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THE EFFECT OF CROP ROTATION ON THE STRUCTURE OF THE FUNGAL COMMUNITY COLONISING RICE STRAW RESIDUES IN PADDY RICE CULTURED SOIL IN THE MEKONG DELTA OF VIETNAM

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ABSTRACT

In this study, we compared fungal population between the rice monoculture and the polyculture on fungal population. 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). A fungal primer pair (ITS1–ITS2) was used to amplify internal transcribed spacer (ITS) sequences from total DNA extracts from rice straw residues. Amplified DNA was analyzed by DGGE. Individual bands from DGGE gels were cloned and sequenced. DGGE gels representing the fungal communities of the continuous rice and the rotation site were compared and related to fungal identified from the field.

The results demonstrate that the fungal communities of rice straw residues varied greatly due to the crop rotation. Fungal communities colonizing the rice monoculture were found significantly different from alternating crop of mungbean and baby corn. Cloning and sequencing of bands from the DGGE gels revealed the presence of Ascomycota and Basidiomycota that are common in agricultural soils. It was shown that the colonizing rice straw residues of crop rotation seemed to select the phylum Ascomycetes, while different members of the phylum Ascomycota and Basidiomycota were detected in the rice straw residues of the rice monoculture.

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1 INTRODUCTION

The role of fungi in soil is extremely complex but consider to be fundamental for the soil ecosystem. Most importantly, fungi play an essential role in decomposition of organic residues, nutrient cycling, plant health and development (Bridge *et al*., 2001). Fungi are important for the initiation of bioconversion of lignocellulose residues. Aerobic filamentous fungi such as *Trichoderma reesei* and *Aspergillus niger* produce extracellular cellulolytic enzymes like endoglucanases, cellobiohydrolases (exoglucanases) and beta-glucosidases.

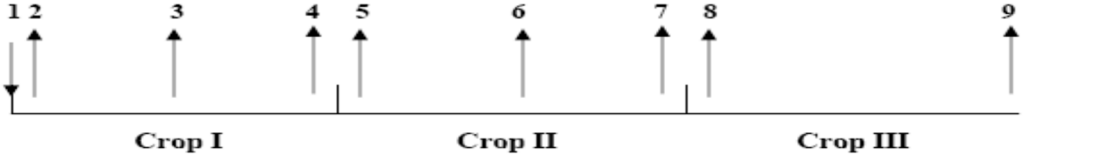
Previous studies have shown that fungi are also present in paddy rice soils (Tonouchi, 2009) and that they as such may also contribute to organic matter decomposition in these ecosystems. However, despite the important role of this group of micro-organisms in degradation of organic matter and nutrient cycling, none of them examined how the fungal community evolves in rotation systems where paddy rice cultivation is alternated by culti-

vation of upland crops versus a paddy rice monoculturing system. In this study, we used DNA samples from the same field set-up to examine whether an alternative practice as crop rotation with upland crops is associated with important changes in the structure of the fungal community colonizing rice straw residues in the soil compared to systems which undergo continuous paddy rice cultivation. The fungal community was studied by Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the fungal Internal Transcribed Spacer (ITS) marker amplified by PCR and sequence analysis of amplified ITS fragments from selected samples.

2 METHODS

2.1 Set-up of field experiment and sampling approach

The experimental field used in this study is located in Cai Lay district, Tien Giang province. The field was designed as a completely randomized block of experimental plots undergoing 4 different crop rotation systems (CRS) with 3 replicate plots per system. Each plot covered an experimental area of 90 m^2 (6 by 15m). The 4 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 fungal community colonizing rice straw according to the time schedule shown in Figure 1. At each time point, 3 litter bags were recovered from each plot.



