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Shifts in microbial communities with increasing soil fertility across a chronosequence of paddy cultivation in subtropical China

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ABSTRACT

An understanding of microbial community assembly and succession are keys to uncovering mechanisms underlying soil fertility development. The dynamics of microbial communities during a paddy soil chronosequence were investigated by phospholipid fatty acid profiling and amplicon high throughput sequencing. The upper 20 cm were sampled from soils after 5, 15, 30 and 100 years of paddy use and from adjacent barren land. Enzyme activities and contents of soil organic carbon, nitrogen and phosphorus of paddy fields strongly increased compared to barren land, and continued to increase at least up to 100 years of paddy cultivation. The increasing soil trophic status favored bacteria over fungi, and fast-growing copiotrophic bacteria gradually replaced slow-growing oligotrophic bacteria. The genus *Ignavibacterium* with versatile metabolism was identified as an indicator of the bacterial community in year 30 and 100. The variations of bacterial α-diversity tended to stabilize, but species richness continued to increase after 30 years of paddy use. The β-diversity indicated that bacterial community structure in paddy fields differed from that in barren land. The soils of 5 and 15 years of paddy cultivation clustered into one group separated from the group formed from the year 30 and 100. Redundancy analysis indicated that two stoichiometric ratios: C/N and C/P were the major factors affecting microbial community succession. We conclude that long-term paddy cultivation resulted in changes in biochemical properties and variations in trophic pattern of microbial communities, corresponding to increasing soil fertility.

1. Introduction

Paddy soils are anthropogenic soils (Antrosols or Technosols according to WRB 2014) and are a major resource for food production. Important pedogenic processes and transformations associated with anthropogenic activities have been identified and are well described concerning paddy soil genesis (Chen et al., 2011; Cheng et al., 2009; Kogel-Knabner et al., 2010; Wang et al., 2015). During the process of paddy cultivation, soil fertility evolution is defined as change in the ability of a soil supplying nutrients to crops (Shang et al., 2014). Soil fertility is mainly regulated by plants and microorganisms (Hartman et al., 2008), and in turn soil properties are the major factor shaping microbial communities (Cline and Zak, 2015; Cui et al., 2012; Wang et al., 2015). However, it is hard to disentangle the relationship between nutrient accumulation and microbial community succession during paddy soil development.

Most previous studies have reported the profiles of soil properties at a given stage of paddy cultivation, and this may conceal the dynamics of soil development (Su et al., 2015). Chronosequence approach provide insight into the rates and directions of soil ecosystem evolution spanning multiple time-scales due to the advantage of space-for-time substitution (Jangid et al., 2013; Jones et al., 2009). The phyla *α-Proteobacteria* and *Verrucomicrobia* are major indicators for two stages of bacterial community succession in paddy soils: a rapid-succession with clear increases in bacterial diversity within the first several decades and thereafter, a long gradual-succession stage lasting for centuries (Cui et al., 2012). Many researchers have depicted the dynamics of soil microbial community succession along chronosequence, and have

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provided various explanations for these successions (Dini-Andreote et al., 2014; Jones et al., 2009). The shifts in bacterial communities were closely aligned with ecosystem development, pedogenesis and vegetative succession (Jangid et al., 2013). Distinct ecological niches are common for soil bacteria, which were suited for either fast-growing copiotrophic bacteria or slow-growing oligotrophic bacteria (Cederlund et al., 2014).

Numerous factors contribute to the succession of microbial communities, including soil nutrients, soil type, vegetable coverage and management (Cederlund et al., 2014; Girvan et al., 2003; Lauber et al., 2009; Sul et al., 2013). The shifts in the soil physical structure, as well as variations in pH and salinity predominate in bacterial community temporal succession in an undisturbed salt marsh chronosequence (Dini-Andreote et al., 2014). It remains unclear what are the main factors controlling the dynamics of microbial community succession in soil under long-term paddy cultivation.

The mechanism underlying the microbial community succession in distinct soil types might be inconsistent (Cline and Zak, 2015; Dini-Andreote et al., 2014; Huang et al., 2015). So, it is necessary to fully explain soil fertility evolution in a particular soil type to provide instructive guidance for paddy cultivation. In subtropical China, paddy soils derived from Quaternary red clay are generally deficient in available nutrients, and fertilization is always used to increase fertility (Li et al., 2000; Zhong and Cai, 2007). Most studies have reported the role of soil nutrient accumulation with the duration of paddy cultivation, but only few have assessed the successional patterns of microbial communities along paddy chronosequence (Cheng et al., 2009; Han et al., 2015).

