 2 mm, mixed again, and subsequently placed in plastic bags for transport.

To standardize the soil moisture of the dry soil samples during storage and prior to use for soil analyses or plant growth experiments, the soils were incubated for 6 weeks in 1-l jars containing a vial of saturated Na_2HPO4 allowing equilibration to a soil water potential of 6.9 MPa.

Table 1[a] Mean annual precipitation and initial soil chemical parameters

Site	Precipitation[b] mm	Clay %	pH[c]	P-water µg kg^{-1}	P-Bray I mg kg^{-1}	CEC[d] cmol kg^{-1}	C org g kg^{-1}	Nmin mg kg^{-1}
Gaya	800	13	4.2	140	2.5	2.8	1.55	9
Kaboli	1,300	16	4.7	1,250	3.8	3.3	6.47	18

[a] Adapted from Buerkert et al. 2000
[b] Average annual rainfall over 5 to 10 years
[c] pH in 0.01 M KCl (1:2.5)
[d] Effective cation-exchange capacity

Growth chamber experiment

One hundred grams of each soil, Kaboli-rotation, Kaboli-continuous, Gaya-rotation, and Gaya-continuous was placed in acid-washed, 95-ml, pine-seedling tubes. Four tubes each were sown to millet, cowpea, and groundnut in Gaya soils and four tubes each to maize, cowpea and groundnut in Kaboli soils. Two seeds pregerminated for 48 h and exhibiting a root radicle were planted per tube. Seedling emergence was generally 1–2 days after sowing. Two tubes of each crop species were harvested at 7 days and again at 14 days after sowing. Plants were grown in a growth chamber with 65% relative humidity at 22°C. The light schedules and light intensity were 15/9 h of 370 μE m^{-2} s^{-1}. Root and soil samples were taken in duplicate of root tips (0.5 cm), of older root (0.5 cm), and of the bulk soil (1 g) from each tube. Roots were sampled using a sterile razor-blade and forceps. Roots were not washed and any soil adhering directly to the roots was included in the sample. Bulk soil was taken from the surface, middle and bottom of the tubes and mixed into an aggregate sample.

DNA extraction

Soil samples of 0.5 g or three root samples 0.5 cm in length, respectively, were placed in a bead-beater tube (BIO 101, Vista, Calif.) and shaken in a Fastprep FP120 bead beater (BIO 101, Vista, Calif.) at 5.5 m s^{-1} for 30 s. Total DNA from soil and water samples was isolated with the Fast-DNA-Kit (BIO 101, Vista, Calif.) as described by the manufacturer.

PCR primers and DGGE analysis

Primers PBRA 338f and PRUN518r were used for total bacterial community amplification (Ovreas et al. 1997). PBRA338f contained a GC clamp on the 5' end as described by Muyzer et al. (1993). A PCR protocol using "Ready-To-Go" PCR beads (Pharmacia Biotech, Piscataway, N.J.) with 20 ng template, and 5 pmol each primer added per tube. The PCR program was as follows: 1 cycle of denaturation at 94°C for 5 min, followed by a touch down PCR (94°C for 1 min, 53°C for 1 min, decreased by 1°C every cycle until 55°C, 72°C for 3 min), 20 cycles of 94°C for 1 min, 72°C for 3 min, and 1 cycle of 72°C for 10 min.

Ammonia-oxidizing community DNA was amplified using a nested PCR approach. Initially a 1.5-kb fragment was amplified using primers pA and pH (Edwards et al. 1989). PCR reactions employed Ready-To-Go PCR beads with 20 ng template, and 5 pmol each primer with the following PCR protocol: 1 cycle of denaturation at 94°C for 2 min, 30 cycles of 94°C for 30 s, 55°C for 60 s, plus 1 S/cycle at 72°C and one final extension at 72°C (Kowalchuck et al. 1997). After PCR, 20-μl samples were run on 1% low melt agarose gels and bands corresponding to 1.5 kb excised. Bands were placed in sterile Eppendorf tubes with 20 μl sterile deionized water. Eppendorf tubes were incubated at 65°C for 1 h and 5 μl solution were used for PCR with CTOf189-GC and CTO654r primers (Kowalchuk et al. 1997). Primer CTOf189 included a GC clamp as described by Muyzer et al. (1993).