Crop I	Crop II	Crop III	
Rice	Rice	Rice	CRS1
Rice	Rice	Baby corn	CRS2
Rice	Rice	Mungbean	CRS3
Baby corn	Rice	Mungbean	CRS4

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 PCR and DGGE analysis

Fungal ITS1 regions were obtained by a nested PCR approach. In a first PCR, the ITS1-5S rRNA gene-ITS2 ribosomal DNA region of fungi is amplified using primers ITS1F-GC (Gardes and Bruns, 1993) and ITS4 (White *et al*., 1990) as described by Anderson *et al*. (2003). The product of this reaction was diluted (1/50) and used as template in a second PCR with primer set ITS1F-GC/ITS2 (Gardes and Bruns, 1993; Anderson *et al*., 2003) to generate the product for DGGE analysis that covers the ITS1 region. The ITS1 region is more variable than the ITS2 region. Moreover, primer set ITS1F-GC/ITS2 appears to be more selective, producing less non-fungal sequences (Melo *et al*., 2011). Primer sequences are shown in Table 1. Each PCR reaction mixture contained 1.25 U Taq polymerase (Qiagen), 25 pmol of each primer, 10 nmol of each dNTP (promega), 0.1% BSA (Bovine Serum Albumin) and 1xPCR buffer (Qiagen). PCR reactions were conducted in a Mastercycler apparatus from Eppendorf; Hamburg, Germany. DGGE was performed on an Ingeny phor U-2 system (Leiden, The Netherlands) using gels prepared according to Muyzer *et al*. (1993). 30 µL of the PCR product was loaded onto 6% (w/v) polyacrylamide gel with a denaturing gradient ranging from 20% at the top to 60% at the bottom of the gel in Tris–acetate–EDTA (TAE) buffer. Electrophoresis was performed for 17 h at 60°C and 100 V. After electrophoresis, 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 1: PCR primers used in this study

Primer	ITS	rDNA target Sequence (5'–3')	Reference
ITS1F-GC*	Fungi	CTTGGTCATTTAGAGGAAGTAA	Gardes and Bruns (1993)
ITS2	Fungi	GCT GCG TTC TTC ATC GAT GC	White et al. (1990)
ITS4	Fungi	TCC TCC GCT TTA TTG ATA GC	White et al. (1990)

**ITS1F had the GC clamp CGC CCG CCG CGC CCC GCG CCC GGC CCG CCC sequence.*

2.3 Cloning and sequencing of fungal ITS1 fragments

PCR products were cloned into plasmid vector pCR®2.1-TOPO®, using the TOPO TA Cloning kit (Invitrogen, Merelbeke, Belgium) as described by the manufacturer. To check transformants for the incorporation of the fungal ITS1 gene, a nested PCR was performed consisting of a first PCR with M13f and M13r primers as described by Invitrogen followed by a second amplification step with fungal primers ITS1F-GC and ITS2. DGGE fingerprinting of the amplified fragments were compared with the fingerprints of the fungal corresponding community and appropriate clones were chosen for sequence analysis. The PCR products obtained from the clones were purified with a PCR purification kit (Promega) as described by Promega and subjected to sequencing reactions. Sequencing reactions were performed with the QuickStart DNA sequencing kit (Beckman) and analysed on an automatic sequencer (CEQTM8000, Beckman Coulter, Fullerton, CA, USA). Resulting partial ITS1 gene sequences (about 300 bp) were compared with sequences deposited in GenBank by performing a BLASTN search (Altschul *et al*., 1997).

2.4 Data analysis

Gelcompar II version 4.602 (Applied Math's, Sint-Martens-Latem, Belgium) software was used to construct dendrograms of the fungal ITS 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, 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. 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 Analysis of fungal diversity using UPGMA clustering

None of the samples resulted into a detectable PCR product in the first PCR with primers ITS1F-GC and ITS4. In addition, some samples did not show a PCR product with the nested PCR approach using primers ITS1F-GC and ITS2 in the second PCR. At 14 days of cultivation of crop I, PCR products after the second PCR were only obtained with samples taken from CRS4. In the other cases, for each rotation system, maximum two of the samples show that a detectable PCR product that could be analysed by DGGE.

Figure 2 shows the fungal ITS1 DGGE profiles obtained for the different crop rotation systems during growth of crop I, II and III, respectively, as well as the corresponding UPGMA cluster. Since DGGE profiles were obtained for only two samples and since often a high variability was obtained between two samples of the same rotation system.