We investigated soil microbial community evolution along a paddy chronosequence. For this purpose, four distinct paddy fields and adjacent barren land were identified: estimated as 0, 5, 15, 30 and 100 years of cultivated paddy soil from subtropical China. We hypothesized that soil nutrients were predominant in shaping microbial communities along the paddy chronosequence. We expected that oligotrophic phylotypes would dominate the initial stage of succession (0–15 Years) and be gradually replaced by copiotrophic bacteria during later succession (30–100 Years) with higher soil organic matter (SOM) levels. To validate this, the soil fertility conditions were quantified using nutrient contents: carbon (C), nitrogen (N), phosphorus (P) and potassium (K). Phospholipid fatty acid (PLFA) analysis was used to evaluate the soil microbial community succession along the paddy chronosequence. The 16S rRNA genes were used to identify bacterial community succession along the soil chronosequence. The activities of enzymes β-xylosidase (βX), β-glucosidase (βG), *N*-acetyl-glucosaminidase (NAG) and acid phosphatase (AP) were used to reflect the potential of SOM decomposition because these enzymes integrate information about microbial activity and physicochemical conditions (Blagodatskaya et al., 2016; Razavi et al., 2015; Sinsabaugh et al., 2014, 2008).

2. Materials and methods

2.1. Site description

An investigation was conducted at the Ecological Experimental Station of Red Soil in Yujiang County of Jiangxi Province, China (28°15′30″N, 116°55′30″E). This region is a typical subtropical monsoon climate with mean annual temperature of 17.6 °C and annual precipitation of 1795 mm. The sample plots were several hundreds of meters apart and were originally barren land that were used to cultivate double-cropping rice (*Oryza sativa* L.) over various periods of time spanning one century. All field plots had identical water and fertilizer managements: the plow horizon extended to 17–25 cm depth; routine irrigation and chemical fertilizers (all in kg ha^{-1}: 79 N, 79 P_2O_5 and 79 K_2O) were applied in each growing season; and straw of early rice was incorporated into soil after harvesting (Li et al., 2005). Ages of the 15-, 30- and 100-year soils were checked with published literature (Li et al., 2005, 2003). Ages of the 5-year soil were identified based on historical information with much help from experienced local experts. Each paddy chronosequence and the adjacent barren land consisted three field plots selected at random from local region.

2.2. Soil sampling and analyzing chemical properties

Each sample consisted five surface soil (0–20 cm) cores collected at randomly from each field plot. All soils were derived from Quaternary red clay. After removing root debris, each bulk sample was shock-frozen in a dry ice box and transported to the laboratory. One portion of each sample was freeze-dried and stored at −80 °C for PLFA and amplicon high throughput sequencing analyses and the other portion was stored at field moisture content at 4 °C for later determination of enzyme activities and microbial biomass C. The air-dried at room temperature was prepared for chemical analysis.

The chemical properties of soils were determined using the conventional methods described by Lu (1999). Soil pH was assayed using a pH meter (FE30, Mettler-Toledo, CH) with 1:2.5 soil:water suspension. Soil organic C (SOC) was determined by $K_2Cr_2O_7$—H_2SO_4 oxidation. Total and available N were measured as Kjeldahl-N; total P and available P were assayed by HF–$HClO_4$ digestion and sodium bicarbonate extraction (molybdenum blue method), respectively; total K and available K were determined by HF-$HClO_4$ digestion and ammonium acetate extraction (flame photometer), respectively.

