Effects of open drainage ditch design on bacterial and fungal communities of cold waterlogged paddy soils

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Abstract

A field experiment established in 1980 was conducted to evaluate the effects of open drainage ditch applied for water removal on bacterial and fungal communities of cold waterlogged paddy soils in 2011. In this experiment, traditional plate counting and temperature gradient gel electrophoresis were employed to characterize the abundance and diversity of soil bacterial and fungal communities. Four different distances from the open drainage ditch, 5, 15, 25 and 75 m with different degrees of drainage were designed for this study. Maximum populations of culturable aerobic bacteria and fungi were at 15-m distance while minimum populations were at 75-m distance. Significant differences (p < 0.05) in fungal populations were observed at all distances from open drainage ditch. The highest diversity of the bacterial community was found at a distance of 25 m, while that of the fungal community was observed at a distance of 5 m. Sequencing of excised TGGE bands indicated that the dominant bacteria at 75-m distance belonged to anaerobic or microaerobic bacteria. Relationships between microbial characteristics and soil physicochemical properties indicated that soil pH and available nitrogen contents were key factors controlling the abundance of culturable aerobic bacteria and fungi, while soil water capacity also affected the diversity of fungal community. These findings can provide the references for better design and advanced management of the drainage ditches in cold waterlogged paddy soils.

Key words: abundance, bacterial and fungal communities, diversity, open drainage ditch.

Introduction

Cold waterlogged paddy soils (CWPS) are a type of low-yield paddy field widely distributed in southern China. In Fujian Province, CWPS occupy about 128,000 hectares (ha), accounting for 12.0% of the total area of paddy fields, and about 47.7% of the total low-yield paddy area in the whole province (Li et al., 2011). Low productivity of cold waterlogged paddy (CWP) fields is attributed to their physical and chemical factors, such as high groundwater table, poor drainage conditions, low soil temperature, excessive amount of reducing substances, poor aeration conditions, and low contents of available nutrients (Cheng, 1984). Obviously, only through proper drainage can CWPS become favorable for rice growth. Once drained, excess water in agricultural soils is rapidly removed, the moisture in paddy soil decreases greatly, and the groundwater table is lowered. Thus, drainage promotes cohesion of soil particles, increases bulk density and hardness of soil (Cheng, 1984), improves soil aeration conditions, and accelerates oxidation of soil organic matter (SOM) contents. Thereby, drainage which serves as an important water management strategy, not only improves the physical properties of paddy soils, but also promotes soil fertility.

In China, most farmers have conventionally employed some techniques for land drainage, such as open drainage ditch, mole drainage, tile drainage, and their combinations. Agricultural drainage ditches are essential for removal of surface and ground waters to allow for crop production in poorly drained agricultural landscapes (Nee-
Ditches range in size from small depressed channels designed primarily for carrying surface runoff to major channelized streams draining large watersheds and regional groundwater (Needelman et al., 2007). In fact, the ditches serve not only to prevent flooding so as to reduce crop stress through the rapid removal of excess water, but also to dry the soils to facilitate operation of farm machineries.

Although it is well known that drainage ditches can bring about noticeable shifts in soil quality and rice growth in poorly drained agricultural land, little is known as to their effect on soil microorganisms, which play the major role in soil ecosystems. Soil microbial function governs to mediate nutrient cycling, organic matter decomposition, and soil aggregate formation (Kamaa et al., 2011).

Good understanding of soil microbial communities will allow us to identify the relationships between ditch drainage, soil fertility and rice productivity. CWP field with an open drainage ditch was established in 1980 in the middle of paddy field in Shunchang, Fujian Province in China. Thus, this study aimed to use cultivation-dependant method and molecular techniques, including DNA-extraction and PCR/TGGE-analysis, to characterize the abundance and diversity of soil bacterial and fungal communities at four different distances from open drainage ditch.

Materials and Methods

Site descriptions and soil samplings

The study site is located at the Shunchang Long-Term Experimental Station (LTES, since 1980) of Cold-Waterlogged Field Improvement, Fujian Academy of Agricultural Sciences (26°42’N, 117°42’E), Fujian Province, China. The elevation of the LTES is 262 m above sea level. It has subtropical monsoon humid climate with annual average temperature of 18.5 °C, precipitation of 1691.3 mm and sunshine of 2,292 h. The CWPS is derived from quaternary sediment and classified as a type of gleyic paddy soil. It has subtropical monsoon humid climate with annual average temperature of 18.5 °C, precipitation of 1691.3 mm and sunshine of 2,292 h. The CWPS is derived from quaternary sediment and classified as a type of gleyic paddy soil.