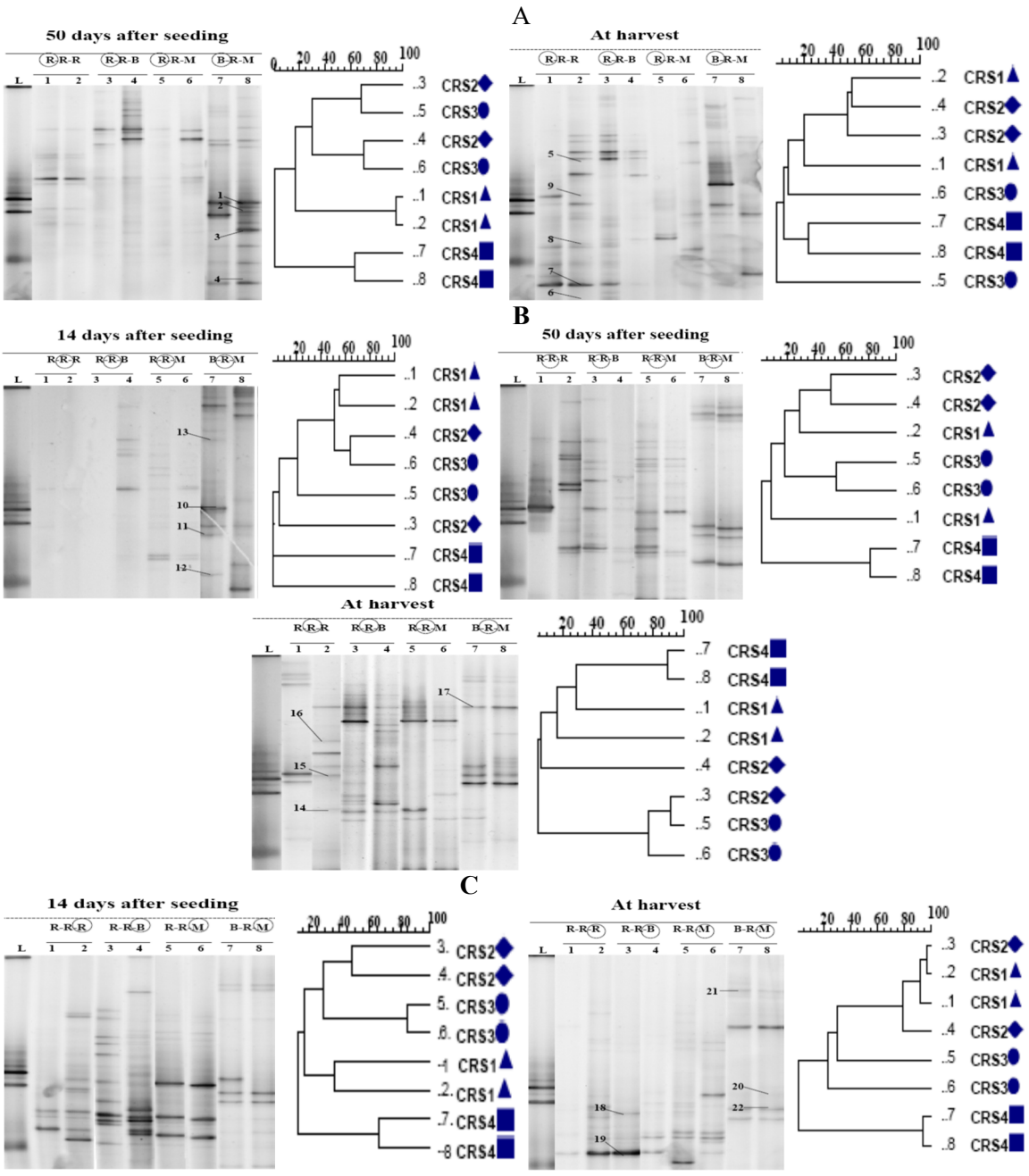


Fig. 2: Fungal ITS1 DGGE fingerprints of the fungal community colonizing rice straw residues (left) and results of the corresponding UPGMA cluster analysis (right) in 2 replicate field plots undergoing the different crop rotation systems during cultivation of crops I (A), II (B) and III (C) at 14 days 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 ITS1 DGGE ladder. Bands marked with numbers (from 1-22) correspond to ITS1 regions recovered in the ITS1 clone library analysis. In the PCA and UPGMA plots, the symbol used corresponds 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

