Molecular characterization of soil fungal communities in paddy soils

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Abstract
Soil fungal communities are important for soil ecosystem functioning. In particular, they are the most important group of organisms involved in decomposing organic matter, especially for resistant compounds such as lignin. Therefore, fungi have an important impact on the decomposition of straw in soil.
In paddy soils, fungal communities are predominant only after drainage and during post-harvest fallow conditions as they need oxygen which is limited under flooded conditions. To investigate the influence of soil management practices on fungi in paddy soils, the period of drainage after flooding was simulated in soil microcosms. In addition, different rice straw applications were tested in an incubation experiment. Molecular analysis including DNA-extraction and PCR/DGGE-analysis was used to characterize the soil fungal communities in these experiments. The resulting dendrograms show clear shifts and correlations between the simulated water stages and rice straw applications.

Key Words
Soil fungal communities, molecular microbiology, DGGE, 18S rDNA, paddy soil.

Introduction

Knowledge about fungi in paddy soils is less compared to other environments, although fungi might play an important role in the decomposition of rice straw, which is up to now almost completely burned. The abundance of fungi in flooded paddy soils is much lower than in upland soils (Kyuma 2004). In general, microorganisms in paddy soils are affected by rapid changes of their habitats caused by the changes between flooding and drainage (Lennartz *et al*. 2009).

There is a great need in soil science to develop novel methods allowing a detailed description of soil fungal diversity without cultivation to improve the understanding of soil fungi and their ecology. Therefore, nucleic acid extraction approaches have become more widely applied in investigations of soil fungi in recent years (van Elsas *et al*. 2000). Direct DNA extraction from soil, coupled with polymerase chain reaction amplification and community profiling techniques, has been proved successful in investigations of bacterial ecology and promises progress for describing the taxonomic and functional characteristics of soil fungal communities (Anderson and Cairney 2004).

The aim of this study is to apply techniques of molecular microbiology to analyse shifts in soil fungal communities in paddy soils. Therefore, two experiments will be established, simulating (i) the period of drainage during cultivation of rice on differently-textured paddy soils and (ii) different rice straw applications on a clay-rich paddy soil.

Methods

Microcosm experiment
A microcosm experiment was set up in a climate chamber in order to simulate a total vegetation period of rice under controlled conditions. Therefore, undisturbed soil microcosms (25 cm in diameter, 30 cm height) were taken at three selected sites in China (Table 1). Soil sampling for the molecular characterization during soil drainage was performed at 111 days after transplanting (DAT) and 118 DAT. These dates are representing the last day under flooded conditions and the day after one week of drainage, respectively. Soil samples were stored at -20 °C until further processing.

Incubation experiment
In order to investigate the microbial response of different rice straw applications in paddy soil an incubation experiment was set up. Therefore, 50 g of homogenized soil (HC) was mixed with shredded rice straw (S) or burned rice straw (bS) and incubated in 250 ml bottles at 25 °C for seven weeks (Table 2). Soil samples were taken after one day and 44 days of incubation for all simulated treatments.

Table 1. Soil description.

Soil	Location	Cultivation	Parent material	Classification (WRB)	Sand (%)	Silt	Clay	Texture (USDA)
LC	Liu Jia, Yujiang County, Jiangxi Province, PR China	Flooded rice - rice rotation	Red Sandstone	Hydragric Anthrosol	61.3	26.2	12.5	Sandy loam
MC	Sun Jia, Yujiang County, Jiangxi Province, PR China	Flooded rice - rice rotation	Quaternary Red Clay	Anthraquic Cambisol	32.7	44.0	23.3	Loam
HC	Jinjiaba, Wujiang County, Jiangsu Province, PR China	Rape - flooded rice rotation	Alluvial Clay	Stagnic Anthrosol	0.9	58.1	41.0	Silty clay

Table 2. Incubation experiment setup.

Treatment	Description	Rice straw (g/kg)
0S	without rice straw	0
bS	burned rice straw *	8.5*
1× S	1× rice straw	8.5
2× S	2× rice straw	17.0

* amount of 1× rice straw before burning; FC: field capacity

Characterization of soil fungi using DGGE

Extraction and purification of DNA from soil samples was carried out using the FastDNA SPIN Kit for Soil (Q-BIOgene) with a modified protocol. The procedures of polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) were modified according to Vainilo and Hantula (2000) using 18S rRNA-based primer pairs. DGGE-gels were documented and dominant bands were selected for subsequent sequencing. Dendrograms were constructed from the distance similarity matrix computed by the Nei-Li option and followed by the neighbour joining tree-construction method (Hampl *et al*. 2001; Nei and Li 1979).

