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EFFECT OF CROP ROTATION ON THE DIVERSITY OF THE BACTERIAL COMMUNITY COLONIZING RICE STRAW RESIDUES IN PADDY RICE CULTURED SOIL IN THE MEKONG DELTA OF VIETNAM

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ABSTRACT

In this study, the influence of crop rotation on the microbial diversity was investigated over three crops. The studied crop rotation systems were rice-rice-rice (CRS1), rice-rice-baby corn (CRS2), rice-rice-mungbean (CRS3) and baby corn-rice-mungbean (CRS4).Litter bags containing rice stems were inserted into the soil and recollected at different time points for analysis, and they were used to compare with the diversity of the bacterial community colonizing the rice straw by means of 16S rRNA gene based Denaturing Gradient Gel Electrophoresis (DGGE), UPGMA analysis and Shannon index(H) comparison.The results showed that the bacterial diversity colonizing rice straw residues were significantly different in composition in the (CRS4) rotation system compared to those in the 3 other systems.There were not significant differences in bacterial Shannon index during cultivation of crop I. In crop II and crop III, the CRS4 rotation system showed a H index higher than this of recorded in the CRS1, CRS2 and CRS3 treatments.The average H over the whole experiment was highest for the CRS4 system (H=1.13) and significantly different compared to those for the other 2 systems CRS2(H=1.03) and CRS3(H=1.05), indicating that crop rotation with two upland crops was a vital key to the improvement of the Shannon index.

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1 INTRODUCTION

Bacteria are the most abundant and diverse group of microorganisms in soil (Gans *et al*., 2005). Agricultural management practices that have been shown to affect microbial community structure, including bacterial diversity composition, include crop rotation, crop monocultivation and addition of inorganic and organic nutrients (Ferris *et al.,* 2004;Saison *et al.,* 2006; Carrera *et al.,* 2007;Govaerts *et al.,* 2007). Although several studies have reported on the effect of agricultural practice type on the bacterial community structure in paddy rice field (Henckel *et al.,* 1999;Lueders and Friedrich, 2000) with some studies focusing on the community associated with decomposition of crop residues (Weber *et al.,* 2001; Akasaka *et al.,* 2003), none of those studies looked at the effect of crop rotation systems that include the intermittent cultivation of upland crops. In this study, the effect of implying a long-term crop rotation approach that included the cultivation of at least one upland crop, on the bacterial diversity as catalysts of crop residues, in rice paddy soils in the Mekong Delta of Vietnam was investigated. We examined whether the intermittent cultivation of an upland crop in a

paddy rice cultivation schedule is associated with important changes in the structure of the bacterial community colonizing rice straw residues in the soil compared to systems which undergo continuous paddy rice cultivation. Therefore, a field experiment was set-up in Cai Lay district in the Mekong Delta where differences in soil fertility are being studied between experimental plots undergoing either rice mono-cultivation or rice rotated with different upland crops. Litter bags containing rice straw residues were buried in the soil and the bacterial community colonizing the rice straw residues were periodically analyzed and compared. Bacterial community analysis was performed by Denaturing Gradient Gel Electrophoresis fingerprinting (DGGE) of bacterial 16S rRNA gene sequences amplified by targeted PCR. The experiment included full cycles of crop rotation. Within cycle, three crops were cultivated on the same plot. The rice straw residue was inserted during seeding of the first crop.

2 METHODOLOGY

2.1 Field experiment design and sampling approach

The experimental field used in this study is located in Cai Lay district, Tien Giang province. The soil at the field site was classified as Eutric Gleysol (WRB 2006). The principal characteristics of the soil are listed in Table 1.