During cultivation of crop I, clear differences in DGGE profiles were found between samples taken at day 14 (data not shown) and at day 50 for CRS4 (baby corn-rice-mungbean) on the one hand and the 3 other systems (CRS1, CRS2 and CRS3) on the other hand (Fig. 2A). As mentioned above, at day 14, PCR products were only obtained for samples derived from CRS4 (data not shown). For samples taken at day 50, UPGMA clustered the fungal community profiles into two groups consisting of (i) CRS4 and (ii) CRS1, CRS2 and CRS3. The CRS4 profiles of samples taken at 50 days shared only 0.4 % similarity with the DGGE profiles of the other three CRSs. Furthermore, UPGMA clustered the profiles of systems CRS2 and CRS4 separately from those of CRS1 (16.9 % similarity). Also at harvest of crop I, UPGMA suggested differences in fungal community structure between CRS4 on the one hand and CRS1, CRS2 and CRS3 on the other hand but no separation between profiles of CRS1, CRS2 and CRS3 samples could be noted.

During cultivation of crop II, which was rice in all 4 rotation systems, similar trends were observed as during cultivation of crop I (Fig. 2B). At day 14, UPGMA and PCA still separated the profiles of CRS4 from those of CRS1/CRS2/CRS3. However, at day 14 of crop II, only a few bands were detected in the CRS1/CRS2/CRS3 profiles. At day 50 and at harvest, clear CRS1/CRS2/CRS3 profiles were again obtained and both in UPGMA, profiles originating from CRS4 clustered differently from those originating from the other 3 systems. Moreover, although less obvious in UPGMA

Figure 2 shows that the ITS1 DGGE profiles of the fungal community colonizing the residual rice straw during cultivation of crop III which is an upland crop in the CRS2/CRS3/RS4 systems and paddy rice in the CRS1 system. Trends similar to those found during cultivation of crop I and II are continuing with CRS4 profiles clustering differently from those of the other 3 systems in both UPGMA. In addition, at both day 14 day of cultivation and at harvest, separate clusters can be observed for CRS1, CRS2 and CRS3.

3.2 Analysis of fungal diversity using Shannon diversity index (H)

H calculated based on the fungal ITS1 DGGE profiles were different between crop rotation systems (Table 3). During cultivation of crop I, the highest average H was found for the CRS4 system, i.e., the system in which crop I was an upland crop but differences were not significant except between CRS4 and CRS3. During cultivation of crop II, which was paddy rice in all systems, H was again the highest in CRS4 but again without significance between the systems. During growth of crop III (which was an upland crop in CRS2, CRS3 and CRS4), H was higher in the rotation systems with the upland crop compared to CRS1, but without apparent significance between the systems. Overall over all crops, CRS4 showed the highest H and CRS1 the lowest H.

Table 3: Average and standard deviation of the Shannon diversity index H calculated from the fungal ITS1 DGGE profiles for the different crop rotation systems

Rotation system	CROP*			Year+
	I	II	III	
CRS1	0.52±0.005^{ab}	0.51±0.108^{a}	0.60±0.100^{a}	0.54±0.067^{a}
CRS2	0.59±0.154^{ab}	0.57±0.025^{ab}	0.73±0.024^{a}	0.63±0.034^{ab}
CRS3	0.38±0.087^{a}	0.72±0.083^{ab}	0.62±0.079^{a}	0.57±0.025^{a}
CRS4	0.64±0.160^{b}	0.77±0.043^{b}	0.69±0.044^{a}	0.70±0.072^{b}

**: average H ± standard deviation of H values obtained for all samples taken during three cultivation of the indicated crop (I, II or III) (3 sampling times for each crop with two 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 2 replicates per sampling time point)

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

3.3 Sequence analysis of ITS1 fragments

Samples selected for ITS1 sequence analysis were those obtained at 50 days of cultivation of crop I in system CRS4 (designated as CL23), at harvest of crop I in system CRS1 (designated as CL27), at day 14 of crop II in CRS4 (designated as CL46), at harvest of crop II in CRS4 (designated as CL70), at harvest of crop II in CRS1 (designated as CL63), at day 14 of crop II in CRS2 (designated as CL100) and at harvest of crop III in CRS4 (designated as CL106). Hence, samples CL23 and CL106 were obtained during periods of drained conditions and cultivation of upland crops while samples CL27, CL46, CL63 and CL70 and CL100 were associated with paddy rice cultivation and flooded conditions. For each sample, approximately 40-50 white colonies were obtained in the cloning procedure. These

clones were analyzed by DGGE to identify ITS1 region fragments matching dominant bands in the corresponding DGGE community profiles, sequenced and their sequence analyzed by BLASTN. Only 22 of the 48 clones showed a match in BLASTN search. In Fig. 2, bands corresponding with those clones are accordingly numbered while results of the BLASTN analysis are shown in Table 4.

