Plant and Soil **264:** 325–334, 2004.

Legume rotation effects on early growth and rhizosphere microbiology of sorghum in West African soils

P. Marschner[1], R.G. Joergensen[2], H.-P. Piepho[3] & A. Buerkert[4,5]
[1]*Soil and Land Systems, School of Earth and Environmental Sciences, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia.* [2]*Department of Soil Biology and Plant Nutrition, Nordbahnhofstr. 1a, University of Kassel, 37213 Witzenhausen, Germany.* [3]*Department of Bioinformatics, Institute of Plant Production and Grassland Science, University of Hohenheim, 70593 Stuttgart, Germany.* [4]*Institute of Crop Science, Steinstr. 19, University of Kassel, D-37213 Witzenhausen, Germany.* [5]*Corresponding author**

Received 5 April 2003. Accepted in revised form 26 January 2004

Key words: AM fungi, DGGE, nematodes, microbial biomass, microbial community structure

Abstract

Cereal yield increases in legume rotations on west African soils were the subject of much recent research aiming at the development of more productive cropping systems for the mainly subsistence-oriented agriculture in this region. However, little has been done to elucidate the possible contribution of soil microbiological factors to these rotation effects. Therefore a pot trial was conducted using legume rotation and continuous cereal soils each from one site in Burkina Faso and two sites in Togo where cropping system experiments had been conducted over 4 yrs. All soils were planted with seedlings of sorghum (*Sorghum bicolor* L. Moench). From 21 days after sowing onwards relative growth rates in rotation soils were higher than in the continuous cereal soils, resulting in between 69 and 500% higher shoot dry matter of rotation sorghum compared to sorghum growing in continuous cereal soils. Across sites rotation soils were characterized by higher pH, higher microbial N and a lower microbial biomass C/N ratio and, with the exception of one site, a higher fungal biomass in the rhizosphere. The bacterial and eukaryal community structure in the soil, assessed by denaturing gradient gel electrophoresis (DGGE), differed between sites. However, only at one site differed the bacterial and the eukaryal community structure in the rotation soil significantly from that in the continuous cereal soil. Although the results of this study confirmed the marked plant-growth differences between sub-Saharan legume-rotation soils and their continuous cereal counterparts they also showed the difficulties to differentiate possible microbiological causes from their effects.

Introduction

With scarce productive farmland facing increased pressure from a growing population, cereal-legume rotations have been repeatedly suggested as an effective means to increase productivity on sandy mineral-soils of sub Saharan west Africa. The dominant crops in this region are pearl millet (*Pennisetum glaucum* L.) in the drier northern Sahelian zone, being replaced by sorghum (*Sorghum bicolor* L. Moench) and then maize (*Zea mays* L.) towards the wetter southern Sudanian and Guinean zones. Under local farm management these cereals are irregularly interplanted with cowpea (*Vigna unguiculata* L.) and groundnut (*Arachis hypogaea* L.). Regular cereal-legume rotations have been shown to increase yields in cereals and recommended to local farmers, often without a clear understanding of the causes responsible for rotation effects on soil fertility. Recent field and laboratory studies have indicated that for some of the legume-rotations on West African soils involving groundnut, yield increases in subsequently planted sorghum or maize can be explained by a combination of (i) higher early soil nitrogen (N) released from decomposing legumes roots; (ii) higher availability and uptake of phosphorus (P) through increased pH and phosphatase

*FAX No: +49-5542-981230. E-mail: buerkert@uni-kassel.de

activity and enhanced early colonisation with arbuscular mycorrhizae (AM); and (iii) decreased root infestation with plant-parasitic nematodes (Alvey et al., 2001; Bagayoko et al., 2000).

Little research has been conducted on these soils to determine the degree to which changes in soil microbiological properties such as quantitative or qualitative shifts in bacterial and fungal populations may directly or indirectly contribute to the observed rotation-induced increases in cereal growth. It is well known that plant species differ in their microbial composition of the rhizosphere (Graystone et al., 1998; Marschner et al., 2001a). The observed rotation effects on west African soils may be due to a change in the structure of the rhizosphere microorganisms, which could result in a relative increase of microbial species with positive effects on plant growth. Such positive effects could include increased nutrient mobilisation, as in the case of phosphate-mobilising species or a decreased population density of deleterious species. The increased colonisation by AM fungi in rotation soils (Bagayoko et al., 2000) may be a key factor in such changes as it has been shown that AM colonisation can have a strong effect on the rhizosphere microflora (Marschner et al., 2001b; Posta et al., 1994).

The aim of this study was to examine the chemical and microbiological properties of three different West African soil during early growth of sorghum in order to assess the role of soil microbiology in the observed growth enhancing effects of legume-rotations on cereal dry matter.