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10-fold serial dilutions of soil suspensions were prepared for spread plate counting. 10⁻³-10⁻⁵ dilutions were used for bacteria counting with beef extract peptone medium (Jiang et al., 1995), and 10⁻³-10⁻⁴ dilutions were prepared for fungi counting with rose bengal medium (Smith and Dawson, 1944). Plates with 0.1 mL of inoculum were inverted and incubated at 28 ± 2 °C for 3 d for bacteria and 5 d for fungi. After incubation, a plate with a countable number of colonies (between 30 and 300) was selected. The number of colonies was counted and the number of microbes in 1 g of dry soil was calculated.

DNA extraction from soils and PCR amplification

Soil DNA was extracted using the SDS-based DNA extraction method(Zhou et al., 1996). For bacteria, the 16S rDNA V3 fragments were amplified using the fD1/rD1 (Weisburg et al., 1991) and F341GC/R534 (Muyzer et al., 1993) primers with nested PCR. Amplification using primer pair fD1/rD1 was performed in 50-µL reaction mixtures, including 5 µL 10 x PCR buffer with (NH₄)₂SO₄, 3 L 25 mM MgCl₂, and 4 µL 2.5 mM dNTPs. Then, 0.5 µL Taq DNA polymerase (5 U µL⁻¹, MBI Fermentas, Canada), 1 µL 20 µM each primer, 1 µL DNA template (10-50 ng)
were prepared. A touchdown PCR strategy was employed as follows: 5 min at 94 °C, 20 cycles of 1 min at 94 °C, 45 s at temperatures decreasing from 65 °C to 55 °C, 2 min at 72 °C, 10 cycles of 1 min at 94 °C, 45 s at 55 °C, 2 min at 72 °C, and final extension for 5 min at 72 °C. PCR amplification of a 16S rDNA V3 fragment using primer pair F341GC/R534 was carried out in a 25 μL containing 2.5 μL PCR buffer, 1.5 μL MgCl₂, 2.8 μL dNTPs, 0.5 μL Taq DNA polymerase, 0.5 μL each primer, 1 μL DNA template (Amplicons resulted from fD1/rD1 were diluted 1:100). Concentrations of PCR components and cycling conditions were the same as above. For fungi, fragments of 18S rRNA gene were amplified with primer pair FR1-GC/FF390 as described by Vainio and Hantula (2000). All PCR components (50 μL) were the same as fD1/rD1 reactions except for PCR buffer (10 x buffer with KCl) and primer pair. The PCR procedure was as follows: 3 min at 95 °C, then 35 cycles of 30 s at 95 °C, 45 s at 50 °C, 1 min at 72 °C, and final extension for 10 min at 72 °C (Beauregard et al., 2010). All reactions were carried out using a Whatman Biometra T1 96-well Thermocycler. Primer sequences are listed in Table 1.

TGGE and diversity measurements

PCR products were loaded on 0.45-mm thick 8% denaturing gels (8% polyacrylamide gel (Acr/Bis = 37.5:1), 1 x TAE (40 mM Tris-Cl, 1 mM EDTA), 2% glycerol, and 8 M urea). TGGE was performed using a TGGE system (Whatman Biometra, Germany). All gels were run at 130 V for 3 h with 1 x TAE buffer. Temperature gradient was optimized at 56-69 °C for bacteria and 49-61 °C for fungi. After electrophoresis, gels were silver-stained using the procedures devised by Bassam and Caetano-Anollés (1993). Gel images were captured using the Cannon camera and analyzed by the Bio-rad Quantity One software. The diversity indices, including richness index and Shannon index, were calculated according to TGGE patterns, respectively. Each resolved band was considered as a specific phylotype. The pixel intensity of each band detected by Quantity One software represented the abundance of a specific phylotype for diversity estimations. The Shannon-Weaver indices ($H'$) (Shannon and Weaver, 1963) were calculated using the following Eq. (1):

$$H' = -\sum p_i \ln p_i$$  (1)

where $p_i = n_i/\Sigma n_i$, $n_i$ is the abundance of the $i$th phylotype per lane, and $\Sigma n_i$ is the total abundance of all phylotypes per lane. In theory, $H'$ values range from 0 (only one species present in the sample) to 5 (all species in the sample are represented by the same number of individuals), but in fact, $H'$ values usually lie between 1.5 and 3.5 for ecological data and rarely exceed 4.0 (Seaby and Henderson, 2006). In general, more disturbed and less stable environments have lower $H'$ values.