Most of the recovered ITS1 gene sequences were related to ITS1 gene sequences of fungi belonging to the phyla *Ascomycota* and *Basidiomycota*, two subphyla *Pezizomycotina* and *Saccharomycotina* and 8 different fungal families. At least for the identified DGGE bands, sequence information confirmed the existence of differences in fungi community composition in the different systems and the fungal community structure dynamics in time within as system as previously indicated by the DGGE results. Moreover, sequences obtained from flooded samples were different from those obtained from drained samples (containing upland crops). The fungal ITS sequences were associated with eight different fungal families. Members of the families *Lasiosphaeriaceae* (bands 1, 2, 10 and 19), *Hypocreomyceae* (band 3), *Chaetomiaceae* (band 4), *Dipodascaceae* (bands 11, 13 and 21), *Sporormiaceae* (bands 12 and 20), *Saccharomyceae* (band 17) and *Nectriacea* (band 22) appeared in the CRS2 and CRS4 systems while members of the families *Chaetomiaceae* (band 7), *Lasiosphaeriaceae* (bands 6 and 15) and *Trichocomaceae* (bands 9) appeared in the CRS1 system. Interestingly, uncultured *Basidiomycota* (bands 5, 14 and 16) were only identified in the CRS1 system. The family *Lasiosphaeriaceae* appeared in both samples from rotation systems including an upland crop (CRS2 and CRS4) and in paddy rice monoculture systems (CRS1). The families *Hypocreomyceae, Dipodascaceae, Sporormiaceae, Saccharomyceae* and *Nectriacea* appeared only in the CRS2 and CRS4 systems while the family *Trichocomaceae* were only identified in CRS1.

Table 4: Results of BLASTN analysis of ITS1 gene sequences recovered from the clone libraries. Sequence length was approximately 300 bp

No	Closest relative	Accession No	% similarity	Taxon- Phylum-Family
CL23 (crop I, day 50, CRS4)				
1	*Podospora intestinacea*	AY999121.1	84	Ascomycota-*Lasiosphaeriaceae*
2	*Cercophora mirabilis*	EF197069.1	89	Ascomycota- *Lasiosphaeriaceae*
3	*Fusarium equiseti*	FJ439592.1	90	Ascomycota-*Hypocreomyceae*
4	*Zopfiella latipes*	AY999129.1	90	Ascomycota-*Chaetomiaceae*
CL27 (crop I, at harvest, CRS1)				
5	Uncultured *Basidiomycota*	GU328618	88	Uncultured *Basidiomycota*
6	*Apodus oryzae*	AY681200.1	92	Ascomycota-*Lasiosphaeriaceae*
7	*Zopfiella latipes*	AY999129.1	92	Ascomycota- *Chaetomiaceae*
8	Uncultured soil fungus	DQ980572.1	85	Uncultured soil fungus
9	*Paecilomyces inflatus*	GU566291.1	79	Ascomycota- *Trichocomaceae*
CL46 (crop II, day 14, CRS4)				
10	*Podospora intestinacea*	AY999121.1	85	Ascomycota- *Lasiosphaeriaceae*
11	*Dipodascaceae* sp.	EF060596.1	86	Ascomycota- *Dipodascaceae*
12	*Westerdykella dispers*	DQ468016.1	86	Ascomycota- *Sporormiaceae*
13	*Dipodascaceae* sp.	EF060596.1	86	Ascomycota- *Dipodascaceae*
CL63 (crop II, after harvest, CRS1)				
14	*Thielavia* sp	EU620166.1	83	Ascomycota-*Chaetomiaceae*
15	Uncultured *Lasiosphaeriaceae*	FN689696.1	83	Ascomycota-*Lasiosphaeriaceae*
16	Uncultured *Basidiomycota*	GU328619	92	Uncultured *Basidiomycota*
CL70 (crop II, at harvest, CRS4)				
17	*Candida tropicalis*	EU288196.1	97%	Ascomycota- *Saccharomyceae*
CL100 (crop III, at harvest, CRS2)				
18	*Westerdykella purpurea*	FJ624258.1	87	Ascomycota-*Sporormiaceae*
19	*Arnium gigantosporum*	FJ196771.1	92	Ascomycota-*Lasiosphaeriaceae*
CL106, (crop III, at harvest, CRS4)				
20	*Pycnidiophora* sp	FJ903343.1	87	Ascomycota-*Sporormiaceae*
21	*Dipodascaceae* sp.	EF060596.1	87	Ascomycota- *Dipodascaceae*
22	*Gibberella moniliformis*.	AM946177.1	97	Ascomycota- *Nectriaceae.*