2.3. Soil enzyme activity assays

The activities of βX, NAG, βG and AP were quantified fluorometrically using MUB-linked substrates: 4-MUB-β-D-xylopyranoside, 4-MUB-*N*-acetyl-β-D-glucosaminide, 4-MUB-β-D-glucoside and 4-MUB-phosphate, respectively (DeForest, 2009; German et al., 2011; Saiya-Cork et al., 2002). First, soil suspension was prepared by adding 1 g of fresh soil to 125 mL of 50 mM acetate buffer (pH 5.0) and homogenized for 2 min with an OMNI mixer (OMNI, LA, USA). Next, 200 μL of soil suspension was added to wells that each contained 50 μL of 10-μM MUB (4-methylumbelliferone) solution, 200-μM MUB-linked substrate, and acetate buffer. They severed as sample assay, quench standard, and soil control. Then 200 μL of acetate buffer was dispended in wells that each contained 50 μL of MUB solution, substrate solution, and acetate buffer. They served as reference standard, substrate control, and blank control. The reaction mixtures were thoroughly mixed before incubating in darkness at 20 °C for 2 h. After incubation, 10 μL of 1.0 M NaOH solution was added to each well to stop the reaction. Fluorescence immediately was measured using a microplate fluorometer (SpectraMax i3x, Molecular Devices, CA, USA) with 365-nm excitation and 450-nm emission filters (Saiya-Cork et al., 2002). Absolute enzymatic activities were expressed in units of nmol h^{-1} g^{-1} and calculated by the following equations (DeForest, 2009):

$$\text{Activity}(\text{nmol h}^{-1}\text{g}^{-1}) = \frac{\text{Net fluorescence}\times 125\text{mL}}{\text{Emission coefficient}\times 0.2\text{mL} \times \text{Time(h)} \times \text{Soil mass(g)}} \quad (1)$$

where

$$\text{Net fluorescence} = \left(\frac{\text{Sampl eassay} - \text{Soil control}}{\text{Quenchcoefficient}}\right) - \text{Substratecontrol} \quad (2)$$

$$\text{Emission coefficient}(\text{fluorescence nmol}^{-1}) = \langle \text{H2}\rangle\langle \text{H2}\rangle\frac{\text{Reference standard}}{0.5\text{nmol}} \quad (3)$$

$$\text{Quench coefficient} = \frac{(\text{Quenchstandard} - \text{Soil control})}{\text{Reference standard}} \quad (4)$$

The 125 mL in the first equation refers to the volume of soil

suspension and 0.5 nmol in the third equation is the amount of MUB standard added to a well. Fluorescence values were means from the four analytical wells subtracted from the blank. Soil mass (g) is the oven-dried weight.

2.4. Phospholipid fatty acid analysis

A modified Bligh–Dyer method (Bossio et al., 1998) was used to extract PLFAs. In the first step, 3 g of freeze-dried soil sample was added into a Teflon centrifuge tube with 15.2 mL of buffer (chloroform:methanol:phosphate at 1:2:0.8 volume ratio) to extract the PLFAs. The second step was separating polar lipids from extracted fatty acids in chloroform using a silica-bonded phase column (SPE-Si, Supelco, Poole, UK) with chloroform, acetone and methanol in that order. In the third step, polar lipids were transformed into fatty acid methyl esters (FAMEs) in the reaction of alkaline methanolysis trans-esterification. In the last step, FAMEs were quantified using nonadecanoic acid methyl ester (19:0) as the internal standard. They were determined by a gas chromatograph mass spectrometer (Agilent 7890, Santa Clara, CA, USA) and a MIDI Sherlock Microbial Identification System (MIDI, Newark, DE, USA) following the manufacturers' instructions. The PLFA contents were expressed as nmol g^{-1}.

PLFAs were separated into various taxonomic groups according to previously published PLFA biomarker data (Bossio et al., 1998; Frostegård et al., 1993; Green and Scow, 2000). In particular, the polyunsaturated PLFAs 18:2ω6c and 18:1ω9c were chosen as fungal biomarkers (Kaiser et al., 2010). The fatty acids 10Me16:0, 10Me17:0 and 10Me18:0 were biomarkers of actinomycetes (Green and Scow, 2000). The PLFAs 15:0, 16:0, 17:0 and 18:0 were biomarkers for general bacteria (Zelles et al., 1997). We used i15:0, a15:0, i16:0, i17:0 and a17:0 as biomarkers for Gram-positive bacteria; and 16:1ω7c, 18:1ω7c, cy17:0ω7c and cy19:0ω7c for Gram-negative bacteria (Green and Scow, 2000).