Table 1: Chemical and physical properties of the soil at the Cai Lay field site

Depth (cm)	pH_{H2O} (1:2.5)	P total (%P_2O_5)	N total (%)	OM (%C)	CEC cmol(+)/kg	Sand (%)	Silt (%)	Clay (%)	Texture
0-15	5.1	0.14	0.24	2.57	22.3	2.4	30.9	66.7	Clay
15-25	6.1	0.06	0.11	1.40	26.6	1.4	32.5	66.1	Clay

The field was designed as a completely randomized block of experimental plots undergoing four different crop rotation systems (CRS) with three replicate plots per system. The four applied rotation systems were (1) CRS1: rice (Crop I) - rice (Crop II) - rice (Crop III), (2) CRS2: rice (Crop I) - rice (Crop II) - baby corn (Crop III), (3) CRS3: rice (Crop I) - rice (Crop II) - mungbean (Crop III) and (4) CRS4: baby corn (Crop I) - rice (Crop II) - mungbean (Crop III). Litter bags (nylon material with a pore size of 200 ηm) were filled with 5 g of dried rice straw residues and buried, prior to seeding of the first crop, into the soil at a depth of around 10 cm. Before inserting, the bags with rice straw were sterilized at 121°C for 20 minutes. The litter bags were periodically recovered from the soil for 16S rRNA gene based DGGE analysis of the bacterial community colonizing rice straw according to the time schedule shown in Figure 1. At each time point, three litter bags were recovered from each plot.

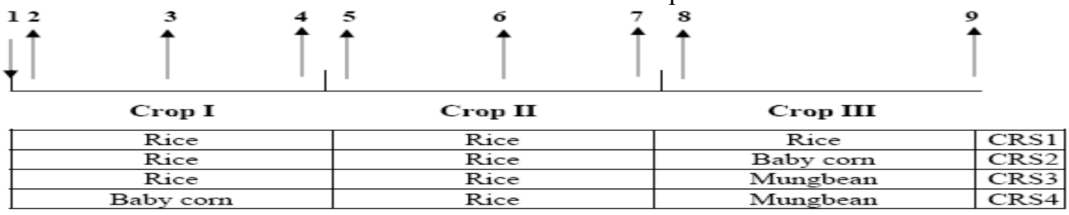


Fig. 1: Time schedule showing the recovery of litter bags from the Cai Lay field experiment

The arrow indicated with 1 refers to litter bags inserted before the start of the experiment. Arrows indicated with 2, 5 and 8 refer to sampling of litter bags after 14 days of cultivation of crops I, II and III, respectively. Arrows indicated with 3 and 6 refer to sampling of litter bags after 50 days of cultivation of crops I and II, respectively. Arrows indicated with 4, 7 and 9 refer to sampling of litter bags at harvest of crops I, II and III, respectively.

2.2 Total DNA extraction, PCR amplification and DGGE analysis

Total community DNA was extracted from the incubated rice straw residues by a method modified from Boon *et al.* (2000).

PCR amplification and DGGE analysis

For PCR amplification of bacterial 16S rRNA gene fragments, primer pair F984-GC and R1378 was used. The primers used in this study are shown in Table 2.

DGGE of bacterial 16S rRNA gene fragments was performed on an Ingeny phor U-2 system (Leiden, The Netherlands). Electrophoresis was performed for 15 h at 60°C and 120 V. DGGE gels were stained for 30 min with 1xSYBR Gold (Molecular Probes, Leiden, The Netherlands) and photographed on a UV transilluminator with a GeneLink camera system (SYNGENE, Cambridge, UK).

Table 2: PCR primers used in this study

Primer	16S rDNA target Sequence (5'–3')	Reference
F984-GC* Bacteria	GC.-AACGCGAAGAACCTTAC	Nubel et al., (1996)
R1378 Bacteria	CGGTGTGTACAAGGCCCGGGAACG	Nubel et al., (1996)