3.4 Fungal community dynamics under continuous rice cropping versus crop rotation

Understanding the structure of soil fungal communities under different rotation systems practices is important for the development of sustainable crop production systems. In this study, analysis of different rDNA non-coding ITS1 spacer fragments created through PCR successfully depicted and distinguished soil fungal communities under four different crop rotation systems. Selected PCR products were cloned and sequenced and the cloned sequences were aligned with published sequences in the NCBI BLAST nucleotide database. Non-coding rDNA spacer regions, such as the ITS1, benefit from a fast rate of evolution, resulting in greater sequence variation between closely related species compared with the more conserved coding regions of the rRNA gene cluster. Thus, fungal ITS sequences generally provide higher taxonomic resolution than sequences generated from rRNA coding regions (Lord *et al.,* 2002; Anderson *et al.,* 2003).

At the beginning of experiment, i.e., 14 days after seeding crop I, no PCR fragments were obtained with samples from CRS1, CRS2 and CRS3, even using a semi-nested PCR approach, suggesting that in those rice straw samples the number of fungal cells was below the limit of detection of the used PCR approach. In contrast, amplification with CRS4 samples was successful. On the other hand, at day 50, clear PCR signals were obtained with DNA from all samples. The main reason behind the apparent low abundance of fungi in the CRS1/CRS2/CRS3 samples taken at day 14 of crop I might be that on that moment colonization of the rice straw residues was still very poor especially under the water logged paddy rice soil of systems CRS1, CRS2 and CRS3. Previously, Reichardt *et al*. (2001) described that the biomass of fungi, as primary microbial decomposers of crop residues, showed a negative correlation with the soil-water content. Also Sugano *et al.* (2007) demonstrated that flooding affected the eukaryotic communities irrespective of the presence of rice straw. In contrast, the aerobic conditions in CRS4 might have resulted in more rapid growth and colonization of the rice straw residues by fungi. Interestingly, at day 50 of crop I and further on during cultivation of crop II and III, also in the flooded paddy rice systems, clear fungal ITS1 PCR signals were obtained showing that even under anaerobic conditions, fungal communities stably colonized the rice straw. Moreover, changing management practice from upland crop to paddy rice cultivation with flooded conditions, did apparently not affect colonization of the rice straw by fungi as shown in the CRS4 crop rotation system. The results presented here also indicate that there may have been a high level of spatial heterogeneity among the soil fungal communities within and between sampling locations and also within a single soil sample. Similar observations were made by others (Ettema and Wardell, 2002; Franklin and Mills, 2003; Ranjard *et al*., 2003).

Overall, the results from the fungal community profiles are similar to those of bacterial and actinomycetes profiles (Dung *et al.*, 2011). During growth of crop I, crop II and crop III, often major differences between the different systems and especially between CRS4 and the other 3 systems, were observed. Moreover, often differences between CRS1 and CRS2 and CRS3, were observed even when similar cultivation conditions were employed, i.e., under conditions of paddy rice cultivation for instance during growth of crop I and II. These results, as for the other two microbial groups, indicate the effect of crop rotation with upland crops on fungal community structure even in case of paddy rice cultivation conditions. As for the other two microbial groups, the differences observed during growth of crop I can be explained by the cultivation of the previous crop including crop type itself and associated cultivation conditions or by the overall rotation cropping system. On the other hand, we cannot exclude that the differences observed during growth of crop II between CRS1, CRS2 and CRS3 might have been affected by the state of degradation after cultivation of crop I. The composition of the rice straw will change during incubation. Moreover, during growth of crop II, differences between CRS1, CRS2 and CRS3 were less obvious compared to the crop I cultivation period. Also differences observed between CRS4 and the other 3 systems during growth of crop II might be explained by differences in state of degradation reached after crop I. The differences observed in fungal community profiles during cultivation of crop III were again more obvious between CRS1 and the other 3 systems which can be explained by the complete different soil cultivation conditions. Interestingly, there were also major differences between CRS4, CRS3 and CRS2 during growth of crop III despite the fact that crop III was in all systems an upland crop indicating that either slight differences in cultivation condition (for ex. type of plant) or the previous cultivation conditions affected fungal community structure. The current plant species however seem not to play a role since different fungi community structures were found in CRS3 and CRS4 (both containing mungbean during cultiva-