Finally, cluster analysis according to the TGGE patterns was employed to produce the dendrograms by the unweighted pair-group method with arithmetic average (UPGMA) method using Quantity One software.

16S/18S rRNA genes sequence determination

The dominant bands in the TGGE gels with the same mobility were excised, incubated overnight in 10 mM Tris-HCl (pH 8.0) to elute DNA, re-amplified as described above, and electrophoresed with TGGE. Band excision, PCR, and TGGE were repeated until a single band was present. PCR products generated from TGGE bands were amplified with primers without GC-clamp at the 5'-end. Purified PCR products were ligated into the pMD19-T vector (TaKaRa Biotechnology, Dalian, China) according to the manufacturer’s instructions. Four positive clones per band were used for DNA sequencing. The obtained partial environmental 16S/18S rRNA gene sequences were deposited in the European Nucleotide Archive database under accession numbers HE867098-HE867106 and compared to the sequences in NCBI GenBank database using the BLAST 2.2 program.

Statistical analysis

One-way ANOVA was employed to analyze the difference of all indices at each distance. Post hoc tests for each variable were made using LSD comparisons. The Pearson correlation analysis was utilized to determine the correlations between all the measured parameters. Signifi-

Table 1 - Primer used in this study.

| Target group | Primer | Sequence (5' → 3') | Length of amplicon (bp) |
|--------------|--------|-------------------|------------------------|
| Bacteria     | fD1    | GAGTTTGATCCTGGCTCAG | 1533                   |
|              | rD1    | AGAAAGGAGGTGATCCAGCC |                     |
|              | F341GC | GC clamp¹-CCTACGGGGAGGCACGAG | 234       |
|              | R534   | ATTACCGGGCGCTGCTGG   |                        |
| Fungi        | FR1-GC | GC clamp²-AICCATCTAATCGGT | 430       |
|              | FF390  | CGATAACGAACGAGACCT  |                        |

¹GC clamp represents CGC CCG CCG CCG CCG CCG CGC GGG GGG GCG GCA CGG GGG G.
²GC clamp represents CCC CCG CCG CCG CCG CCG CGC GGG GGG GGG GGG GGG GCA CGG GCC G.
cant differences for all statistical tests were evaluated at the level of $p \leq 0.05$ unless noted otherwise. All data analyses were conducted with the SPSS software (SPSS for Windows, Version 13.0, Chicago, IL).

**Results**

**Soil chemical properties and number of microbes in relation to distance**

pH of all soils were slight acid. SWC and SOM contents ranged from 58.5 to 70.6% and from 3.03 to 4.64%, respectively (Table 2). From ANOVA analysis, soil pH, AN and AK contents decreased gradually with increasing distance from the open drainage ditch and were significantly greater ($p < 0.05$) at 5-m than at 75-m distance. SOM contents were the lowest at 25 m distance and the highest at 75-m distance. SWC increased gradually with increasing distance and was significantly lower at 5-m than the other distances ($p < 0.05$). There were no significant differences ($p < 0.05$) in soil AP contents detected at all distances. The highest populations of culturable aerobic bacteria and fungi were at 15-m distance and the lowest at 75-m distance. Moreover, significant differences ($p < 0.05$) in fungi populations were also observed at all distances.

According to Pearson’s correlation analyses, the numbers of culturable aerobic bacteria and fungi showed significantly positive correlation with soil pH (i.e., $R = 0.669$, $p < 0.05$ and $R = 0.730$, $p < 0.01$, respectively) and AN contents (i.e., $R = 0.741$, $p < 0.01$, and $R = 0.721$, $p < 0.01$, respectively) but not with SWC, SOM and AP contents (Table 3). In addition, fungi counts also showed significantly positive correlation with AK contents (i.e., $R = 0.844$, $p < 0.01$).