Materials and methods

Soils and experimental conditions

In December 1998 soil samples were taken from a sorghum/groundnut experiment in the Sudanian zone of Burkina Faso on a Haplustalf at Kouaré (11°59′ N, 0°19′ E; 850 mm average annual rainfall) and in December 1999 from maize/groundnut experiments in the Guinean zone of Togo on an isohyperthermic Plinthic Kanhaplustult at Koukombo (10°17′ N, 0°23′ E; 1100 mm) and an isohyperthermic Plinthustalf at Kaboli (8°45′ N, 1°35′ E; 1300 mm). Clay content (%), cation exchange capacity ($cmol_c\ kg^{-1}$), base satuation (%) and organic C ($g\ kg^{-1}$) were 15, 2.8, 99 and 5.2 in the Kouaré soil; 5, 1.9, 97 and 3.7 at Koukombo and 16, 3.3, 71 and 6.5 at Kaboli, respectively (Buerkert et al., 2000). At each site a split-plot experiment had been conducted over the past 4 yrs with fertility treatments as mainplots and cropping systems as subplots (Bagayoko et al., 2001). The four subplots, which consisted of two replicates of each, a legume rotation (legume after cereal) and a continuous cereal (cereal after cereal) treatment, were sampled for the present study. Throughout the previous four years all selected plots had received 500 kg ha^{-1} of cereal crop residues, which were broadcast as surface mulch before the onset of the rainy season in March of each year. The soil was taken from 0–0.2 m depth and pooled for both replicates leading to two treatments (‘rotation soil’ and ‘continuous soil’ for each site), air-dried and sieved to 2 mm, packed in plastic bags and shipped to Germany where it was stored dry in the dark at about 15 °C. In spring 2001 each of the six soils (three sites by two cropping system histories) was filled into pots with 3 kg per pot and four replicates. All pots were covered with a thin plastic film into which holes were punched to allow for gas exchange, and incubated at 10% moisture (w/w) for 24 h.

Subsequently, five one-day-old pre-germinated seeds of sorghum (*Sorghum bicolor* (L.) Moench) were planted and thinned to three seedlings at five days after sowing (DAS). All pots were arranged randomly in a fully automatic growth chamber (Heraeus, Balingen, Germany) with the following day/night regime: 14/10 h, 30/25 °C and 40/60% relative humidity. The photon flux density during the day was about 480 μE m^{-2} s^{-1}. The pots were re-randomised and watered daily with deionized water.

Determination of plant and root properties, soil sampling and sample analysis

To monitor seedling development over the course of the experiment, a growth curve was established using a combination of destructive and non-destructive measurements. At 7 days after planting (DAS) three of the five seedlings were clipped at the ground after total shoot length had been measured to the nearest millimeter. Non-destructive length measurements of the remaining two seedlings per pot were taken at 8 and 10 DAS followed by a combined non-destructive and destructive sampling of one of the remaining seedlings at 13 DAS. Further non-destructive readings occurred at 21, 26, 27, 29, 33 and 35 DAS and the final harvest of the remaining seedling at 36 DAS. All destructively harvested plant material was dried to constant

weight at 65 °C and its dry matter (DM) weight was determined.

At time-points with only non-destructive measurements, seedling shoot dry matter (DM) was estimated from total shoot length (LENGTH) by a regression approach. Initial analysis of the data revealed a variance-mean relationship for DM, so data were log-transformed to stabilize the variances. The DM vs. LENGTH relationship changed during ontogenesis, as was confirmed by a bivariate plot of DM vs. LENGTH at the three different non-destructive measurement dates (results not shown). Thus, a response surface model (Dean et al., 1999) was fitted containing an interaction with DAS. The model was in the form:

$$\log(\mathrm{DM}) = \mathrm{intercept} + a_1^* \mathrm{LENGTH} + a_2^* \mathrm{LENGTH}^2 + b_1^* \mathrm{DAS} + b_2^* \mathrm{DAS}_2 + c^* \mathrm{LENGTH}^* \mathrm{DAS},$$

where a_1, a_2, b_1, b_2 and c are regression coefficients.

The data revealed no significant pot or autocorrelation effects after fitting the covariates (results not shown), therefore a model with independent errors was used. First the response surface model was fitted for all six site × cropping system combinations simultaneously to allow an initial selection of terms. Subsequently, the terms were tested in separate analyses of variance (ANOVA) for each site × cropping system combination. To guard against overfitting, F-tests of an ANOVA table were Bonferroni-adjusted to control the family-wise type I error.

The mean of LENGTH at dates with only non-destructive measurements was calculated and used in the estimated regression equation to obtain log(DM) estimates. Standard errors and 95% confidence intervals for the log(DM) estimates were computed using the delta method, also known as method of statistical differentials (Johnson and Kotz, 1969), assuming approximate normality of the estimates. Estimates and confidence limits were then back-transformed to the original scale. All computations were done using the GLM, MIXED and NLMIXED procedures in SAS (SAS, 1990). The joint analysis of variance revealed significant linear and quadratic effects in LENGTH and DAS, whereas the interaction term LENGTH*DAS was not significant after Bonferroni adjustment (Dean and Voss, 1999). The selected models were used to estimate DM values for all time points.