**contained GC clamp: CGC-CCG-GGG-CGC-GCC-CCG-GGC-GGG-GCG-GGG-GCA-CGG-GGGG at the 5' site*

2.3 Data analysis

Gelcompar II version 4.602 (Applied Math's, Sint-Martens-Latem, Belgium) software was used to construct dendrograms of the bacterial 16S rRNA gene DGGE fingerprints by the Pearson's correlation unweighted-pair group method using arithmetic averages (UPGMA). The diversity of the microbial community was described by the Shannon index of general diversity H (Shannon and Weaver, 1963), using the densitometric curves of the DGGE profiles according to the formula:

$$H = - \sum (n_i/N) \log (n_i/N).$$

Where n_i is the height of the peak and N the sum of all peak heights in the densitometric curve (Boon *et al*., 2002).One-way ANOVA analysis was performed with SPSS for Windows release 13.0 (SPSS for Windows, Version 13.0, USA) to compare statistically the Shannon index of different treatments. Differences between values at $P< 0.05$ were considered significantly different.

3 RESULTS AND DISCUSSION

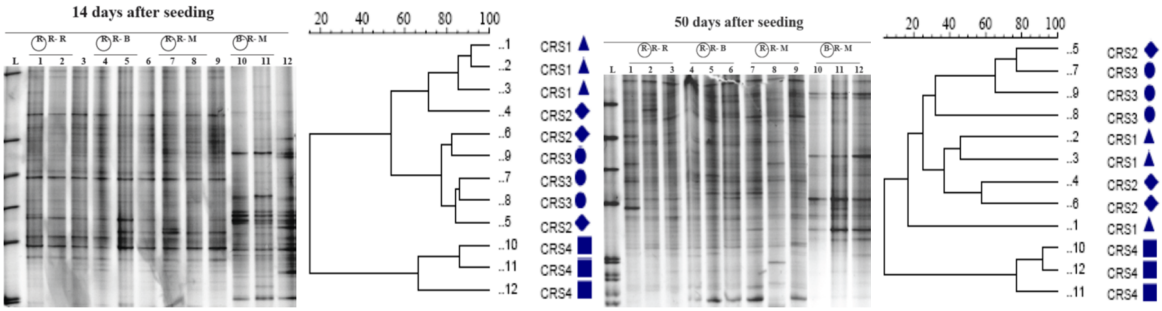
3.1 Results

3.1.1 Dynamics of the bacterial community colonizing rice straw residues in soil undergoing various crop rotation systems during three crops

The different DGGE banding patterns were examined in two ways, i.e., UPGMA cluster analysisand Shannon diversity index (H) analysis. UPGMA were done per sampling time. Figure 3A, 3B and 3C show the bacterial 16S rRNA gene DGGE profiles obtained for the different crop rotation systems during growth of crop I, II and III, respectively and the corresponding UPGMA results. Examining the DGGE profiles and the dendrograms resulting from the UPGMA cluster analysis, the major conclusions could be made:

Clear differences were found at particular sampling times between plots undergoing different treatments. The clearest difference was observed between plots undergoing crop rotation system CRS4 (baby corn- rice- mungbean) on the one hand and the three other systems on the other hand. This was particularly evident during the cultivation of crop I and to a lesser extent during cultivation of crop II. Crop I was paddy rice for all systems except for CRS4 where it was baby corn. During cultivation of crop I, profiles from replicate plots of treatment CRS4 taken at a particular time point, were highly similar with a similarity level among three replicates of 66.4, 77.1 and 57.0% for samples taken at day 14, at day 50 and at harvest, respectively. In contrast, the replicates of CRS4 were only 3.2-15% similar to profiles of CRS1, CRS2 and CRS3 at all sampling points(Figure 3A). In addition, at day 14, the replicates of CRS1, CRS2 and CRS3 clustered as separate subgroups. During cultivation of crop II, which was paddy rice in all 4 systems, the profiles of CRS4 formed a separate cluster while profiles originating from CRS1, CRS2 and CRS3 clustered together in the UPGMA analysis for samples taken at day 14 and day 50 (Figure 3B). Profiles from CRS4 were only 10.4% and 38.5% similar to profiles originating from the other 3 rotation systems at day 14 and day 50, respectively