**Microbial community structure associated with ditch distance**

TGGE analysis was performed using three replicate samples from each distance in the CWP field. TGGE results demonstrated that the number of bands in eubacterial 16S rDNA amplified fragment was higher than that in fungal 18S rDNA amplified fragment from all distances except for 5-m distance (Figure 2). For bacteria, TGGE patterns showed regular changes associated with the distance from the open drainage ditch. Some bands (Figure 2a, from 1B to 7B) of 16S rRNA gene gradually became stronger with increase in distance, but some others gradually turned fainter such as band 1b, and even disappeared such as band 2b and 3b (Figure 2a). However, the TGGE profile of fungi showed abrupt shift from 5-m to 15-m distance. 18S rRNA gene bands were abundant at a distance of 5 m, but few at distances of 15 m, 25 m and 75 m. Moreover, there were some specific bands at a certain distance, such as 1F (Figure 2b) at 75 m, and 2F at 25 m (Figure 2b).

Cluster analyses of the TGGE profiles generally distinguished the bacterial and fungal communities into two major clusters (Figure 3). In the bacterial community, those soils at distances of 5, 15 and 25 m with relatively similar banding patterns clustered together, but those at 75-m distance formed another cluster (Figure 3a). The dendrogram of clustering for the fungal community was distinctly different from that for the bacterial community. Those soil properties and microbial populations at different sampling sites.

| Site | pH     | SWC (%) | SOM (%)  | AN (mg/kg) | AP (mg/kg) | AK (mg/kg) | Bacteria ($10^4$ cfu/g) | Fungi ($10^3$ cfu/g) |
|------|--------|---------|----------|------------|------------|------------|-------------------------|----------------------|
| 5 m    | 6.73 ± 0.10 a | 58.5 ± 2.2 b | 3.87 ± 0.27 ab | 159.4 ± 9.4 a | 6.72 ± 4.06 a | 51.6 ± 15.7 a | 10.60 ± 1.09 ab | 3.94 ± 0.82 b |
| 15 m   | 6.57 ± 0.25 ab | 68.0 ± 6.8 a | 3.61 ± 1.36 ab | 152.82 ± 1.5 b | 2.95 ± 0.41 a | 48.5 ± 9.4 a | 13.61 ± 4.39 a | 4.88 ± 0.29 a |
| 25 m   | 6.12 ± 0.21 bc | 69.5 ± 4.9 a | 3.03 ± 0.18 ab | 130.2 ± 12.4 bc | 3.13 ± 0.23 a | 37.8 ± 15.7 a | 7.98 ± 1.75 bc | 2.60 ± 0.39 c |
| 75 m   | 5.79 ± 0.47 c | 70.6 ± 3.7 a | 4.64 ± 0.18 a | 111.3 ± 13.2 c | 3.04 ± 1.60 a | 12.7 ± 0.8 b | 5.16 ± 0.27 c | 0.23 ± 0.05 d |

*Values are given as means ± S.D. (n = 3); values within a column followed by different lowercase letters are significantly different (n = 3, LSD, $p < 0.05$).

**Table 3 - Pearson’s correlation between soil properties, soil available nutrients and microbial populations.**

| pH | SWC (%) | SOM (%) | AN (mg/kg) | AP (mg/kg) | AK (mg/kg) | Bacteria ($10^4$ cfu/g) | Fungi ($10^3$ cfu/g) |
|----|---------|---------|------------|------------|------------|-------------------------|----------------------|
| pH  | 1.000   | -0.590*| 1.000      |            |            |                         |                      |
| SWC | -0.073  | -0.205  | 1.000      |            |            |                         |                      |
| SOM |         |         | 1.000      |            |            |                         |                      |
| AN  | 0.763**| -0.549  | 0.080      | 1.000      |            |                         |                      |
| AP  | 0.325   | -0.399  | 0.151      | 0.409      | 1.000      |                         |                      |
| AK  | 0.667*  | -0.453  | -0.365     | 0.610*     | 0.508      | 1.000                   |                      |
| Bacteria | 0.669* | -0.459  | -0.103     | 0.741**    | 0.122      | 0.497                   | 1.000                |
| Fungi | 0.730**| -0.379  | -0.442     | 0.721**    | 0.181      | 0.844**                 | 0.749**              |