DGGE gels were made from 8% (wt/vol) acrylamide stock solutions (acrylamide/bis acrylamide solution 37.5:1) containing 0% and 100% denaturant (7 M urea and 40% formamide, vol/vol). DGGE was performed with acrylamide gels containing a linear gradient ranging from 20% to 70% denaturant (7 M urea and 40% formamide, vol/vol) for bacterial DNA, and a gradient of 40–50% for ammonia-oxidizing community DNA. The gels were run for 4 h at 200 V with a DcodeTM Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, Calif.). Microbial community structure was analyzed for both bacterial and ammonia-oxidizing communities.

Statistical analysis

DGGE banding patterns of soil or root samples were analyzed by converting the banding patterns to densiometric graphs with peaks representing individual taxonomic units, which could then be differentiated by R_f values and peak area, to calculate the Shannon index or to perform statistical analyses, including correspondence analysis or principle component analysis, as described by Yang and Crowley (2000).

Results

Eubacterial community diversity

Gaya

In contrast to rhizoplane bacterial communities, bulk soil communities resulted in a smear of amplified sequences in the DGGE gel, and thus bulk soil samples from both sites were not analyzed further. Species richness (number of bands) for all rhizoplane samples as represented by the 16S rDNA banding profiles numbered 23 at 7 days and 30 at 14 days of crop growth. Seven-day-old rhizoplane samples from continuous soil grown plants contained 13–17 16S rDNA bands, whereas rhizoplane communities for plants grown in rotation soil contained 9–14 bands. At 14 days, the total number of unique bacterial groups or 16S rDNA bands from all samples increased, but there was an overall decrease in the average number of bacterial species per plant species, ranging from 9 to 11 DNA bands per plant species in continuous soil and 5 to 7 DNA bands per plant grown in rotation soil. Microbial species richness was greater in the continuous than in rotation soil. However, the bacterial community structures on plant roots from rotation soils were more diverse between samples of the same plant type and demonstrated distinct crop species effects on community structure. The

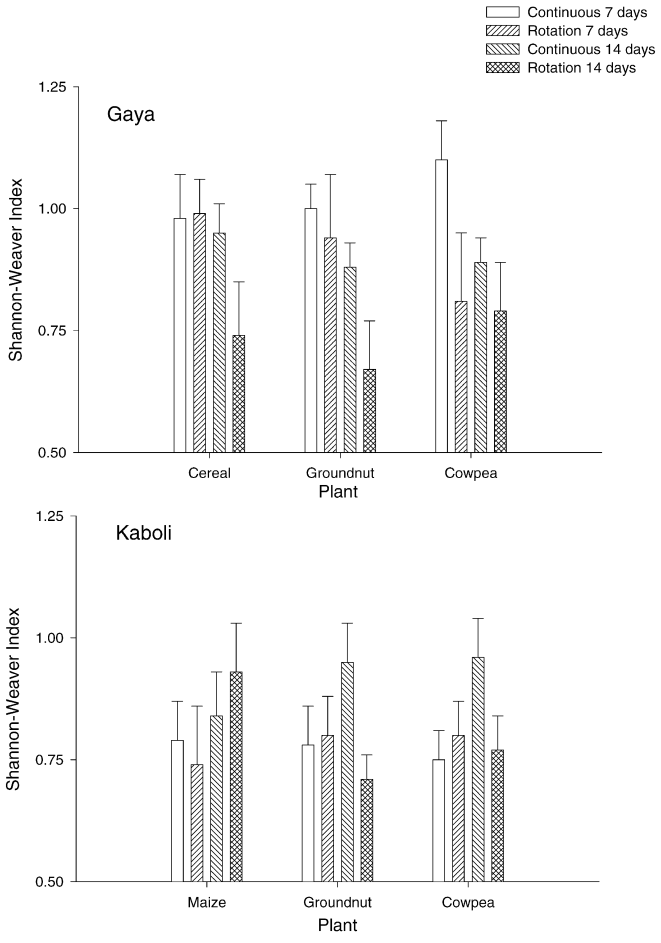


Fig. 1 Effect of crop rotation on the diversity of dominant bacterial communities as measured by the Shannon index