A



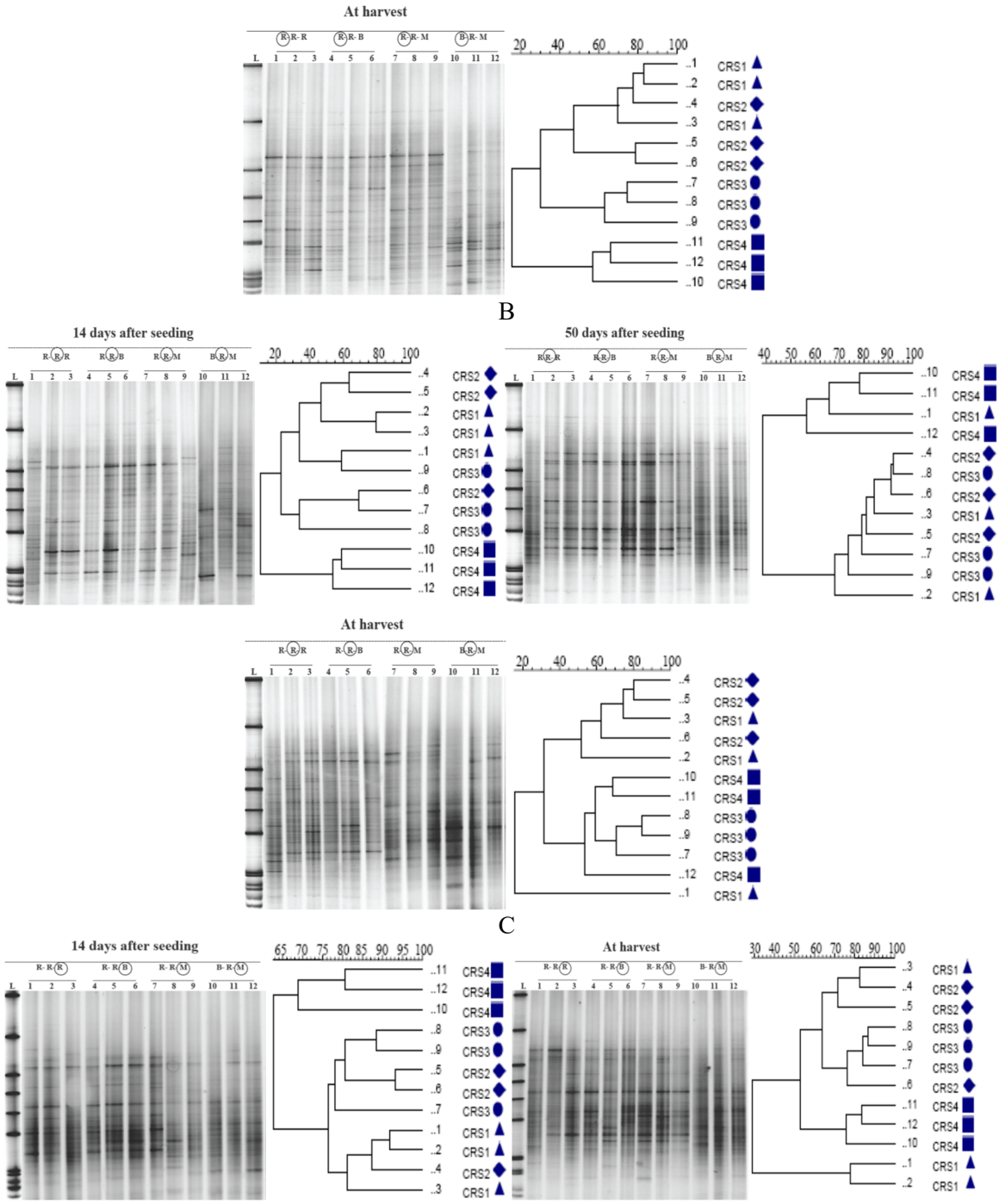


Fig. 3: Bacterial 16S rRNA gene DGGE fingerprints of the bacterial community colonizing rice straw residues and results of the corresponding UPGMA cluster analysis in three replicate field plots undergoing the three crop rotation systems during cultivation of crops I (A), II (B) and III (C) at 14 days of crop cultivation, at 50 days of crop cultivation and at crop harvest. In the DGGE fingerprints, the profiles are marked as follows: CRS1 (R-R-R, rice-rice-rice); CRS2 (R-R-B, rice-rice-baby corn); CRS3 (R-R-M, rice-rice-mungbean) and CRS4 (B-R-M, baby corn-rice-mungbean) with each lane representing a replicate plot. The actual crop (R for rice, M for mungbean and B for baby-corn) under cultivation on the moment of sampling is surrounded. The profile in lane L corresponds to the 16S rRNA gene DGGE ladder. In the UPGMA plots, the symbols used correspond with a particular rotation system as follows:(▲), CRS1; (◆), CRS2; (●), CRS3 and (■), CRS4. The number mentioned before/together with the indicated system corresponds to the lane number in the DGGE fingerprint gel

On the other hand, samples taken at harvest of crop II showed a different clustering (Figure 3B). Two main clusters could be recognized: one group consisting of profiles from CRS1 and CRS2 and a second group consisting of profiles from CRS3 and CRS4. The similarity between the two groups was 31.2%. From the start of crop III, the CRS4 community showed a profile more similar to those of samples obtained from the other rotation systems (Figure 3C).

During cultivation of crop III, profiles from samples taken at 14 days of cultivation, were classified in two main groups, i.e., one group containing CRS4 profiles and one group containing the profiles from CRS1, CRS2 and CRS3 (Figure 3C).

3.1.2 The Shannon diversity index (H)