2.5. DNA extraction, PCR amplification and amplicon high throughput sequencing

DNA was isolated from 0.5 g of fresh soil samples using a FastDNA™ SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions. Primers (FP16S 5′-GTGCCAGCMGCCGCGG-3′ and RP16S 5′-CCGTCAATTCMTTTRAGTTT-3′) were used to amplify the genes targeting regions V4–V5 of 16S rRNA genes. Both the forward and reversed primers were added a unique 8-bp barcode at its 5′ end, so each sample was tagged by this barcode in the PCR. For each reaction, 20 μL of mixture was prepared and included 1 × reaction buffer, 0.3 mM dNTP, 0.25 μM F primer, 0.25 μM index primer, 1 U of Taq DNA polymerase (TaKaRa, Japan) and 1 μL of diluted template. The cycling program was 98 °C for 30 s, 11 cycles of 98 °C for 10 s, 65 °C for 30 s and 72 °C for 30 s and a final 5-min extension at 72 °C. For each sample, triplicate reaction mixtures were pooled together and purified using a QIAquick gel extraction kit (QIAGEN, Germany). The bar-coded PCR products from different samples were pooled in equimolar amounts and then sequenced using the pair-end method on the Illumina MiSeq platform at Genesky (Shanghai, China).

Sequencing raw data were merged by FLASH (Magoc and Salzberg, 2011) and then processed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (version 1.9.0) (Caporaso et al., 2010). Briefly, primers, barcodes, low-quality sequences (quality score $Q < 25$ or sequences shorter than 200 bp) or any unresolved nucleotides were discarded from analysis. High-throughput sequencing data were assigned to operational taxonomic units (OTUs) using UPARSE pipeline (Edgar, 2013) with a similarity threshold of 97%, and chimeric sequences were identified and removed. In total, there were 937,452 high-quality sequences obtained in 15 samples with 35,680–84,808 sequences per sample (mean 54,306). Each sample was rarefied to the same number of reads (35,680) for downstream analyses. OTU assignments were used to calculate the taxon frequencies for each sample. The Chao1 and ACE richness indices, Shannon diversity index, and a weighted UniFrac matrix (Lozupone and Knight, 2005) were calculated using the MOTHUR program (http://www.mothur.org).

2.6. Data analyses

One-way ANOVA with Tukey's test was conducted using SPSS ver. 16.0 for Windows (SPSS Inc., Chicago, IL, USA) to determine significant differences among paddy chronosequence ($P < 0.05$). Principal coordinate analysis (PCoA) based on a weighted UniFrac distance was performed to assess the temporal variation in phylogenetic β-diversity among different stages of succession. The PLFA and OTU datasets were Hellinger transformed before its downstream analysis. To test the influence of soil chemical parameters on community structure, forward selection was used on redundancy analysis (RDA) to select a combination of these variables that explained most of the variation observed in the bacterial 16S rRNA gene matrix and PLFAs. The correlations between soil chemical factors and microbial communities were tested by Monte Carlo permutations test (999 permutations). These analyses were performed in R (version 3.2.5; R Development Core Team) using vegan package for PCoA and RDA.

The indicators of bacterial communities in different stages of paddy cultivation were identified by the method of LEfSe (linear discriminant analysis effect size) (Segata et al., 2011). This method is available from the online interface at http://huttenhower.sph.harvard.edu/lefse/

3. Results

3.1. Changes in soil chemical properties during paddy soil chronosequence

Soil properties in paddy fields strongly increased compared to barren land. The pH value increased by 0.3 units within 5 years of paddy use compared to barren land, but thereafter decreased slightly to a stable 5.09 by 100 years. Most nutrient contents increased along the paddy chronosequence; but total K slightly decreased (Table 1). Soil organic C content increased by 1.4 times in 100-year soils compared to year 5. Total N and available N contents followed identical shift patterns to SOC, and increased by 1.4 and 1.6 times, respectively. Total P and available P contents slightly decreased during 5–30 years, thereafter gradually increasing to 0.95 g kg^{-1} and 74.6 mg kg^{-1}, respectively. The ratio of organic C to total N significantly increased by one fold within 5 years of paddy use compared to barren land, and thereafter remained at the same level. The ratio of organic C to total P significantly increased by 1.5 times in year 5 compared to barren land, and continued to increase to 37.7 in year 30.

The β-xylosidase (βX), β-glucosidase (βG), *N*-acetyl-glucosaminidase (NAG) and acid phosphatase (AP) activities strongly increased by 1.2–9.8 times within 5 years of paddy use compared to barren land. They significantly increased by 0.6–1.8 times in year 30 compared to year 5. The βX evidently increased by 1.6 times in year 30 compared to 100-year soil (Table 1).