At final harvest total root length was estimated by the gridline-intersection method (Tennant, 1975) and shoot N was analyzed with a macro-N-analyzer (Heraeus, Bremen, Germany).

Chemical and microbiological characterization of the soils after 35d

The soil was divided into a 'bulk' and a 'rhizosphere' component whereby the rhizosphere soil was defined as the soil adhering to the roots after gentle shaking (Alvey et al., 2001). Soils were analyzed for pH (1:2 H_2O), water soluble P (P-water) and P-Bray1 by standard procedures. Colonization by AM fungi was assessed after staining by the gridline-intersection method and the number of plant-parasitic nematodes was determined for each planted pot according to Bagayoko et al. (2000).

Microbial biomass C and biomass N were estimated by fumigation-extraction (Brookes et al., 1985; Vance et al., 1987). One portion was fumigated for 24 h at 25 °C with ethanol-free chloroform. Following fumigant removal, the soil was extracted by horizontal shaking at 200 rev min^{-1} with 0.5 M K_2SO_4 for 30 min and filtered (Schleicher and Schuell 595 1/2). The non-fumigated portion was extracted similarly. Organic C in the extracts was measured as CO_2 by infrared absorption after combustion at 850 °C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany). Microbial biomass C was calculated as follows: Microbial biomass C = E_C/k_{EC}, where E_C = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and $k_{EC} = 0.45$ (Wu et al., 1990). Total N in the extracts was measured as NO_2^* by chemoluminescence detection after combustion at 850 °C using a Dimatoc 100/Dima-N automatic analyser. Microbial biomass N was calculated as follows: Microbial biomass N = E_N/k_{EN}, where E_N = (total N extracted from fumigated soils) – (total N extracted from non-fumigated soils) and $k_{EN} = 0.54$ (Brookes et al., 1985).

Ergosterol was extracted from 5 g moist soil with 100 mL ethanol for 30 min by oscillating shaking at 250 rev min^{-1} and filtered (Whatman GF/A) (Djajakirana et al., 1996). Quantitative determination was performed by reversed-phase HPLC analysis at 26 °C using a column of 125 × 4 mm Sphereclone 5 μ ODS II with a Phenomenex guard column (4 × 3 mm). The chromatography was performed isocratically with methanol (100%) and a detection resolution of 282 nm (Dionex UVD 170 S).

For DNA analysis all plastic ware and solutions were sterilized before use. Approximately 300 mg of soil were placed in screw cap tubes containing glass beads of different sizes (0.1 to 4 mm). Seven hundred and fifty μL extraction buffer (per liter: 4 mol

guanidine thiocyanate, 0.2 mol NaOacetate, 0.025 mol EDTA, 1 mol KOacetate and 25 g PVP) and 150 μL 10% Na-laurylsarcosyl were added before the samples were homogenized in a bead beater (Fast-Prep, Model FP120, Bio101, Vista, USA) at 5.5 m s^{-1} for 30 s. After centrifugation at 14000 RCF for 10 min, 300 μL of the supernatant were transferred into a fresh microcentrifuge tube. The samples were shaken for 10 min in presence of 150 μL ethanol, 300 μL NaI solution (per liter: 0.15 mol Na_2SO_3, 6 mol NaI) and 50 μL Binding Matrix (Bio101). The samples were centrifuged at 6000 RCF for 1 min and the supernatant discarded. The pellet was washed twice with a wash buffer (per liter: 10 mmol TrisHCl, 0.5 mmol EDTA, 5 mmol NaCl and 500 ml ethanol). After evaporating the remaining ethanol at 70 °C, the pellet was resuspended in 150 μL of ultrapure water and incubated at 70 °C for 4 min. The samples were centrifuged at 14000 RCF for 3 min and 100 μL of the supernatant containing the extracted DNA were transferred into a fresh tube. The DNA samples were stored at -20 °C for further analysis.

The microbial community structures in the soil were characterized by the denaturing gradient gel electrophoresis (DGGE). Bacterial 16S rDNA genes were amplified using the primer set F984 and R1378 (Heuer et al., 1997). Eucaryotic DNA were amplified using the primer pair NS1 (GTAGTCATATGCTTGTCTC) and NS2 (GGCTGCTGGCACCAGACTTGC) (White et al., 1990). A GC clamp was attached to the forward primer to prevent complete separation of the strands in the DGGE gel. For PCR, 5 μL of a tenfold dilution of the DNA extract were added to 20 μL of PCR reaction mix composed of 0.2 μL Taq polymerase (Qbiogene, Illkirch, France), 2 μL dNTPs (2 mM each) (Boehringer, Mannheim, Germany), 2.5 μL 10X PCR buffer (Qbiogene, Illkirch, France), 0.4 μL of each primer and 14.5 μL ultrapure water.