tion of crop III). Previously, it has been demonstrated that crop species have a pronounced effect on the composition of the microbial communities (Olsson and Alstrom, 2000; Berg *et al*., 2002). Otherwise, also the different state of degradation of the rice straw residues at the start of crop III cultivation might have affected fungal community structure. In a recent literature review it was concluded that plant type, soil type, and soil management all contribute to soil microbial community structure, and that complex interactions were involved (Garbeva *et al*., 2004). Previously, including legumes into crop rotations with cereals has been found to affect fungal community structure (Alvey *et al*., 2003; Oehl *et al.,* 2003). Similarly, Mathimaran *et al*. (2007) found that crop rotation of baby corn with crotalaria as compared to monocropping of baby corn significantly affected the community composition of arbuscular mycorrhizal fungi, but not their density and species diversity. Donnison *et al*. (2000) also found that changes in the composition of soil fungal communities were related to changes in plant productivity and composition. Previous studies have shown that shifts in the structure of bacterial and fungal communities can be associated with changes in a number of soil properties including soil texture **(**Girvan *et al.,* 2003), soil pH (Blagodatskaya and Anderson, 1998; Fierer and Jackson, 2006) and soil nitrogen availability (Frey *et al*., 2004). Fungi may be more sensitive to shifts in vegetation type than soil bacteria, particularly mycorrhizal fungi that form symbiotic associations with specific plant types (Heinemeyer *et al.,* 2004) or Basidiomycota which are involved in decomposing lignified plant detritus (Bardgett and McAlister, 1999). Similarly, Wu *et al*. (2007) provided evidence that land and crop management practices can differentially affect the diversity and composition of soil fungal communities and root colonizing fungal communities.

As found for the bacterial and actinomycetes community, the fungal community structure also changed in time during the cultivation of a specific crop. Apparently, changes in agricultural management concomitant with a change in cultivated crop, does not solely explain the dynamics in fungal community structure. Likely, changes in fungal community structure, at least within the cultivation period of a certain crop, is due to changes in rice straw composition during the degradation process and the proliferation of specialist degraders depending on the composition. This insinuates that also among fungi a metabolic specialization exists depending on the composition of organic residue. Rovira and Vallejo (2002) previously suggested fast degradation by the microbial communities of substrates of high quality which causes them only to be present in the beginning of the degradation process. Other studies also indicated that residue quality and amount influence the soil fungal community structure (Nicolardot *et al*., 2007). The residues of high C/N ratio favor soil fungi and enhance macroaggregate formation (Bossuyt *et al*., 2001). However, it is important to notice that one and the same organism can be involved in the degradation and transformation of organic substances of different origin and composition (Vancura *et al*., 1988).

The ITS1 sequences obtained in this study could be associated with 8 different fungal families belonging to either the Ascomycota (20 clones of 22 of the sequences) or Basidiomycota (2 clones of 22 of the sequences) phylum. However, sequences of 26 clones did not match any of the known sequences and, therefore, could not be identified. This was not surprising considering that only 5 - 10% of an estimated 1.5 million fungal species have been described (Hawksworth and Rossman, 1997; Hawksworth, 2001). Another explanation is that the number of sequences of ITS1 regions in the public databases is limited. Our results compare with a recent report by Xuan (2007) who studied the fungal diversity on rootless stubble of rice plants from two different sites (Hoa An and Vinh Nguon) in the Mekong Delta in which 66% of isolated fungi were identified as Ascomycota and 11% as Basidiomycota. Cahyani *et al*. (2004) reported that especially members of the phylum Basidiomycota were present during the composting process of rice straw. ITS1 sequences associated with members of the fast growing Zygomycota phylum were not detected in our experiment which agrees with results reported by Hagn *et al*. (2003) who found that Zygomycetes are not that frequently isolated from agricultural soil in general and arable soil in particular. The finding that the Ascomycota phylum apparently constituted the abundant fungal phylum colonizing the rice straw residues in the CRS2 and CRS4 crop rotation system was not that unexpected since Ascomycetes are the largest group of the true fungi (Larena *et al*., 1999). Most of them are saprophytic and live on dead organic material, which they help to decompose. However, Ascomycetes also cause plant diseases, varying from powdery mildews to rots, cancers and vascular wilts (Oehl *et al.,* 2003). Members belonging to the families *Hypocreomyceae, Dipodascaceae, Sporormiaceae, Saccharomyceae* and *Nectriaceae* were detected on rice straw residues of CRS2 and CRS4 but not on rice straw residues of CRS1. In contrast, ITS1 sequences associated with the family