Results

The amount of extracted soil fungal DNA varied between the investigated soils according to their clay content (LC>MC>>HC). The primer combination NS1f/FR1 (Pennanen *et al*. 2001) was suitable to amplify 18S rDNA regions. The PCR-products of both experiments were used for the DGGE-analysis. The resulting DGGE-gel (Figure 1a) shows differences in the pattern and intensities of bands of all soils, treatments, and irrigation stages representing shifts in the soil fungal community. The dendrogram of the gel-analysis shows a close relation of the soils LC and MC (Figure 1b). In both soils the fungal diversity between the wet and dry stage differs within a cluster. The HC soil of the microcosm experiment forms a cluster with the treatments without or burned rice straw application in the incubation experiment. The rice straw applications (1× and 2×) are representing an own cluster which is separated from all other samples in the dendrogram. In the incubation experiment, both investigated incubation times have an influence on the soil fungal diversity as they form own clusters for the treatments with rice straw (1× and 2×) and without rice straw (0 and burned), respectively.

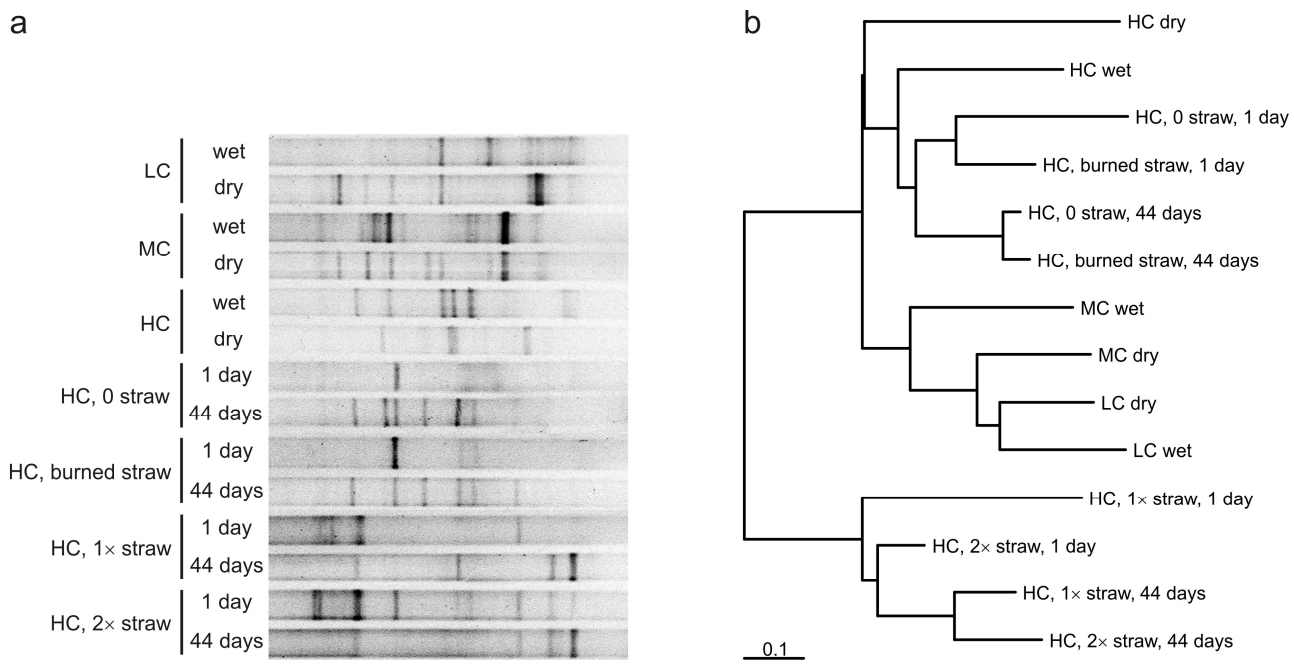

Figure 1. 18S rRNA-based analysis of fungi in the experiments. (a) DGGE gel; (b) dendrogram of the DGGE-analysis.

Conclusion

The results of the DNA-based DGGE-analysis showed shifts of soil fungal communities in both experiments. Within the microcosm experiment the discrimination of the differently-textured soils was clear. The differences between the HC soil and the soils with lower clay content (LC and MC) were very distinct when DGGE-analysis was applied. In the incubation experiment, the differences between the treatments with rice straw (1× and 2×) and the treatments without or burned straw were very clear after DGGE-analysis. Within these treatments, the incubation time had a noticeable influence on the soil fungal community.

The simulated water stages in the microcosm experiment had an impact on the shift of the soil fungal community during the period of drainage. As it has been reported by Kyuma (2004), flooded paddy soils contain less fungi compared to upland soils. As fungi are the most important microorganisms for the decomposition of straw (Finlay 2007), there is more research needed on the occurrence of soil fungi under flooded conditions and after incorporation of rice straw.

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