

# Effects on microbial communities of long-term application of organic matter and conversion to upland condition of paddy field: Estimation by eDNA analysis

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## Abstract

Microorganisms and their activities in paddy field soil play important roles for rice production and soil fertility. Effects of long-term application of organic matter and conversion to upland on microbial community structures were investigated in Japanese paddy fields by molecular ecological techniques (eDNA analysis) for better understanding of their roles in paddy field soil. The effect of long-term application of organic matter (rice straw compost) on denaturing gradient gel electrophoresis (DGGE) patterns of 16S rRNA genes of bacterial communities and 18S rRNA genes of fungal communities was small. DGGE banding patterns from 18S rRNA genes of fungal communities in paddy-upland rotational plots and upland plots converted from paddy fields were different from those for plots continuously cropped with paddy rice. These results indicated that the microbial communities in paddy fields were not influenced by long-term application of organic matter, but were affected by conversion to upland fields from paddy fields.

## Key Words

Community, eDNA, fungi, bacteria, paddy field, upland

## Introduction

Rice cultivation in flooded paddy fields is a good and important system of crop production in the monsoon area of Asian countries. Many kinds of physical, chemical and biological functions of soil contribute to growth of rice plants in paddy fields. Soil microorganisms and their activities play important roles in those functions, especially various metabolic reactions in soil such as mineralization of soil organic nitrogen and decomposition of rice straw and compost applied to soil, which support rice production as well as maintain the fertility of paddy soil. Therefore, it is important to elucidate microbial community structures in paddy fields in order to understand the roles of microorganisms.

In the previous studies, we showed the stability of bacterial communities in paddy field soil throughout a year including the cultivation periods of rice under flooded conditions and of wheat under upland conditions by the denaturing gradient gel electrophoresis (DGGE) analysis based on DNA and RNA (Kikuchi *et al.*, 2007). In this communication, we present effects of long-term application of organic matter (rice straw compost) and conversion to upland on microbial communities in Japanese paddy fields revealed by molecular ecological studies (eDNA analysis).

## Materials and Methods

### Study site

Three experimental fields with long-term application of organic matter (for 32-44 years), Aomori Prefectural Agriculture and Forestry Research Center in Kuroishi, Aomori, Japan (latitude 40°38'N, longitude 140°34'E; Anthraquic Kuroboku soils; Melanaquand) (Kuroishi), National Agricultural Research Center for Tohoku Region in Daisen, Akita, Japan (latitude 39°29'N, longitude 140°29'E; Gray Lowland soils; Typic Fluvaquents) (Omagari) and National Agricultural Research Center for Kyushu Okinawa Region in Chikugo, Fukuoka, Japan (latitude 33°12'N, longitude 130°29'E; Gray Lowland soils; Endoquepts) (Chikugo), were used and soil samples were taken from the plots applied with chemical fertilizer (CF) and with rice straw compost plus chemical fertilizer (RSC) for the analyses of soil bacterial and fungal communities in 2006 and 2008. The fields were continuously cropped with only rice in Kuroishi and Omagari or under double cropping with rice and wheat in Chikugo. The samples collected from paddy-upland rotational fields in the

National Agricultural Research Center for Tohoku Region and upland fields converted from paddy fields in the National Agricultural Research Center for Kyushu Okinawa Region in 2008 and 2009 (Table 1) were also subjected to the analysis of soil fungal communities.

**Table 1. Cropping system of fields under paddy-upland rotation in Omagari and conversion to upland in Chikugo.**

|                | Plot                  | 1968-1981 |    | 1982-1999 |    | 2000-2002                |    | 2003-2004 |    | 2005-2007 |    | 2008-2009 |    |    |    |    |    |    |    |    |    |    |
|----------------|-----------------------|-----------|----|-----------|----|--------------------------|----|-----------|----|-----------|----|-----------|----|----|----|----|----|----|----|----|----|----|
| <b>Omagari</b> | Paddy-upland rotation | R         |    | S         |    | R                        |    | S         |    | R         |    | S         |    |    |    |    |    |    |    |    |    |    |
| <b>Field A</b> | Cont. paddy           | R         |    | R         |    | R                        |    | R         |    | R         |    | R         |    |    |    |    |    |    |    |    |    |    |
|                |                       | -89       | 90 | 91        | 92 | 93                       | 94 | 95        | 96 | 97        | 98 | 99        | 00 | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 |
| <b>Omagari</b> | Rot.1                 | R         | S  | R         | R  | S                        | R  | S         | R  | S         | R  | S         | R  | R  | S  | R  | S  | R  | S  | R  | S  | R  |
| <b>Field B</b> | Rot. 2                | R         | S  | S         | R  | S                        | S  | S         | R  | S         | S  | S         | R  | S  | S  | R  | S  | S  | R  | S  | S  | R  |
|                | Cont. paddy           | R         | R  | R         | R  | R                        | R  | R         | R  | R         | R  | R         | R  | R  | R  | R  | R  | R  | R  | R  | R  | R  |
|                |                       | Plot      |    | -2004     |    | 2005 - 2009              |    |           |    |           |    |           |    |    |    |    |    |    |    |    |    |    |
| <b>Chikugo</b> | Upland converted      |           |    | R         |    | S (-W) (Double cropping) |    |           |    |           |    |           |    |    |    |    |    |    |    |    |    |    |
|                | Cont. paddy           |           |    | R         |    | R                        |    |           |    |           |    |           |    |    |    |    |    |    |    |    |    |    |