The bacterial DNA was amplified in a Trio Thermocycler (Biometra, Göttingen, Germany) with 35 cycles of 1 min denaturation at 94 °C, 1 min at 55 °C for primer annealing and 2 min at 72 °C for primer extension. In the first cycle the denaturation phase was extended to 5 min at 94 °C to prevent annealing of the primers to non-target DNA. The 35 cycles were followed by a final step of 10 min at 72 °C and cooling at 10 °C (Heuer et al., 1997). The eukaryotic DNA was amplified in the same thermocycler by the following program: 5 min at 94 °C, followed by 10 min at 80 °C and 35 cycles of 30 s at 94 °C, 45 s at 45 °C and 90 s at 72 °C followed by a final step of 10 min at 72 °C and cooling at 10 °C. Successful amplification was verified by electrophoresis in 1.8% (wt/vol) agarose gels with SYBR green I nucleic acid staining (FMC Bio Products, Rockland, USA). DGGE was performed with 8% (wt/vol) acrylamide gels containing a linear chemical gradient ranging from 35% to 55% (7 M urea and 40% (vol/vol) formamide). The gels were allowed to polymerize overnight. DNA samples containing 20 μL of the PCR products were subjected to electrophoresis in 1$\times$ TAE buffer at 60 °C and at a constant voltage of 150 V for 5 h (BIO-RAD Dcode systems; Biorad, Germany). After electrophoresis, the gels were stained for 30 min with a SyBR green I nucleic acid stain (FMC Bio Products, Rockland, USA; 10,000 fold diluted in 1$\times$ TAE) and photographed under UV light with a video imaging system. Band detection and quantification of band intensity was performed using Image Master (Pharmacia Biotech, New Jersey, USA). The PCR product of a standard bacterial mix consisting of a mixture of pure culture DNA extracts from *Escherichia coli, Pseudomonas fluorescens, Bacillus megaterium* and *Gordonia* sp. was added twice on each gel. Two bands of this bacterial mix were assigned the same Rf value in all gels. The Rf value of a given band therefore reflects its position with respect to these two bands of the standard bacterial mix. DNA band intensity was normalized by dividing the band intensity of each band by the mean band intensity of the gel. Therefore band position and intensity are expressed as relative values. Each band represents a species or a group of species having rDNA sequences with similar melting behavior. The band intensity indicates the relative abundance of the group under these PCR conditions.

Community structures based on relative band intensity and position were analyzed by performing a redundancy analysis with Monte Carlo permutation tests (CANOCO 4.0, Microcomputer Power, Ithaca, USA). The Monte Carlo tests were based on 1000 random permutations of the data. Community similarities were graphed by using ordination plots with scaling focused on inter-sample differences with species scores divided by the standard deviation (Jongman et al., 1995). Factors potentially affecting community structure such as soil type, cropping system (rotation or continuous), soil compartment (rhizosphere or bulk) and shoot dry weight were used as environmental data. The results were plotted using only the significant variables ($P \leq 0.05$). As the DGGE banding patterns of some samples were very weak, these were not included in the data analysis. The community

Table 1. Shoot N concentration, total root length, mycorrhizal colonisation, infestation with plant-parasitic nematodes and root ergosterol of 35 d old sorghum in soils from continuous and rotation systems at Kouaré, Koukombo, and Kaboli, West Africa; data are means of 4 replicates, CV = coefficient of variation

	Shoot N (mg g^{-1})	Root length (m)	Mycorrhizal colonisation (%)	Nematodes (100 g soil)$^{-1}$	Root ergosterol (mg g^{-1})
Treatment means					
Kouaré					
Continuous	17	16	60	220	0.7
Rotation	24	66	28	150	0.3
Koukombo					
Continuous	16	12	49	163	0.3
Rotation	18	20	53	55	0.1
Kaboli					
Continuous	21	7	52	106	1.5
Rotation	21	18	61	176	0.5
			Analysis of variance		
Source					
Site	0.003	<0.001	0.559	0.335	0.013
Rotation	0.009	0.028	0.520	0.394	0.018
Site × Rotation	0.046	0.007	0.164	0.204	0.320
CV (%)	13	30	45	69	90

structures of all three soils were first analyzed together to assess whether they were site-specific. To study the community structures more closely, the DGGE banding patterns of the three soils were then analyzed separately.

Results

Plant growth

Differences in shoot growth appeared after 21 DAS when sorghum in rotation soil, hereafter referred as to 'rotation sorghum', started to grow significantly faster than sorghum in pots filled with soil from 'continuous sorghum' plots (Figure 1). After 35 d shoot dry matter of rotation sorghum was 6-fold, 2.2-fold and 1.7-fold that of continuous sorghum in Kouaré, Koukombo and Kaboli soils, respectively. The shoot dry matter was highest in the Kouaré soil and lowest in the Koukombo soil. Shoot N concentration was consistently higher in plants of rotation soils than in those growing in continuous soils, particularly at Kouaré (Table 1). Shoot dry matter was closely correlated with root dry matter ($r = 0.96$, $P < 0.01$) and root length ($r = 0.89$, $P < 0.01$). Root length after 35 d was greater in Kouaré soil than in the two other soils. In all three soils, root growth was higher in the rotation soils than in the continuous soil (Table 1). Similarly to shoot growth effects, rotation increased root growth three-fold in Kouaré soil, but only two-fold in the other two soils. Rotation had only small and inconsistent effects on root colonisation by mycorrhizal fungi and nematodes. Nematode infestation was lower in rotation soils from Kouaré and Koukombo but higher in soil from Kaboli (Table 1). Fungi-derived root ergosterol was consistently greater in soils from continuous than in those from rotation sites, and it decreased in the order Kaboli > Kouaré > Koukombo (Table 1). There was no relationship between mycorrhizal colonization and root ergosterol.