The Shannon diversity index (H) was used to compare the bacterial diversity between the different samples based on the DGGE data and to determine whether crop rotation affected bacterial diversity. Averages of H calculated for all profiles obtained during cultivation of either crop I, II or crop III and corresponding standard deviations are listed in Table 3.

Table 3: Average and standard deviation of the Shannon diversity index H calculated from the bacterial 16S rRNA gene profiles for the different crop rotation systems during three crops

system	CROP*			Year+
	I	II	III	
CRS1	1.11±0.02*[a]	1.04±0.05*[ab]	1.03±0.07*[a]	1.07±0.02+[ab]
CRS2	1.11±0.09[a]	0.93±0.06[a]	1.04±0.04[a]	1.03±0.04[a]
CRS3	1.07±0.11[a]	1.05±0.02[ab]	1.04±0.02[a]	1.05±0.04[a]
CRS4	1.07±0.06[a]	1.13±0.10[b]	1.20±0.08[b]	1.13±0.04[b]

**: average H ± standard deviation of H values obtained for all samples taken during cultivation of the indicated crop (I, II or III) (3 sampling times for each crop with three replications per sampling time point).*

+: average H ± standard deviation of H values obtained for all samples of the indicated rotation system (CRS1, CRS2, CRS3 and CRS4) (8 sampling times for each system and 3 replicates per sampling time point)

[a] :Values with different letters in the same column indicate significant different values (p<0.05; with Duncan test)

Overall, H ranged from 0.93 to 1.13. For the samples taken during cultivation of crop I, H ranged from 1.07 to 1.11 but no significant differences could be noted between the different crop rotation systems. However, for the samples taken during cultivation of crop II and crop III, H was highest for the CRS4 samples. Moreover, the difference in H between crop III samples from rotation system CRS4 and crop III samples taken from the 3 other systems was significant. In addition, average H over the whole cycle 1 was highest for the CRS4 system and significantly different compared to the overall average H calculated for the other 3 systems.