3.2. Shift in microbial community composition along the paddy soil chronosequence

The amount PLFA contents and actinomycete, fungal and bacterial PLFAs increased by 2.8–10.2 times within 5 years of paddy use compared to barren land (Fig. 1). They significantly increased by 0.9–1.5 times in year 30 compared to year 5, and thereafter the fungal PLFA contents significantly decreased by 42% in year 100 compared to year 30. The ratio of fungal to bacterial PLFA significantly decreased by 64% within 5 years of paddy use compared to barren land. Thus, we concluded that fungal PLFAs dominated the barren land, and bacterial PFLAs dominated the paddy chronosequence. RDA indicated that C/N ($F = 29.53$, $P = 0.001$), Available P ($F = 11.54$, $P = 0.002$) and C/P

Table 1
Shifts in soil chemical properties along paddy soil chronosequence.

Items	0Y	5Y	15Y	30Y	100Y
pH	4.94 ± 0.07c	5.25 ± 0.03a	5.14 ± 0.05ab	5.06 ± 0.04bc	5.09 ± 0.02b
SOC (g kg^{-1})	2.01 ± 0.03d	11.29 ± 1.70c	12.12 ± 0.36c	17.35 ± 0.88b	26.67 ± 0.65a
TN (g kg^{-1})	0.40 ± 0.01d	1.10 ± 0.11c	1.27 ± 0.01c	1.72 ± 0.08b	2.66 ± 0.06a
Available N (mg kg^{-1})	23.28 ± 1.23d	78.40 ± 9.57c	98.00 ± 5.34c	123.73 ± 9.57b	205.8 ± 9.72a
TP (g kg^{-1})	0.26 ± 0.03c	0.56 ± 0.07b	0.51 ± 0.02b	0.46 ± 0.02b	0.95 ± 0.03a
Available P (mg kg^{-1})	0.55 ± 0.21d	19.54 ± 4.31b	12.57 ± 0.58bc	12.03 ± 0.81c	74.56 ± 2.40a
TK (g kg^{-1})	18.27 ± 1.75a	15.06 ± 1.70a	10.99 ± 0.10b	10.50 ± 0.10b	9.14 ± 0.13b
Available K (mg kg^{-1})	71.67 ± 14.46	73.33 ± 8.82	86.67 ± 24.17	64.17 ± 7.41	105.83 ± 11.02
C/N	5.0 ± 0.1b	10.1 ± 0.6a	9.5 ± 0.2a	10.1 ± 0.1a	10.0 ± 0.2a
C/P	8.0 ± 0.9d	19.9 ± 1.1c	24.0 ± 0.8bc	37.7 ± 0.9a	28.2 ± 1.2b
N/P	1.6 ± 0.2c	2.0 ± 0.1c	2.5 ± 0.1b	3.7 ± 0.1a	2.8 ± 0.1b
βX (nmol h^{-1} g^{-1})	10.7 ± 2.7d	23.6 ± 4.1 cd	30.8 ± 3.8bc	43.2 ± 4.3b	113.2 ± 7.1a
βG (nmol h^{-1} g^{-1})	28.1 ± 5.2c	304.3 ± 56.4b	391.9 ± 16.3b	836.9 ± 67.9a	678.5 ± 30.6a
NAG (nmol h^{-1} g^{-1})	26.5 ± 6.0d	70.9 ± 8.0c	76.2 ± 9.6c	133.5 ± 10.3b	145.1 ± 18.0 a
AP (nmol h^{-1} g^{-1})	259.6 ± 35.4c	744.9 ± 52.3b	749.4 ± 105.4b	1214.6 ± 32.2a	1210.8 ± 55.8a

All values are means ± standard errors, n = 3. Different lowercase letters in a row indicate significant difference ($p < 0.05$) along soil chronosequence. 5Y, 15Y, 30Y, 100Y represent paddy chronosequence, 0Y is barren land. SOC: soil organic carbon; TN: total nitrogen; TP, total phosphorus; TK, total potassium; C/N: ratio of SOC to TN; C/P: ratio of SOC to TP; N/P: ratio of TN to TP; βX: β-xylosidase; βG: β-glucosidase; NAG: *N*-acetyl-glucosaminidase; AP: acid phosphatase.

($F = 6.5$, $P = 0.008$) were the major explanatory factors for the shift in microbial community composition along the paddy chronosequence (Fig. 2).