Soil chemical properties

Soil pH was lower in the soil from Kaboli than in soils from the two other sites (Table 2). In all three soils, the pH of the rotation soil was higher than that of the continuous soil. Water extractable P was lowest in the Kaboli soil and, across sites, it was 2-77% higher in rotation than in continuous soils. With 11 μg P g^{-1} soil in the Kouaré soil and 7.0 μg P g^{-1} soil in the other two soils P-Bray concentrations were very low even

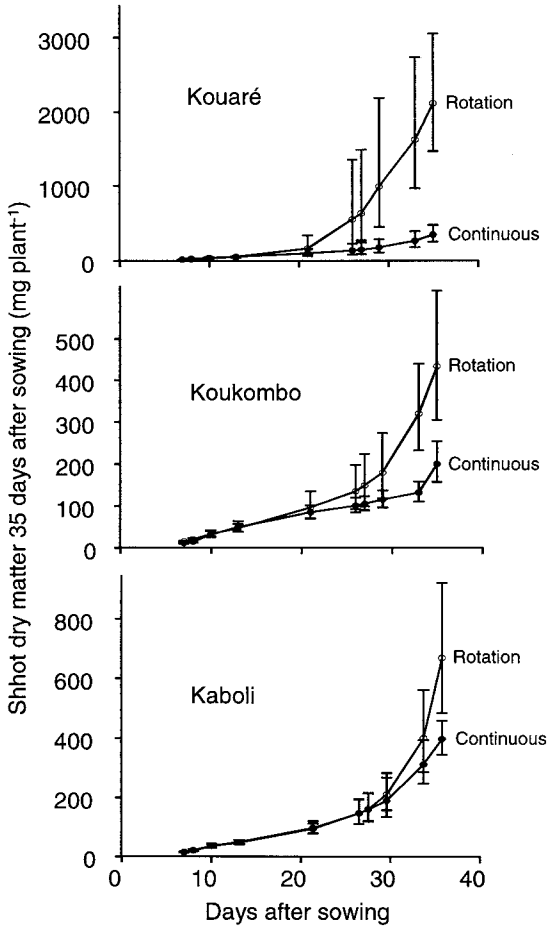

Figure 1. Shoot dry matter of sorghum in continuous cereal and legume rotation soil from Kouaré, Koukombo and Kaboli. Shoot dry matter was either determined directly or derived from shoot length by regression analysis. Data points are means of four measurements with vertical bars representing the upper and lower limits of confidence intervals.

in rotation soils. Compared to continuous soils, P-Bray increased with rotation at Kouaré and Kaboli, but this was barely the case in the soil from Koukombo. The increase in P-Bray was closely correlated with the increase in soil pH ($r = 0.79$, $P < 0.01$) and the increase in root length (0.81, $P < 0.01$). The content of extractable organic C was consistently greater in soils from rotation than in those from continuous cereal plots, particularly at the Kouaré site. It was similar in the soils from Kouaré and Kaboli, but significantly lower in those from Koukombo.

Soil microbial biomass indices

The ergosterol concentration in the rhizosphere soil was approximately 50% lower than bulk soil ergosterol and showed a considerably higher coefficient of variation between the replicates reflecting inconsistent site and rotation effects (Table 3). In the soils from Kouaré and Kaboli the rhizosphere ergosterol concentration was higher in the rotation soils than in the continuous soils. In contrast the bulk soil ergosterol concentrations were higher in soils from continuous plots than in those from rotation plots, except for Kaboli.

Microbial biomass C ranged from 49 to 104 μg g^{-1} soil without consistent rotation effects and was highest at the Kaboli site (Table 3). Microbial biomass N ranged from 4.0 to 19.2 μg g^{-1} soil and was higher in the rotation soils especially from the Kouaré site. A close, but still poorly understood linear relationship was observed between microbial biomass N and root length ($r = 0.79$, $P < 0.01$). The microbial biomass N level was lowest in soils from the Koukombo site. The C-to-N ratio of the microbial biomass decreased in the order Koukombo > Kaboli > Kouaré and was lower in the rotation than in the continuous soils. The bulk soil ergosterol-to-microbial biomass C ratio varied between 0.8 and 2.2% without significant rotation effects, but decreasing in the order Kouaré > Koukombo > Kaboli (Table 3).

Microbial community structure

When interpreting RDA plots a few important points should be considered. Each community is represented by a symbol. The distance between symbols (communities) is used as a measure of similarity in that symbols that lie close together have a similar community structure whereas a large distance between symbols indicates large differences in community structure. It should also be noted that the x axis corresponds to the first eigenvalue and explains a higher percentage of the total variance than the y axis. In the present study, approximately 76% of the total variance was explained by these two axes.