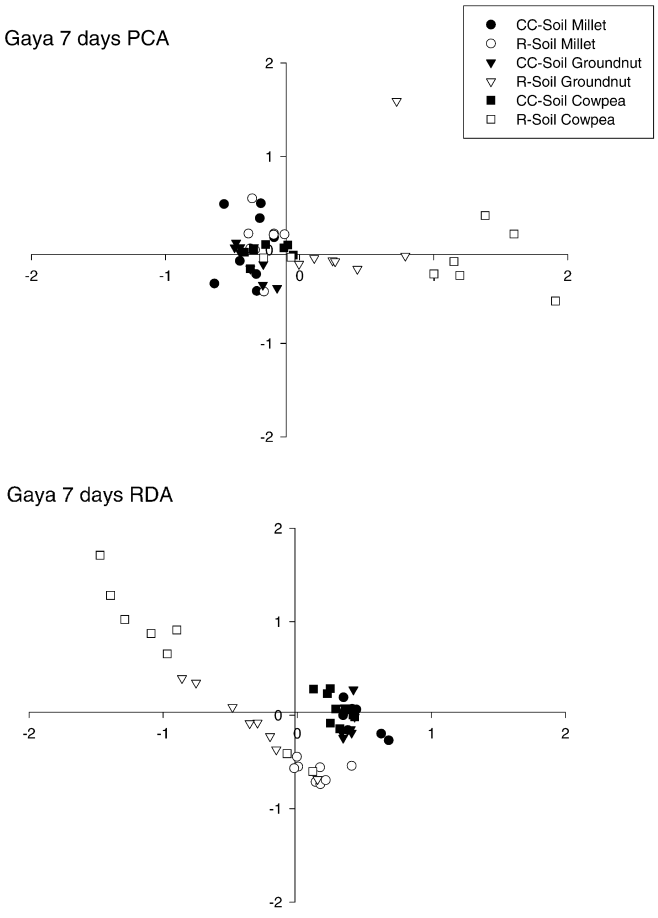


Fig. 2 Principle components analysis (PCA) and redundancy analysis (RDA) ordination diagrams of bacterial communities associated with the rhizoplane of 7-day-old millet, groundnut and cowpea plants as affected by crop rotation in Gaya soil (*R* rotation, *CC* continuous cereal)

Shannon index (H) for all crops ranged from 0.67 to 1.1. Diversity (H) values were larger at 7 days than at 14 days, with the most distinct difference being between the rhizoplane microbial communities of plants grown in continuous soil compared with the rotation soil grown crops, 0.88–0.95 and 0.67–0.79, respectively (Fig. 1).

Kaboli

The results of the analysis of microbial diversity in rhizoplane communities were similar to those at Gaya but less pronounced. Slightly higher total numbers of bacterial species were observed at 14 days than at 7 days after sowing (DAS). The total number of species as determined by R_f increased from 25 at 7 days of crop growth to 28 species at 14 DAS for Kaboli samples. At 7 days, individual rhizoplane communities contained 6–8 dominant bacterial species for both rotation soils and continuous soils. After 14 days, continuous soil grown crops had slightly higher diversity with 9–11 dominant species per sample, whereas the number of rhizoplane species from rotation crops ranged from 6 to 10 species per root sample. There was very little difference in the H value for rhizoplane communities in all crops from either rotation or continuous soils at 7 days. However, at 14 days there was a decrease in the H value for groundnut and cowpea in rotation soil versus continuous soil, whereas there was an increase in H for maize (Fig. 1). Effects of cropping systems on the H value were much larger than those of crop species.

Statistical analysis

Gaya

The analysis of DGGE banding patterns revealed significant cropping system effects on rhizoplane community structure and species composition. When the rhizoplane microbial communities were analyzed by principle components analysis (PCA) at 7 DAS, the *x* and *y* axis explained 47.3% of the variation in the data (Fig. 2). Most of the groundnut and cowpea samples grown in rotation soil clustered away from the continuous soil grown samples. The rhizoplane bacterial community of the