Trichocomaceae and uncultured Basidiomycota were only found on rice straw residues of CRS1. Previously, Xuan (2007) found that the family *Hypocreaeceae*, *Nectriaceae, Sordariaceae, Sporormiaceae and Trichocomaceae* was present in rice straw decomposition in paddy rice soil of two experimental sites in the Mekong Delta. Members of the *Trichocomaceae* family were also found to be responsible for rice straw decomposition in paddy rice field soil by Sugano *et al*. (2007). Members of the families *Saccharomyceae, Hypocreaeceae* and *Trichocomaceae* have also been associated with composting of rice straw (Cahyani *et al.,* 2004). Some members of the family *Trichocomaceae* are important causes of food spoilage and biodeterioration, while others are widely used in biotechnological applications (Pitt and Hocking, 1997). They grow quickly and produce large numbers of conidia which are easily dispersed. As a result, these fungi occur over a wide range of environments (Cahyani *et al*., 2004; Xuan, 2007; Sugano *et al*., 2007; Tonouchi, 2009). Furthermore, a major proportion of the identified fungal populations in the CRS1, CRS2 and CRS4 systems both in drained soil and flooded soil seem to belong to the family *Lasiosphaeriaceae*. This result agrees with the results of Huhndorf *et al*. (2004) who showed that the family the *Lasiosphaeriaceae* is by far the largest and most morphologically diverse family in the order of *Sordariales*. Some members of the family *Nectriaceae* are potential pathogens of rice in the developing rice period (Chaverri *et al*., 2002). Species belonging to the family *Dipodascaceae*, only found in CRS4 rotation system, have a widespread distribution, and are found in decaying plant tissue, or as spoilage organisms in the food industry. Also the family *Sporormiaceae* has a cosmopolitan distribution and its members are saprobic on coprophilous and rotting vegetation (Cannon and Kirk, 2007).

Comparable investigations support the assumption that crop rotation (CRS2 and CRS4) and monoculture (CRS1) practices in agriculture influence the composition of the microbial communities. Oehl *et al*. (2003) investigated the impact of land use on the diversity of arbuscular mycorrhizal fungi (AMF). The highest AMF species number and diversity was found in the low input, organically managed arable land under crop rotation. They identified 18 AMF species from land under crop rotation, and 8–13 AMF species from land under baby corn monocropping using trap cultures. van Elsas *et al*. (2002) used DGGE analysis to show a clear effect on the bacterial and fungal microflora of arable land under oat–baby corn rotation, baby corn monoculture, and permanent grassland. The microbial diversity was higher in arable land under crop rotation than under monoculture, and higher in grassland than in arable land. Collins *et al*. (1992) examined the inclusion of summer fallow and a legume crop in along-term rotation experiment. They reported that soil microbial biomass C and N as well as populations of bacteria, fungi and actinomycetes were significantly higher under monoculture wheat than under wheat fallow rotation. Macini and Caputo (2009) have confirmed those finding, showing that diversity of fungal communities in potato sites (monoculture) was significantly lower than that in rotation sites. In addition, fungal communities in rotation sites showed lower Berger–Parker dominance than those in the potato sites, suggesting that rotation sites had a higher diversity as well as a better fungal community balance than potato sites.

4 CONCLUSIONS

Our data suggest that agricultural management practices based on crop rotation including the cultivation of upland crops affect the fungal community composition in that soil. Since fungi play a major role in organic residue recycling, differences in fungi diversity might affect the decomposition process. Moreover, our shows that the inclusion of paddy rice in such rotation systems does not necessarily affect fungi diversity in the next crop.

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