When the rhizosphere DGGE banding patterns of all three soils were analysed together the bacterial community structure differed between the three soils ($P = 0.08$) and between rotation and continuous soils ($P = 0.06$; Figure 2). Although Figure 2 suggests the same for the eukaryotic community, these differences were not significant ($P > 0.10$). The subsequent site-specific analysis revealed that the community structure of both bacteria and eukarya was significantly affected by rotation only in the Kaboli soil ($P < 0.05$).

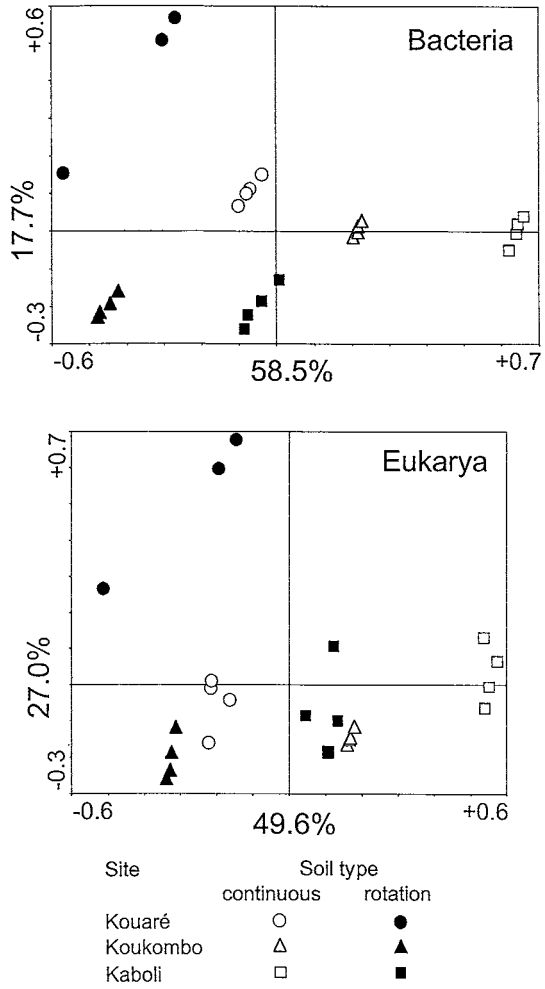

Figure 2. Redundancy analysis plots of the 16S and 18S rDNA DGGE profiles of the bacterial and the eukaryal community structures in the rhizosphere of sorghum in rotation and continuous soils from Kouaré, Koukombo and Kaboli in west Africa. Values on the axes indicate the percentage of total variation explained by the axes. Each community is represented by a symbol. The distance between symbols (communities) is used as a measure of similarity in that symbols that lie close together have a similar community structure whereas a large distance between symbols indicates large differences in community structure. It should also be noted that the *x* axis corresponds to the first eigenvalue and explains a higher percentage of the total variance than the *y* axis.

Discussion

The present study confirmed the positive effect of rotations on growth of sorghum in three different soils from West Africa. It revealed that rotation effects may be largely explained by changes in soil chemical properties such as pH and availability of N and P. Changes in microbiological properties also appear to play a role but their effect is strongly site-specific.

The magnitude and timing of rotation-induced increases in sorghum dry matter measured in this pot study (Figure 1) are very similar to those observed under field conditions (Bagayoko et al., 2000). A positive effect of rotation on shoot and root growth was observed in all three soils eventhough they differed markedly with respect to maximum DM. The larger confidence intervals in the rotation soils compared to the continuous cereal soils reflect the different TDM levels in the two cropping systems leading to similar coefficients of variation.

The highest shoot DM in the rotation soil was obtained in the soil from Kouaré, which also had the highest concentration of total N and available P. The Kaboli and Koukombo soils were both lower in available nutrients. In contrast to earlier studies (Alvey et al., 2001; Bagayoko et al., 2000), mycorrhizal colonization was not affected by rotation and nematode density was lower in rotation soils compared with continuous soils in only two of three soils (Table 1). The lack of a consistent effect on nematode density compared to the field results of Bagayoko et al. (2000) may be explained by the short duration of the experiments presented here and the prolonged storage of the soil after collection in West Africa which is also reflected in the relatively low nematode density determined.

There were several properties of the rotation soils that could explain the increased seedling growth in all three soils. Compared to the continuous soils, the pH of the rotation soils was higher. The pH increases may be due to a still unknown adaptation mechanism of groundnut to mineral soils poor in P or the effects of macromolecules such as pectinates (polygalacturonates) in the decomposing cell walls of previously growing legume tissue (Yan and Schubert, 2000). In these soils the increased pH would lead to increased P availability (as shown in the higher concentration of water soluble P).