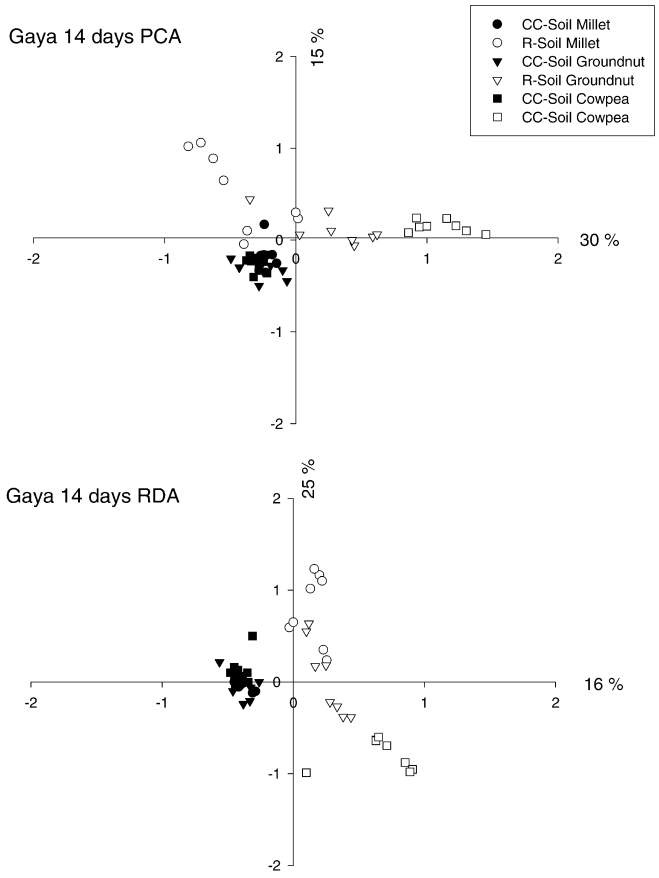


Fig. 3 PCA and RDA ordination diagrams of bacterial communities associated with the rhizoplane of 14-day-old millet, groundnut and cowpea plants as effected by crop rotation in Gaya soil (*R* rotation, *CC* continuous cereal)

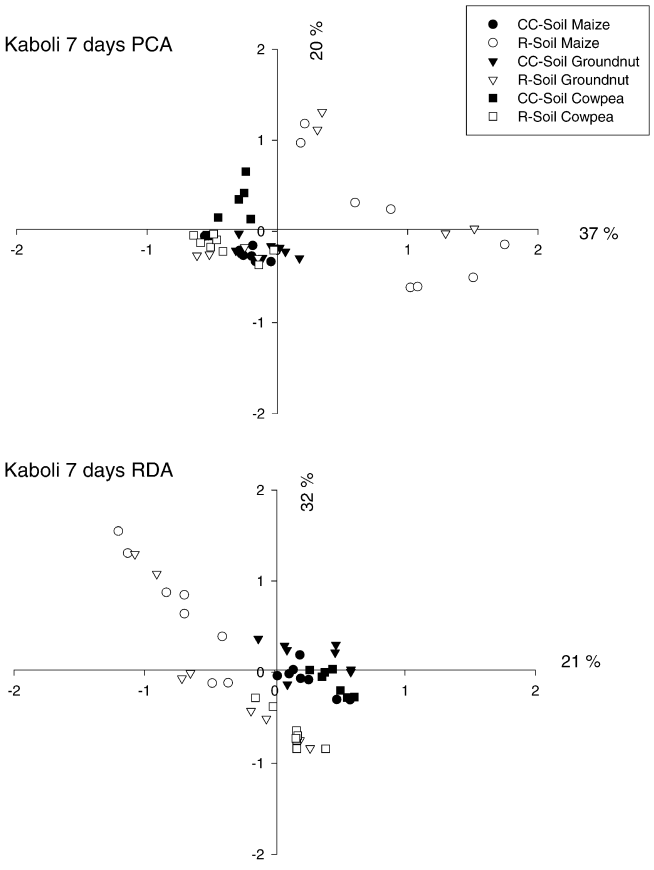


Fig. 4 PCA and RDA ordination diagrams of bacterial communities associated with the rhizoplane of 7-day-old sorghum, groundnut and cowpea plants as effected by crop rotation in Kaboli soil (*R* rotation, *CC* continuous cereal)

cowpea rotation soil showed the most unique assemblage of bacterial species for the crops tested in the Gaya soil at 7 DAS. Redundancy analysis (RDA), in which cropping system was included as an environmental variable (rotation or continuous cereal) explained a total of 41% (p =0.005) of the variation in the data (Fig. 2). Performing RDA with crop species as an additional variable did not improve the overall explained variation in community diversity but allowed the determination of the relative importance of the different crop species and cropping system by p value estimation. Of the three crops examined, cowpea had the most significant effect (p =0.01) on rhizoplane species distribution. Although the p values for plant species were relatively low, the RDA plot using the different crop species as variables was only able to explain 24% of the total variation in the species data (data not shown) and demonstrated the minor affect of crop species on bacterial community structure in our system.