*Significant at the 0.05 level; **significant at the 0.01 level.
samples at distances of 15, 25 and 75 m, except for sample 25I, were assigned to one cluster, while those at 5-m distance and sample 25I were detected in another cluster (Figure 3b). The richness and Shannon indices for bacteria and fungi both showed the minimum values at 15-m distance (Table 4). The bacterial and fungal Shannon-Weaver indices ($H'$) at four distances ranged from 2.772 to 3.071 and from 2.055 to 3.148, respectively (Table 4). The highest diversity ($H' = 3.071$) of bacterial community was found at a distance of 25 m, while that ($H' = 3.148$) of the fungal community was observed at a distance of 5 m, which was significantly different from the diversity at the other distances ($p < 0.05$, Table 4).

Nucleotide sequencing and analysis

Seven dominant bacterial bands (1B, 2B, 3B, 4B, 1b, 2b, and 3b) in Figure 2a and two specific fungal bands (1F and 2F) in Figure 2b were excised for sequence analysis. The BLAST analysis of the 16S rDNA sequences derived from TGGE gel bands classified these sequences into six main groups: *Chloroflexi* (1B), *Deltaproteobacteria* (2B), *Acidobacteria* (3B and 3b), *Betaproteobacteria* (4B), *Gammaproteobacteria* (1b) and *Alphaproteobacteria* (2b) (Table 5). Furthermore, band 2B was highly similar to the *Geobacteraceae* (96%) which was the closest cultivated relative of hits in GenBank database, and band 4B was highly similar to the cultivated relative *Curvibacter* sp.

| Treatment | Richness | Shannon ($H'$) |
|-----------|----------|---------------|
|           | Bacteria | Fungi | Bacteria | Fungi |
| 5 m       | 31 ± 1a  | 38 ± 3a | 2.886 ± 0.126b | 3.148 ± 0.098a |
| 15 m      | 29 ± 3b  | 15 ± 4b | 2.772 ± 0.109b | 2.055 ± 0.225b |
| 25 m      | 32 ± 1a  | 21 ± 10b | 3.071 ± 0.023a | 2.149 ± 0.754b |
| 75 m      | 30 ± 1a  | 18 ± 4b | 2.917 ± 0.053ab | 2.244 ± 0.113b |

*Values are given as means ± S.D. (n = 3); values within a column followed by different lowercase letters are significantly different (n = 3, LSD, p < 0.05).*
ATCC 700892 (98%), while other bacterial bands had no similar cultivated hits in GenBank database (Table 5). The two fungal 18S rDNA sequences fell within the phylum Chytridiomycota (1F) and Zygomycota (2F), respectively (Table 5). Based on the closest cultivated hit of GenBank database, bands 1F and 2F were similar to Chytriomyces poculatus (98%) and Lepidostroma rugaramae (89%), respectively (Table 5).

Noticeably, the bacterial bands (1B, 2B, 3B, and 4B) in Figure 2a getting stronger and stronger from good drainage (5 m) to poor drainage (75 m) and finally leading to dominant microorganisms at 75-m distance, were most similar to the sequences retrieved from waterlogged environments or flooding zones, like lake sediment, rice paddy soil, wetland and etc (Table 5). However, bands 1b, 2b and 3b in Figure 2a gradually turning fainter or disappeared with increase in distance started from the open drainage ditch, were most similar to the organisms found in grassland soil and dry land without water logging (Table 5). Furthermore, the specific 18S rDNA sequences (1F and 2F) derived from dank soils of 25-m and 75-m distances in Figure 2b were highly similar (> 96%) to microorganisms in aquatic environments like eutrophic lake, stream sediment (Table 5).