The soil microbial biomass C concentrations (Table 3) are in the range of west African soils reported by Wick et al. (1998, 2002), but relatively low in comparison to other subtropical soils with higher organic C and pH (Wichern et al., 2003). This may also, in part, be due to the prolonged storage under dry conditions. Microbial biomass C-to-N ratios above 10 have rarely been observed and indicate nutrient deficiency or insufficient availability of nutrients, such as N or P (Joergensen and Castillo, 2001; Salamanca et al., 2002). The very low content of extractable organic C (Table 2) in combination with high microbial biomass C-to-N ratio at the Koukombo site suggests

Table 2. Soil pH in water, water extractable P, P-Bray, and 0.5 M K_2SO_4 extractable organic C in bulk soil from continuous and rotation sites at Kouaré, Koukombo and Kaboli, west Africa, 35 days after planting of sorghum seedlings; data are means of 4 replicates, CV = coefficient of variation

	pH (H_2O)	P-water	P-Bray	K_2SO_4 extractable C (μg g soil^{-1})
Treatment means				
Kouaré				
Continuous	6.9	1.2	6.6	51
Rotation	8.0	1.6	11.0	94
Koukombo				
Continuous	6.7	1.1	6.9	14
Rotation	6.8	1.9	7.1	26
Kaboli				
Continuous	5.9	0.5	5.6	62
Rotation	6.2	0.8	7.0	82
Analysis of variance				
Source				
Site	<0.001	0.016	<0.001	<0.001
Rotation	<0.001	0.040	<0.001	<0.001
Site × Rotation	<0.001	0.678	0.002	<0.001
CV (%)	2	50	14	12

Table 3. Ergosterol in rhizosphere soil, ergosterol, microbial biomass C and biomass N in bulk soil, the microbial biomass C-to-N ratio and the ergosterol-to-microbial biomass C ratio in bulk soil from continuous and rotation sites at Kouaré, Koukombo and Kaboli, west Africa, 35 days after planting of sorghum seedlings; data are means of 4 replicates, CV = coefficient of variation

	Rhizosphere soil Ergosterol (μg g^{-1} soil)	Bulk soil Ergosterol	Microbial biomass C (μg g^{-1} soil)	Microbial biomass N	Microbial biomass C/N	Ergosterol/microbial biomass C (%)
Treatment means						
Kouaré						
Continuous	0.22	1.04	49	7.9	6.2	2.2
Rotation	0.57	0.99	77	19.2	4.5	1.6
Koukombo						
Continuous	0.38	1.07	92	4.0	23.7	1.2
Rotation	0.35	0.81	67	5.5	12.6	1.2
Kaboli						
Continuous	0.44	0.78	104	8.6	12.0	0.8
Rotation	0.56	0.78	85	8.7	9.8	1.0
Analysis of variance						
Source						
Site	0.290	<0.001	0.051	0.002	<0.001	0.001
Rotation	0.051	<0.001	0.605	0.021	<0.001	0.428
Site × Rotation	0.120	<0.001	0.069	0.027	0.002	0.207
CV (%)	42	5	30	46	22	35

that the low soil organic matter may also be an important reason for the high biomass C-to-N ratios. The higher microbial biomass N in the rotation soils may be explained by the greater C input from the larger root biomass. An unusual and noteworthy result of the present experiment is the observation that none of the microbial biomass indices, that is biomass C, biomass N, and the fungal cell membrane component ergosterol were significantly related to each other.

In the bulk soil compartment the concentration of the fungal cell wall component ergosterol was higher in continuous cereal soils than in rotation soils (Table 3). This is in contrast to the rhizosphere compartment, where the ergosterol concentration tended to be higher in rotation soils. However, the relative importance of rhizosphere *versus* bulk soil microbial communities for plant growth is still poorly understood. The ergosterol-to-microbial biomass C ratio was relatively high, 2 to 3 times larger than in German arable soils (Djajakirana et al., 1996) and nearly 5 times larger than in subtropical soils of high fertility (Joergensen and Castillo, 2001; Salamanca et al., 2002).

Microbial communities have been found to be soil or site-specific (Gelsomino et al., 1999; Marschner et al., 2001a). The lack of clear effects in the present study (Figure 2) might be due to the storage duration of the soils, which may have decreased the microbial population or changed its relative composition by favouring species which can survive long-term starvation. On the other hand microbial communities may have developed adaptive mechanisms to cope with the effects of prolonged dessication given the sharp annual drying and wetting cycles characteristic for their natural ecosystems.

Rotation affected the bacterial and eukaryotic community structure in the soil from Kaboli but not in the other two soils. These findings suggest that only in Kaboli soil the positive plant growth response may be related to microbiological changes in the rhizosphere. However, the results of the present study do not allow to clearly differentiate between cause and effect. On the one hand, the changed microbial community structure may have resulted in an increased capacity for microbial P solubilization, increased production of phytohormones by soil microorganisms, higher associative N_2 fixation, increased biomass turnover or a decrease in pathogenic and deleterious microorganisms. On the other hand, the microbial community is strongly dependent on a soil's chemical and physical properties (Gelsomino et al., 1999; Marschner et al., 2001a). Thus, the observed changes in community structure may be the result of rotation-induced changes in these properties and not directly causing the positive growth response of the plants in the rotation soil.