3.3. Changes in α-diversity of soil bacteria over time

The ACE and Chao1 richness indices increased by 76.8 and 75.0% within 5 years of paddy use compared to barren land, respectively. They decreased slightly by 15 years and then increased gradually to stable values at 100 years. The Shannon diversity index clearly increased within 5 years of commencing rice cultivation, decreased slightly at 15 years and thereafter increased gradually to a stable value of 6.5 in year 30 and year 100 (Fig. S1).

3.4. Shift in phylogenetic β-diversity of soil bacterial community along paddy chronosequence

Principal coordinates analysis indicated clear clustering of the bacterial communities in different ages of the paddy use. Barren land evidently separated from paddy soil; 5-year and 15-year soils were clustered together, which differed from the group of 30-year and 100-year soils (Fig. S2).

The bacterial phyla strongly changed following the conversion to paddy soil. Mantel's test indicated a significant correlation between soil bacteria profiles and soil age (r = 0.62, $P < 0.001$). The phyla of

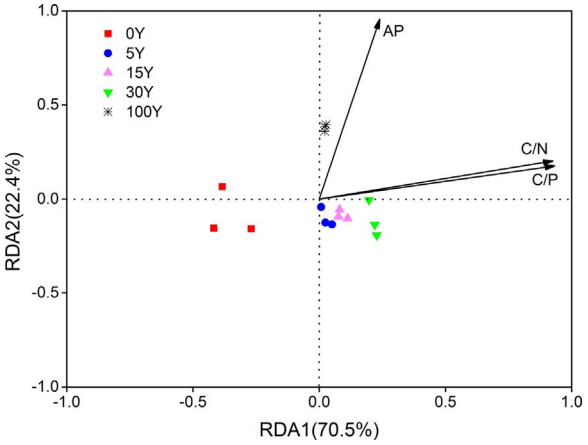

Fig. 2. Redundancy analysis for the relationship between the nutrients and PLFAs. SOC represents soil organic carbon and C/P is the ratio of C to total P. 5Y, 15Y, 30Y and 100Y represent paddy chronosequence, and 0Y is barren land.

Chloroflexi, *Acidobacteria* and *Proteobacteria* dominated the red soil. The proportion of the *Chloroflexi* phylum increased slightly at 5 and 15 years, and then decreased gradually to a stable value at 100 years (Fig. S3a). The proportion of the *Proteobacteria*, *Ignavibacteriae* and

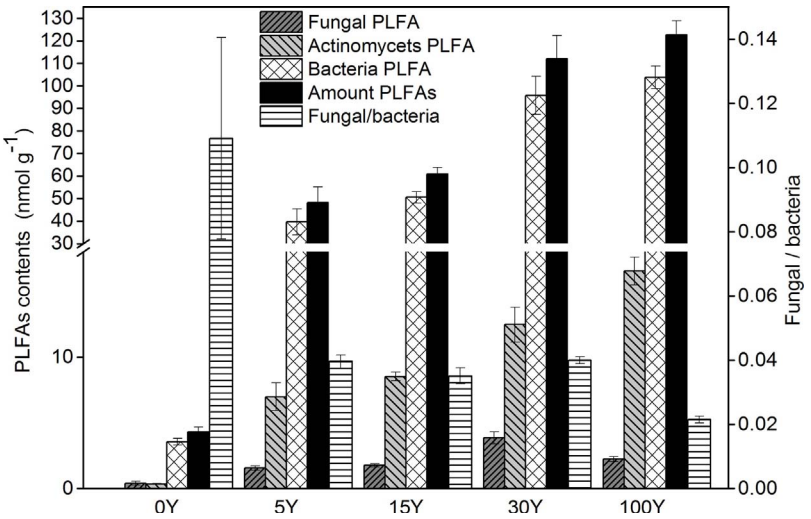

Fig. 1. Total PLFA concentrations and biomarker-PLFAs for bacteria, actinomycetes and fungi of paddy chronosequence. Error bars show the standard deviation. 5Y, 15Y, 30Y and 100Y represent paddy chronosequence, 0Y is barren land.

Nitrospirae phylum in paddy soil was higher than that in barren land (Fig. S3b, e and i). The proportion of the *Acidobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* phylum in paddy soil was lower than that in barren land (Fig. S3c, d, f and g).