Similarly, rhizoplane communities sampled at 14 days in the Gaya soil showed large differences between communities from continuous soil and rotation soil (Fig. 3). Overall differences were more pronounced at 14 than at 7 days. Again, cowpea had the largest selective influence on rhizoplane communities of the three crop species sown in the rotation soil from Gaya, although there were no significant changes in the bacterial communities attributable to crop species. The PCA scatterplot shows cowpea with the tightest cluster of samples located the farthest apart from the origin and from all other samples on the plot. Groundnut and millet samples from rotation soil had a small amount of overlap, but remained distinct from all rhizoplane samples from continuous soil (Fig. 3). Correspondence analysis including cropping system as an environmental variable explained 41% of the variation (Fig. 3). The rhizoplane communities of millet, groundnut and cowpea were more distinct at 14 DAP than at 7 DAP. The RDA analysis including crop species environmental variables yielded p values of 0.005 for cropping system, 0.02 for millet, 0.02 for cowpea, and 0.475 for groundnut. Again the type of crop had a smaller effect on the observed variation between samples than the cropping system. The RDA plot using all environmental variables was only able to explain 27.6% of the variation in the species data on the x and y axis combined. Additionally, there were no differences in the dominant microbial communities for root tips and older root zones.

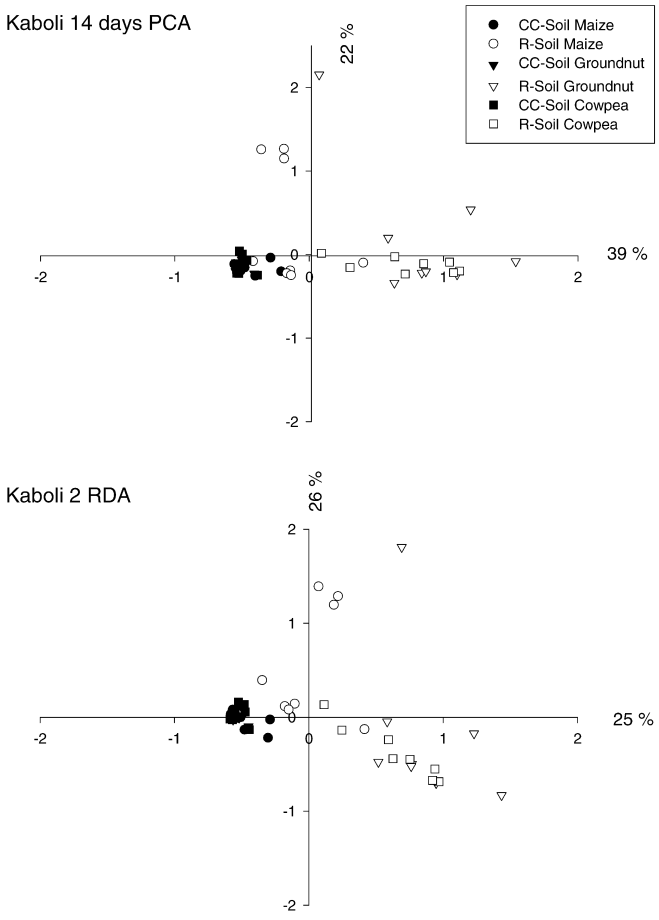


Fig. 5 PCA and RDA ordination diagrams of bacterial communities associated with the rhizoplane of 14-day-old millet, groundnut and cowpea plants as affected by crop rotation in Kaboli soil (*R* rotation, *CC* continuous cereal)

Kaboli

Rhizoplane communities analyzed by PCA in Kaboli soil at 7 DAS showed significant differences in bacterial communities formed for crops grown in continuous soil and rotation soil. There was less total variation in the data at 7 days than at 14 days (Figs. 4, 5). The PCA plot at 7 days was able to explain 55% of the variation in species distribution in all samples. The differences among crop species were not pronounced at 7 days (Fig. 4). Maize, cowpea and groundnut all had p values of 0.005 when added to the RDA model as environmental variables (data not shown). However, they explained little of the variation in community structure; RDA analysis including all crops species as environmental variables explained only 22% of the variation in the data set (data not shown). When cropping system was included as an environmental variable, a total of 37% of the variation was explained by the first two factors (Fig. 4).