Conclusions

The present study showed that the increased growth of sorghum in rotation soils as compared to continuous soil may be explained by a number of factors, some of which apply to all soils while others were site-specific. The rotation soils were characterized by a higher pH, higher microbial N and a lower microbial biomass C/N ratio and with the exception of Koukombo, a higher fungal biomass in the rhizosphere. In the soils from Kouaré and Kaboli, the rotation effect is likely to be caused by a higher N and P availability. Consistent effects of rotation on bacterial or eukaryotic community structure in the rhizosphere were not observed. Nevertheless, the data indicate that rotation effects may have a site-specific microbiological component. The significance of the observed changes in microbial community structure for plant growth remains to be investigated.

Acknowledgements

The authors would like to thank Karen Baumann, Claudia Thieme, Eva Wiegard and Gabi Dormann for technical assistance at sample analysis.

References

Alvey S, Bagayoko M, Neumann G and Buerkert A 2001 Cereal/legume rotations affect chemical properties and biological activities in two West African soils. Plant Soil 231, 45–54.

Bagayoko M, Buerkert A, Lung G, Bationo A and Römheld V 2000 Cereal/legume rotation effects on cereal growth in Sudano-Sahelian West Africa: Soil mineral nitrogen, mycorrhizae and nematodes. Plant Soil 218, 103–116.

Brookes P C, Landman A, Pruden G and Jenkinson D S 1985 Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842.

Buerkert A, Bationo A and Dossa K 2000 Mechanisms of residue mulch-induced cereal growth increases in West Africa. Soil Sci. Soc. Am. J. 64, 346–358.

Dean A and Voss D 1999 Design and analysis of experiments. Springer, Berlin.

Djajakirana G, Joergensen R G and Meyer B 1996 Ergosterol and microbial biomass relationship in soil. Biol. Fert. Soils 22, 299–304.

Gelsomino A, Keijzer-Wolters A C, Cacco G and van Elsas J D 1999 Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. J. Microbiol. Meth. 38, 1–15.

Graystone S J, Vaughan D and Jones D 1998 Functional biodiversity of micorbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and sitka spruce (*Picea sitchensis*). Tree Physiol. 16, 1031–1038.

Johnson N L and Kotz S 1969 Discrete distributions. Wiley, New York.

Joergensen R G and Castillo X 2001 Interrelationships between microbial and soil properties in young volcanic ash soils of Nicaragua. Soil Biol. Biochem. 33, 1581–1589.

Jongman R H, ter Braak C J F and Van Tongeren O F R (Eds.) 1995 Data analysis in community and landscape ecology. Cambridge University Press, Cambridge, UK.

Marschner P, Crowley D E, Yang C-H and Lieberei R 2001a Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol. Biochem. 33, 1437–1445.

Marschner P, Crowley D E and Lieberei R 2001b Arbuscular mycorrhizal infection changes the bacterial 16S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11, 297–302.

Posta K, Marschner H and Römheld V 1994 Manganese reduction in the rhizosphere of mycorrhizal and non-mycorrhizal maize. Mycorrhiza 5, 119–124.

Salamanca E, Raubuch M and Joergensen R G 2002 Relationships between soil microbial indices in secondary tropical forest soils. Appl. Soil Ecol. 21, 211–219.

SAS Institute 1990 SAS/STAT User's Guide, Version 6, 4th edition, Vol. 2. SAS Institute, Cary, NC.

Tennant D 1975 A test of a modified line intersection method of estimating root length. J. Ecol. 63, 995–1001.

White T J, Bruns S, Lee S and Taylor J 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR Protocols. A Guide to Methods and Applications. Eds. M A Innis, D H Gelfand, J J Sninsky and T J White. pp. 315–322. Academic Press, Inc., San Diego, CA.

Wichern F, Richter C and Joergensen R G 2003 Soil fertility breakdown in a subtropical South African vertisol site used as homegarden. Biol. Fertil. Soils 37, 288–294.

Wick B, Kühne R F and Vlek P L G 1998 Soil microbiological parameters as indicators of soil quality under improved fallow management systems in south-western Nigeria. Plant Soil 202, 97–107.

Wick B, Kühne R F, Vielhauer K and Vlek P L G 2002 Temporal variability of selected soil microbiological and biochemical indicators under different soil quality conditions in south-western Nigeria. Biol. Fertil. Soils 35, 155–167.

Wu J, Joergensen R G, Pommerening B, Chaussod R and Brookes P C 1990 Measurement of soil microbial biomass-C by fumigation-extraction – An automated procedure. Soil Biol. Biochem. 22, 1167–1169.

Vance E D, Brookes D C and Jenkinson D S 1987 An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Yan F and Schubert S 2000 Soil pH changes after application of plant shoot materials of faba bean and wheat. Plant Soil 220, 279–287.

Section editor: F.R. Minchin