Based on the phylogenetic β-diversity analysis, the paddy chronosequence were divided into three stages: 1) non-paddy, 2) paddy (< 30 years: including 5 and 15 years) and 3) paddy (≥30 years: including 30 and 100 years). The LEfSe results indicated that *Chloroflexi* phylum and *Acidobacteria-Gp2* class were indicators of bacteria communities in non-paddy soil; the *Anaerolineaceae* family became an indicator of bacteria communities in the initial stage of the paddy chronosequence, and thereafter the *Ignavibacterium* phylum was an indicator (Fig. S4).

3.5. The relationship between soil properties and soil microbial community composition

Soil bacterial communities in non-paddy field were separated from that in paddy fields along the RDA1 axis (82.3%), and the paddy fields cultivated for 5 and 15 years clustered together in the positive section of RDA2 axis (11.1%), while the paddy fields cultivated for 30 and 100 years clustered together in the negative section of RDA2 axis. RDA indicated that C/N (F = 82.00, P = 0.001), C/P (F = 11.13, P = 0.005), TP (F = 4.13, P = 0.038) and TK (F = 4.48, P = 0.038) were major factors to the shift of bacterial communities (Fig. 3).

4. Discussion

Because of heavy weathering and nutrient leaching in subtropical soil, the conversion of dryland or barren land to paddy fields increase land productivity (Li et al., 2005). Compared to barren land, paddy fields profoundly increased content of nutrients within the first 5 years of paddy cultivation. This is likely due to 1) mineral and organic fertilization, 2) high amount of crop residues returned to soil under a double-cropping system, 3) the high clay content in these soils decreases the decomposition of organic matter (Li et al., 2005, 2000). The paddy soil is P-limited and application of P fertilizer increases crop yield and enhances the soil fertility (Zhong and Cai, 2007). With increased duration of paddy use, nutrients gradually accumulated in the paddy chronosequence.

Nutrient cycling and mobilization from organic forms is mainly promoted by a series of enzymes. Enzyme activities profoundly increased during the 30-year and thereafter remains stable until 100 years

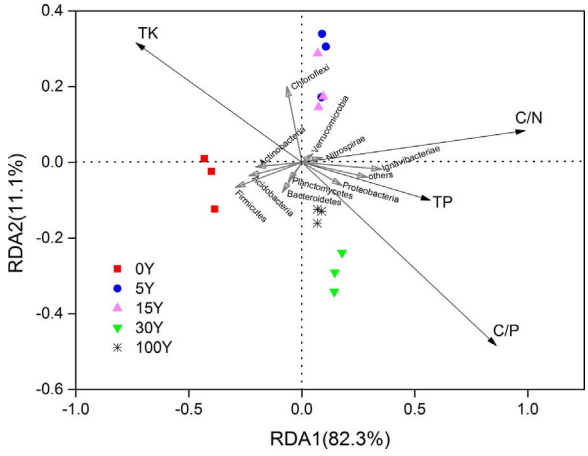

Fig. 3. Redundancy analysis for microbial community succession and its relationships with soil nutrients and stoichiometric ratios: C/P, C/N, TK and TP. 5Y, 15Y, 30Y and 100Y represent paddy chronosequence, and 0Y is barren land. C/P represents the ratio of carbon to total phosphorus; C/N represents the ratio of carbon to total nitrogen; TP represents total phosphorus; Total K represents total potassium.

(Table 1). Generally, it is considered that greater SOM accumulation can lead to increases in activity of enzymes 'simply' by providing a wider range of C and N substrates available to be utilized by a variety of soil microbial groups (Cenini et al., 2016; Sinsabaugh et al., 2009). Jiang et al. (2016) identified the associations between soil enzymes and the abundance of specific bacterial genera, particularly to Chloroflexi and Proteobacteria. In this study, we also found that these enzymes were positively correlated with bacterial PLFA concentrations. With prolonged rice cultivation, the increased soil fertility favored more microbial communities and enzyme production.

The concentrations of PLFAs in paddy soil significantly differed from barren land. In support of this, Bossio et al. (2005) proposed that soil type was the key determinant of bacterial community composition. PLFAs contents increased due to improved soil conditions, such as soil pH increasing by 0.31 and SOC increasing by 4.6 times. Long-term paddy cultivation stabilizes SOC and promotes microbial activity (Liu et al., 2016; Wang et al., 2015). The content of PLFAs, and of fungal and bacterial PLFAs, profoundly increased until 30 years and thereafter (except for a sharp decrease in fungal PLFAs) remained stable until 100 years. One possible explanation is based on the hypothesis that soil bacterial communities showed greater adaptability to local soil properties compared with fungal communities (Fernandez-Calvino and Baath, 2010; Liu et al., 2016).