R, Paddy rice ;S, Soybean ;W, Wheat

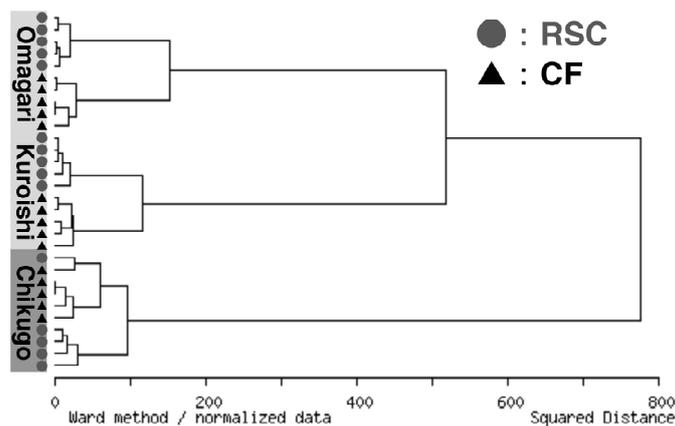
### DGGE analysis

Soil bacterial and fungal communities were analyzed by DGGE of 16S rRNA and 18S rRNA gene fragments, respectively. Variable regions (V3 and V6-V8 for bacteria and V1-V2 for fungi) of 16S rRNA and 18S rRNA gene fragments were amplified with PCR from DNA extracted from the soils and subjected to the DGGE (Muyzer *et al.*, 1993; 1998; Hiramatsu *et al.*, 2007; Morimoto and Hoshino, 2008). Bacterial and fungal communities were compared between the plots based on the analysis of the DGGE banding patterns by cluster analysis and principal component analysis.

### Results

#### *Effect of long-term application of rice straw compost on microbial communities in paddy field soils*

The DGGE banding patterns of 16S rRNA gene fragments in the V3 and V6-V8 regions retrieved from bacterial communities were not greatly different between CF and RSC plots of the experimental fields with long-term application of organic matter in Kuroishi, Omagari and Chikugo. The patterns did not show conspicuous changes throughout the rice growing periods in the respective fields. Figure 1 shows the cluster analysis of the banding patterns of 16S rRNA gene fragments in the V6-V8 regions. Although some differences were found in the patterns among the three locations, the differences were mainly attributed to variations in the band intensities and most of the DGGE bands were commonly present through the samples obtained from the three locations.

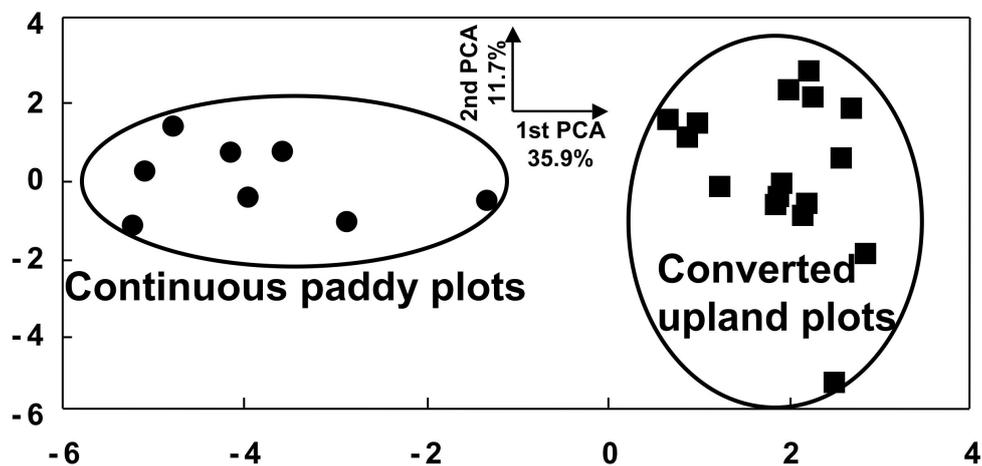


**Figure 1.** Cluster analysis of DGGE pattern of the 16S rRNA gene fragments in the V6-V8 regions retrieved from bacterial communities in the experimental fields (Kuroishi, Omagari and Chikugo) with long-term application of organic matter in 2006 (Hiramatsu *et al.*, 2007). CF, chemical fertilizer plot; RSC, rice straw compost plot.

The DGGE banding patterns of 18S rRNA gene fragments retrieved from fungal communities were also similar between CF and RSC plots of the experimental fields with long-term application of organic matter in Kuroishi, Omagari and Chikugo in 2006 and 2008 (data not shown). The patterns did not show conspicuous changes throughout the rice growing periods in the respective fields. These results show that bacterial and fungal communities in paddy field soil were not influenced by long-term application of rice straw compost and field managements during rice growing periods.

*Effect of conversion to upland of paddy fields on fungal communities*

The DGGE banding patterns of 18S rRNA gene fragments retrieved from fungal communities were different between upland plots converted from paddy fields in Chikugo or paddy-upland rotational plots in Omagari and the plots continuously cropped with paddy rice. Figure 2 shows the principal component analysis of the banding patterns of 18S rRNA gene fragments in Chikugo fields. These results show that fungal communities in paddy field soil were influenced by conversion to upland from paddy fields and were different from the communities in paddy fields.



**Figure 2.** Principal component analysis of DGGE pattern of the 18S rRNA gene fragments retrieved from fungal communities in upland plots converted from paddy field and paddy plots continuously cropped with paddy rice in Chikugo in 2008 and 2009.

**Conclusion**

Composition of bacterial and fungal communities in paddy fields was not influenced by long-term application of organic matter, but was affected by conversion to upland fields from paddy fields. The stable characteristics of soil microbial communities may be attributed to rice cultivation under flooded conditions in paddy fields and may play some important roles in productivity and sustainability of rice cultivation in paddy fields. Thus, it is interesting to elucidate the mechanisms that maintain the stability of microbial communities in paddy field soil.

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