PCA analysis of the rhizoplane communities at 14 DAS explained more variation in the data than at 7 days. The biplot explained 61.5% of the total variation in the species data (Fig. 5). Rhizoplane communities in rotation soil were more clearly distinct from continuous soil rhizoplane communities than they were at 7 days. When cropping system was included as an environmental variable in RDA, the analysis explained considerably more variation in species distribution than at 7 days; 29% of the total variation was due to cropping system with the y axis explaining an additional 25% of the variation in the data (Fig. 5). Crop species effects were smaller at 14 DAS, except for maize (p =0.005), than at 7 DAS. The p values of cowpea and groundnut declined to 0.1 and 0.06 respectively from earlier values of 0.005.

Ammonia-oxidizing bacteria

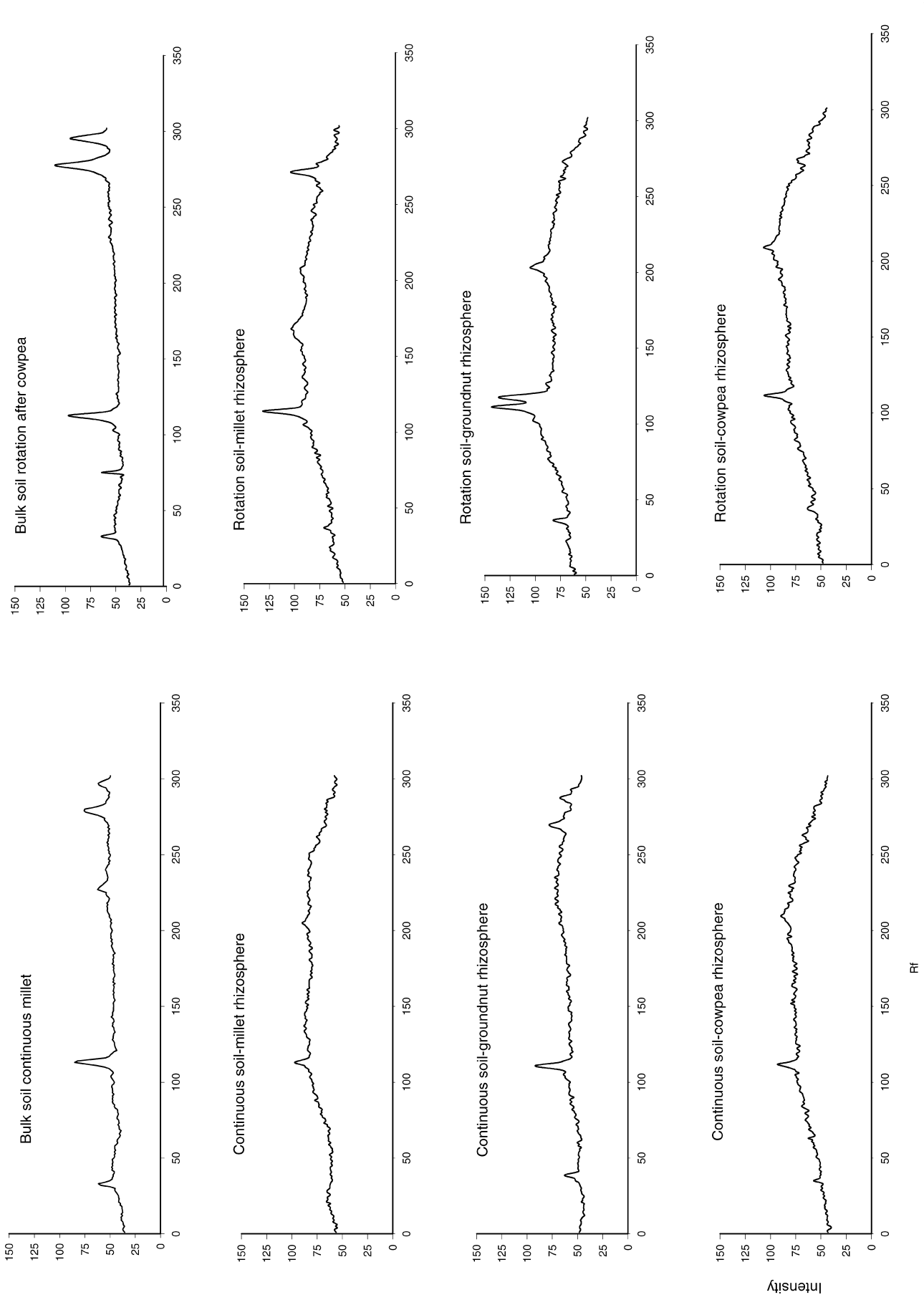
At Kaboli, for both time points, bulk soils and rhizoplane samples contained the same banding patterns, which varied only in intensity (data not shown). Kaboli rhizoplane communities generally had two peaks; one at R_f 110 and a second at R_f 270. This was true irrespective of cropping system and crop species used in the experiment. In contrast, at Gaya very distinct differences in ammonia-oxidizing communities were found to be due to crop species and cropping system. Bulk soil ammonia-oxidizing communities were generally more diverse than rhizoplane samples and gave more intense bands for rotation soil than continuous soil. Rotation soil had an additional peak at R_f 75 that was absent in continuous soil. Millet grown in continuous soil gave one small dominant ammonia-oxidizing band at R_f 110 but no others, whereas millet grown in rotation soil gave two unique bands, one at R_f 110 and another at R_f 270. Groundnut rhizoplane communities contained two peaks, one at R_f 40 and a second at R_f 110, however, groundnut in rotation soil contained four strong peaks at R_f 40, 110, 120, and 200. Cowpea showed no differences due to cropping system (Fig. 6).