### Table 5 - Tentative identification of dominant TGGE bands in TGGE gels and the closet match to the sequence from GenBank database with BLAST.

| Clone (accession no.) | Dominant habitat | Phylogenetic group | Closest relative (accession no.) | Isolation source | Identity (%) | Closest cultivated relative (accession no.) | Identity (%) |
|-----------------------|------------------|-------------------|---------------------------------|-----------------|-------------|---------------------------------|-------------|
| 1B (HE867098)         | 75-m distance    | Chloroflexi       | UB (HQ636245)                   | Lake sediment   | 100         | -                               | -           |
| 2B (HE867099)         | 75-m distance    | Proteobacteria    | UB (HM487998)                   | Lake sediment   | 99          | Geobacteraceae (EF059536)       | 96          |
| 3B (HE867100)         | 75-m distance    | Acidobacteria     | UB (AB660646)                   | Rice paddy soil | 100         | -                               | -           |
| 4B (HE867101)         | 75-m distance    | Proteobacteria    | UB (HM535093)                   | Wetland soil    | 92          | **Curvibacter sp. ATCC 700892** (HM357758) | 98          |
| 1b (HE867102)         | 5-m distance     | Proteobacteria    | UB (EU298759)                   | Prairie soil    | 99          | -                               | -           |
| 2b (HE867103)         | 5-m distance     | Proteobacteria    | UB (JQ649765)                   | Polluted soil   | 100         | -                               | -           |
| 3b (HE867104)         | 5-m distance     | Acidobacteria     | UB (HQ597366)                   | Grassland soil  | 99          | -                               | -           |
| 1F (HE867105)         | 75-m distance    | Chytridiomycota   | UE (JQ689413)                   | Eutrophic lake  | 99          | **Chytriomyces poculatus** (EF443135) | 98          |
| 2F (HE867106)         | 25-m distance    | Zygomycota        | UE (AY689723)                   | Stream sediments | 99         | **Lepidostroma rugaramae** (FJ171731) | 89          |

\*alpha, beta, gamma, delta.
\*UB represents uncultured bacterium.
\*represents no closest cultivated hit in GenBank.

**Relationships between microbial diversity indices and other parameters**

The correlation analyses indicated that the richness indices and Shannon indices of fungi were negatively correlated with SWC (i.e., R = -0.667, p < 0.05 and R = -0.655, p < 0.05, respectively, Table 6), but those of bacteria were not. In addition, the richness indices of fungi was positively correlated with AP contents (i.e., R = 0.581, p < 0.05, Table 6). The Shannon indices of both bacteria and fungi showed significant positive correlation with the TGGE’s band number (i.e., R = 0.648, p < 0.05 and R = 0.954, p < 0.01, respectively, Table 6), but not with the number of culturable cells in Table 2.

### Discussion

Although many studies on the effects of agricultural management practices on soil microbial communities have

### Table 6 - Pearson's correlation between microbial diversity indices, soil properties and soil available nutrients.

| pH  | SWC (%) | SOM (%) | AN (mg/kg) | AP (mg/kg) | AK (mg/kg) | 16S R  | 18S R  | 16S H  | 18S H  |
|-----|---------|---------|------------|------------|------------|--------|--------|--------|--------|
| 16S R | -0.101  | -0.264  | -0.355     | -0.123     | -0.014     | -0.173 | 1.000  | -      | -      |
| 18S R | 0.439   | -0.667* | 0.096      | 0.432      | 0.581*     | 0.361  | 0.159  | 1.000  | -      |
| 16S H | -0.333  | 0.033   | -0.106     | -0.349     | 0.227      | -0.129 | 0.648* | 0.021  | 1.000  |
| 18S H | 0.456   | -0.655* | 0.165      | 0.329      | 0.539      | 0.300  | 0.086  | 0.954** | -0.033 | 1.000  |

*Significant at the 0.05 level; ** significant at the 0.01 level.
^represents Richness index.
^represents Shannon index.
focused on irrigation pattern, fertilization regime, and tillage system (Zhang et al., 2008; Beaugerard et al., 2010; Ceja-Navarro et al., 2010; Islam et al., 2011; Kamaa et al., 2011; Yang et al., 2011), only few studies have been done on ditch drainage. Ditch drainage with associated changes in soil properties led to distinct shifts of abundance and structure of bacterial and fungal communities, which significantly affected the soil ecosystem. Ditch drainage caused decrease in soil pH with increasing distance away from the open ditch (Table 2). Soil pH was one of the most influential factors in soil (Rousk et al., 2010), and strongly affected all chemical, physical and biological soil properties (Andersson et al., 2000; Brady and Weil, 2002; Jones et al., 2009; Rousk et al., 2009), which were further confirmed by our studies. Soil pH was shown to have significant positive correlation with contents of soil AN (R = 0.763, p < 0.01), AK (R = 0.667, p < 0.05), and numbers of viable bacteria and fungi cells (R = 0.669, p < 0.05 and R = 0.730, p < 0.01, respectively, Table 3).