3.2 Discussion

3.2.1 Bacterial community composition dynamics in continuous mono-culture paddy rice system versus paddy rice-upland crop rotation systems during three crops

During three crops, we found that depending on the time point, in differently treated systems, the rice straws were colonized by bacterial diversity with different structures. These differences became apparent in the UPGMA analysis. Large differences were especially found between the community associated with system CRS4 on the one hand and the communities associated with systems CRS1, CRS2 and CRS3 on the other hand. These differences were eminent for all samples taken during the cultivation of crop I and for some samples taken during cultivation of crop II. In addition, differences could also be observed between samples taking from CRS1, CRS2 and CRS3 but only for those samples taken at 14 days of cultivation of crop I. Those results indicated that the crop rotation system one way or another affects the bacterial community colonizing and eventually also degrading the inserted rice straw residues.

The first crop cultivated in rotation systems CRS1, CRS2 and CRS3 were in all cases the same, i.e., paddy rice, and hence the incubation conditions were in all cases identical. Despite this, the structure of the bacterial community differed between the 3 systems for samples taken at 14 days of cultivation. Prior to the rice crop, different crops had been grown on the 3 experimental plots, i.e., rice in case of CRS1, and the upland crops baby corn in case of CRS2 and mungbean in case of CRS3. It indicates that the short term community evolution was affected by the conditions of the soil defined by either the previous crop or by the overall rotation system. For instance, in CRS3, the previous cultivated crop was mungbean. Mungbean is a leg-

ume. Legume-based crop rotation is an effective and often profitable way of supplying N and improving soil properties. By altering the pools and fluxes of C and other chemical elements in the soil, organic and inorganic fertilizers can affect the chemical and microbial properties of soils (Mengel, 1985). On the other hand, the cultivation practice during growth of crop I in CRS4 was completely different from that applied in CRS1/CRS2/CRS3. In the CRS4 system, crop I is the upland crop baby corn while paddy rice was grown as crop I in the CRS1, CRS2 and CRS3 systems. Hence, the conditions in the soil undergoing the CRS4 system were completely different from those in the soil undergoing the other 3 rotation systems. During rice cultivation the plots are saturated with water, while this is not the case during growth of baby corn. It implies that conditions in the soil undergoing the CRS4 system during growth of crop I were mainly aerobic while in the soils undergoing the CRS1, CRS2 and CRS3 systems, mainly anoxic conditions might have prevailed. Drenovsky *et al.* (2004) suggested that soil water content is a major determinant of microbial community composition while Sylvia *et al.* (2005) showed tremendous effect of flooding on microbial community composition. Henckel *et al.* (2001) examined the methanotrophic oxidizing bacteria (MOB) community in deeper rice paddy soil layers after drainage and found that the (MOB) community rapidly changes in structure and colonized deeper soil layers, indicating that soil conditions favorable for MOB including oxygen provision in deeper layers can be rapid after drainage. Differences between the structure of the bacterial community residing on rice residues in drained and flooded paddy soil have also been reported previously (Kimura and Asakawa, 2006;Asakawa and Kimura, 2008; Asari *et al.,* 2008).On the other hand, Kikuchi *et al.* (2007) reported only small differences in bulk soil bacterial community composition in the plow layer of flooded and drainage/upland conditions. Furthermore, prior to the cultivation of baby corn as the first crop, the soil in the CRS4 system had been cultivated with another upland crop, i.e., the legume mungbean, which can have also affected soil characteristics and soil composition and hence community composition as suggested above for explaining the small differences between the CRS1, CRS2 and CRS3 bacterial community composition. Alternatively, we cannot exclude that the actually cultivated crop itself largely affected community structure. The effect of plant species on bacterial community and communities of specific bacterial genera has been documented (Garbeva *et al.,* 2004; Mazzola, 2004).Christensen (1989) suggested that the composition of the plant community can influence the diversity of the soil bacterial community due to the variability in chemical composition of plant exudates. Crop species is a crucial factor for the supply of energy and carbon to the heterotrophic microbial community by producing root exudates (Miethling *et al.,* 2000; Smalla *et al*., 2001).Previously, Lu *et al.* (2002) reported that microbial biomass dynamics in paddy rice soil is largely controlled by organic substances released from rice roots. Finally, the overall rotation system, i.e., all those factors together, might have affected soil conditions including soil fertility, chemical residues, and soil organic matter and hence community composition (Lakin, 2008).