Discussion

Analysis of microbial diversity using DGGE demonstrated that cropping history of the two soils was a major factor affecting the assemblage of microorganisms on the root surface at 7 and 14 DAS. While plant species was a less influential factor on the diversity of rhizoplane communities, it was shown to be more influential in rotation soil. In contrast to the total bacterial communities, very distinct plant effects were found on the ammonia-oxidizing communities in Gaya soil, but not in Kaboli soil.

DGGE analysis of the bulk soil at sowing and again at 7 and 14 DAS resulted in a smear of the amplified

Fig. 6 Denaturing gradient gel electrophoresis (DGGE) gel and line graph profiles of 16S rDNA band patterns resulting from DGGE of ammonia-oxidizing bacteria communities associated with the bulk soil and roots of millet, groundnut, and cowpea grown in Gaya soil


Bulk soil continuous millet
Bulk soil rotation after cowpea
Continuous soil-millet rhizosphere
Rotation soil-millet rhizosphere
Continuous soil-groundnut rhizosphere
Rotation soil-groundnut rhizosphere
Continuous soil-cowpea rhizosphere
Rotation soil-cowpea rhizosphere
Intensity
Rf

sequences in the gel. This indicates a highly complex microbial community with a fairly even distribution. Ovreas and Torsvik (1998) obtained a similar result with complex bulk soil communities from an organic soil and a sandy soil. The inability of a dominant bacterial population to exist in bulk soil from agricultural sites is likely due to the sub-optimal growth conditions with respect to easily degraded carbon sources, making the soil nonconducive for the growth of opportunistic organisms that would then be represented by a few dominant rDNA bands on the DGGE gels. However, when root samples along with adhering soil, representing the innermost soil of the rhizosphere and rhizoplane (as defined by Lynch 1990), were analyzed, distinct bands formed in the DGGE gels, which suggest the selective enrichment of a few species as compared to bulk soil. These bands thus represent a much simpler microbial community than the bulk soil. Mahafee and Kloepper (1997) found the bulk soil and rhizosphere of cucumber to be a continuum of microbial diversity, indicated by shifts in richness while still having some common membership. It is likely that the rhizoplane organisms comprise a subset of the diversity found in bulk soil, especially in subsistence systems where the seed stock comes from the same field.