Soil microbial abundance is strongly influenced by soil physical and chemical properties (Hrelová et al., 1999; Kobarli et al., 2010; Hong et al., 2011), including soil texture, soil aggregate, soil pH, and soil nutrient. However, all of these soil factors can be altered by changes of agricultural managements. In our study, drainage led to obvious change in all detected parameters except for AP along distance from the ditch. Among the detected parameters, soil pH and AN content were significantly and positively correlated with culturable aerobic bacterial counts and fungal counts, suggesting that both soil pH and AN content had marked influence on the growth of bacteria and fungi. Our findings were consistent with those of Brodie et al. (2002) that soil physicochemical factors, such as soil pH or AN content were the principal determinants controlling bacterial community in a field situation. In addition, AK content was significantly and positively correlated with fungi counts, indicating that soil AK content had an important impact on the abundance of culturable fungi in CWPS.

TGGE analysis revealed that some bands (from 1B to 7B) of bacteria became stronger with increasing distance, but some others turned fainter or disappeared (from 1B to 3B)(Figure 2a), which implied that some bacteria proliferated with increasing poor drainage, while some others were suppressed, e.g., at 75-m distance under poor drainage condition, minimum populations of culturable aerobic bacteria were found (Table 2). From good drainage (5 m) to poor drainage (75 m), some aerobic organisms become quiescent or die, and new inhabitants, possibly including facultative (organisms which can function under both aerobic and anaerobic environments) and obligate anaerobic bacteria, took over (Inglett et al., 2005). This was confirmed by the tentative identification of dominant TGGE bands in Table 5, for instance, the 2B band sequence was highly similar to the family Geobacteraceae, which were the obligate anaerobes predominating sedimentary environments (Snoeyen-bos-West et al., 2000; Holmes et al., 2007), and the 4B band sequence was highly similar to Curvibacter sp. ATCC 700892 belonging to microaerobic bacteria (Ding and Yokota, 2010).

Eickhorst et al. (2010) mentioned soil fungal communities are the most important group of organisms involved in decomposing organic matter. In paddy soils, they are predominant only after drainage and during post-harvest fallow conditions as they need oxygen which is limited under flooded conditions. In our study, at 5-m distance with relatively good drainage conditions, the fungal communities were much more diverse than those at other distances with relatively poor drainage conditions (Figure 2b). Additionally, significant differences (p < 0.05) of the diversity indices were noted between 5 m and other distances (Table 4). Furthermore, two clusters of fungal dendrogram clearly distinguished between 5-m and the other distances (Figure 3b). These results could be explained by the close relationship between SWC and ditch drainage, since the significant differences in SWC (Table 2) were identical with the fungal diversity indices (Table 4) and SWC was also significantly and positively correlated with the fungal diversity indices (Table 6).

In comparison with bacteria, fungi seemed to be more easily affected by soil factors. For instance, the fungal abundance was noticeably influenced not only by soil pH and AN content, but also by soil AK, and fungal diversity was significantly affected by SWC and AP contents, while the bacteria diversity was not correlated with SWC and AP. These results suggested that fungal communities were more sensitive to soil factors than bacteria communities. Therefore, changes in fungal community structures in agricultural soil might possibly serve as a sensitive indicator for changes in soil quality due to agricultural management (Kennedy and Smith, 1995; Schneider et al., 2010).

Compared to poorly drained paddy soils at 75-m distance, well drained soils at 5-m distance gave significantly higher values in soil available nutrients, culturable microbes (Table 2) and fungal diversity (Table 4), indicating the improvement of soil properties. In this experiment, the lack of knowledge of rice yields at four different distances was a weak point to judge the functioning of the drainage ditch. Therefore, further study should pay more attention to rice yields, soil characteristics, anaerobic microbial communities, draining rate and their relationships. It will help us know how to manage the drainage ditch.

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