Interestingly, at two sampling time points during growth of crop II, the diversity of the bacterial community in plots undergoing rotation system CRS4 were still significantly different from those taken from plots undergoing rotation systems CRS1, CRS2 and CRS3 despite that in all systems rice was the cultivated crop. This might indicate again that indeed the overall crop rotation system affected the bacterial community colonizing the rice straw. On the other hand, since, prior to the seeding of crop II, the community structure in the plots undergoing the CRS4 system was already different from those in plots undergoing the other 3 systems, this might have affected the further evolution of the community structure during growth of crop II. Moreover, at the end of crop I, due to the different community compositions during growth of crop I, degradation of the straw residues in systems CRS4 might be in a different stage and composition than during degradation of residues in systems CRS1, CRS2 and CRS3. The resulting different substrate composition can have affected community composition during growth of crop II.

During growth of crop III, the communities colonizing the rice straw residues were not significantly different anymore between the different rotation systems, despite that in all 4 systems, a different crop was cultivated. This result might suggest that at least on this step of residues decomposition, the actually cultivated crop or the type of soil management going along with the cultivation of that crop does not affect community structure anymore. During growth of crop III, the straw residues were in all cases already in a far state of decomposition and were colonized by complete different community compared to those proliferating during growth of crop I and crop II as shown by the community composition dynamics. The populations colonizing heavily degraded organic material might be less influenced by the crop rotation system. Apparently,

only communities degrading fresh to medium degraded organic material were affected by the system.

3.2.2 Change in the Shannon diversity index (H)

The Shannon index H applied to DGGE profiles has been used previously in microbial ecology to describe microbial diversity. In case of DGGE, H is based both on the number of DGGE bands and their relative intensities. This would in theory reflect on diversity without the need for cultivation (Eichner *et al.,* 1999). Agricultural management practices have previously been shown to have an impact on soil microbial diversity (Feng *et al.,* 2002). In the present study, the diversity of the bacterial community colonizing the rice straw residues during cultivation of crop I were however not significantly different between the different rotation systems despite the observed differences in community composition. This illustrates the fact that measures of diversity do not necessarily provide information about the composition of the microbial community and hence not about the functionality. For instance, Zak *et al.* (1994) reported that the diversity index of the bacterial community at two sites was the same although the two communities showed a different substrate utilization profile. Within the profile of the bacteria, the variance of H was very low (Boon *et al.,* 2002). On the other hand, during cultivation of crop II, the CRS4 rotation system showed a H higher than this of recorded in the CRS1, CRS2 and CRS3 treatments, indicating that crop rotation with two upland crops was a vital key to the improvement of the Shannon index. Both factors, plant species and land use history, have shown previously clear effects on microbial community diversity (Garbeva *et al.,* 2008). In addition, during the cultivation of crop III, baby corn in CRS2, mungbean in CRS3 and CRS4 was grown in raised bed system with the aerobic condition while rice in CRS1 was cultivated in plain field with anaerobic condition. The of total eubacterial community diversity index (H) of raised bed system was higher compared to plain field (Mittal *et al.,* 2008).

4 CONCLUSIONS

In conclusion, our findings revealed that the rotation of paddy rice cultivation in combination with the cultivation of upland crops affected the diversity of the bacterial community colonizing and degrading rice straw residues buried in the soil on a long-term base. Those differences in community structure seem to be explained by the history of the soil (previous cultivation practice), the time during which the rice straw residues were buried and especially the present environmental conditions including soil water content, plant species and management practices associated with the cultivation of that plant species. As suggested by others, especially soil water content seems to determine rice straw resident bacterial community.

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