The number of bacterial species represented by the number of 16S rDNA bands separated in the gels analyzed here was considerably lower than the 23–30 species reported from other studies (Mahafee and Kloepper 1997; Yang and Crowley 2000). The low diversity measured by DGGE analysis could be partially due to individual DGGE bands representing more than one species. Individual DGGE bands have been shown to be comprised of groups of bacterial species (Yang and Crowley 2000). On the other hand, one bacterium can sometimes produce more than a single band in a DGGE gel. Thus, even though the number of bands is a measure of diversity, it has a somewhat poor resolution at the species level. Higher resolution techniques perhaps involving DNA microarrays or other DNA separation methods such as terminal restriction fragment length polymorphisms may eventually provide more information on the true number of species in rhizosphere samples (Guschin et al. 1997; Liu et al. 1997). Nevertheless, DGGE is highly amenable to statistical analysis of the densiometric curves produced from individual gel lanes, which allows comparison of the relatedness of different samples (Eichner et al. 1999). This statistical approach using correspondence analysis and factor analysis has been used in other studies examining BIOLOG and fatty acid signature data to discern differences attributable to multiple variables (Bossio and Scow 1997; Di Giovanni et al. 1999; Grayston et al. 1998).

Results of the analyses conducted here clearly showed that cropping system had a stronger influence on the composition and intensity of the banding patterns than plant species. This is in contrast to other reports using measurements of metabolic diversity (BIOLOG) or culture-based methodology in which crop-specific effects dominated (Grayston et al. 1998; Merbach et al. 1999). At both measurement intervals and sites, rhizosphere communities from rotation-soil-grown plants were clearly different from those of plants grown in continuous soil (Figs. 3, 4, 5, 6). This is possibly due to the more vigorous crop growth in rotation than in continuous soil and thus to differences in environmental conditions near the roots affected by differential nutrient uptake and carbon exudation. In previous studies of crop rotation effects on West African soils it has been found that legume rotations can lead to pH increases in the bulk and rhizosphere soil (Alvey et al. 2000). It is thus possible that there is a legacy of the leguminous crop versus the cereal crop on the bulk soil community that serves as the inoculum for the root surface thereby creating a different initial soil diversity which leads to the observed differences in bacterial colonization of the root surface.

At both sampling times, 7 and 14 DAS, root microsite location had little influence on the diversity of the microbial communities generally providing nearly identical banding patterns independent of the sampled root portion. This could have been due to the fact that the plants were too young to allow for a succession process to occur which would lead to spatial differences in the microbial communities along the root. These data are in contrast to observations by Yang and Crowley (2000), in which distinct communities were observed at different microsite locations for barley plants subjected to iron deficiency.

The differentiation in the ammonia oxidizers of the two soils and the response of the bacterial community structure to cropping system and plant species was surprising. Ammonia-oxidizing communities were significantly affected both by cropping system and plant species in the soil from Gaya, but not from Kaboli. As ammonia-oxidizing communities affect the NO_3^-/NH_4^+ equilibrium in the soil solution, they may play an important role in the previously measured rotation-induced soil pH increases (Alvey et al. 2000). Plants, in their uptake of N as NH_4^+, generally acidify the rhizosphere, while NO_3^- uptake leads to alkalization. In the more acidic, poorly buffered Gaya soil, rotation-induced changes in the bacterial community composition were clearer than at Kaboli. Ammonia-oxidizing communities in Kaboli soil with its higher pH, higher CEC and organic carbon showed no changes with plant species or cropping system (Fig. 6).

Given the importance of increasing crop yields in subsistence farming systems characterized by a minimal use of mineral fertilizers on marginal soils, further research is required for the optimization of cultural practices that enhance beneficial root-soil interactions leading to higher crop yields. The ability to monitor changes in the rhizosphere microbial communities with respect to management strategies, soil types and crop species chosen offers promise for a better understanding of the causes of differences in crop yields. This research showed that legume rotations can lead to changes in the microbial community composition of a number of crops and that these shifts in community structure can be monitored by their 16S rDNA signatures. Higher resolu-

tion techniques will eventually allow functional analyses of these communities and determination of the possible relationship between phylogenetic structures and the ecological functions important in plant nutrition.

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