With increased duration of paddy cultivation, the improved soil environmental conditions favored the growth of bacteria according to the PLFA data analysis. At the later stage of the paddy chronosequence, the ratio of Gram-positive to Gram-negative bacteria tended to be a stable value of 0.8, which is in line with the trend of Shannon diversity indices of bacterial OTUs (Fig. S1). In this study, high-throughput sequencing data indicated that the bacterial community gradually changed with rice cultivation age. According to the research of Cederlund et al. (2014), distinct ecological niches could explain the presence of the different soil bacteria phyla: fast-growing copiotrophic or slow-growing oligotrophic bacteria. Copiotrophic and oligotrophic microorganisms are discernible by their substrate affinity and growth kinetics for metabolism, and therefore the stability of population density as well as in the efficiency in resource utilization (Ho et al., 2017), among other characteristics and genomic features (e.g. number of rRNA gene operon; Klappenbach et al., 2000). The phylum of *Acidobacteria* is consistently associated with oligotrophic environments (Fierer et al., 2007), which is concordant with our result that *Acidobacteria* predominates in carbon-poor barren land (Fig. S3c). *Actinomycetes* and *Proteobacteria* are recognized as phyla associated to copiotrophy (Fierer et al., 2012), and in support of this, our result indicated that the concentration of biomarker-PLFA for *Actinomycetes* increased with the duration of paddy cultivation (Fig. 1). The trophic status of soil can also be speculated according to the ratio between *Proteobacteria* or *α-Proteobacteria* and *Acidobacteria*, with lower ratios found in oligotrophic environments (Hartman et al., 2008). This idea was also validated by Thomson et al. (2010), who found ratios of *α-Proteobacteria* to *Acidobacteria* of 1.44 and 2.25 in bare and vegetated soils, respectively. Our results indicated that the ratio between the relative abundances of *Proteobacteria* and *Acidobacteria* increased from 0.75 in barren land to 2.21 in paddy fields after 30 years of paddy use. Thus, we concluded that oligotrophic bacteria dominated the initial stage of bacteria community succession, and thereafter gradually changed to be dominated by copiotrophic bacteria in the later stage.

In this study, soil trophic status was suited to bacteria communities throughout the paddy soil chronosequence, which resulted in changes in indicator species. The conversion of barren land into paddy field induced changes in soil redox potential (Kogel-Knabner et al., 2010), and this was the main reason for the initial shift in indicator species. Thereafter, the genus *Ignavibacterium* was identified as an indicator of paddy soil cultivated for more than 30 years − Liu et al. (2012) proposed that this indicator genus with a versatile metabolism could be mixotrophic under certain growth conditions. We conclude that more

benign bacteria occurred with increased duration of paddy cultivation.

The dynamics of succession in the soil bacterial community was associated with variation of soil biochemical properties along the soil chronosequence. The stoichiometric variations of C, N and P significantly influenced microbial community succession along the paddy chronosequence. This result is not consistent with some former reports that proposed pH as the major determining factor in microbial community structure (Cline and Zak, 2015; Fernandez-Calvino and Baath, 2010; Rousk et al., 2010). In the present study, the variation in pH along the paddy chronosequence was not as notable as the stoichiometric variations in the chronosequence. The treated soils were all derived from Quaternary red clay and were deficient in soil nutrients, especially N and P. Long-term fertilization and straw incorporation into soil were incentives for the change in growth of microbial communities, and also promoted nutrient accumulation.

5. Conclusions

Soil nutrient contents and enzyme activities significantly increase with the duration of paddy cultivation. The oligotrophic bacteria were gradually replaced by copiotrophic bacteria, which responded to the increased soil fertility. After 30 years of paddy cultivation, the genus *Ignavibacterium* was identified as an indicator of bacterial communities for fertile paddy soils. The C/N ratio was the major driver for changes of microbial communities between barren land and paddy fields, and the C/P ratio mainly contributed to the shift in bacterial communities during the chronosequence of paddy cultivation. The results implied that fertility evolution of paddy soils was derived from the microbial community succession and nutrient accumulation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.07.031.

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