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ORIGINAL ARTICLE

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Bacterial succession during 500 years of soil development under agricultural use

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Abstract Soil bacterial succession under intensive anthropogenic disturbances is not well known. Using terminal restriction fragment length polymorphisms and 454 pyrosequencing of 16S rRNA genes, this study investigated how soil bacterial diversity and community structure changed under two agricultural land uses (paddy rice and upland cropping) in relation to soil development along a 500-year chronosequence created by intermittent reclamation of estuarine salt marshes. Multivariate analysis revealed orderly changes in soil physicochemical properties and bacterial community structure with time, confirming the occurrence of soil development and bacterial succession. Patterns of soil development and bacterial succession resembled each other, with recent land uses affecting their trajectories but not the overall direction. Succession of bacterial community structure was mainly associated with changes in α-*Proteobacteria* and *Verrucomicrobia*. Two stages of bacterial succession were observed, a dramatic-succession stage during the first several decades when bacterial diversity increased evidently and bacterial community structure changed rapidly, and a long gradual-succession stage that lasted for centuries. Canonical correspondence analysis identified soil Na^+, potentially mineralizable nitrogen, total phosphorous, and crystallinity of iron oxyhydrates as potential environmental drivers of bacterial succession. To conclude, orderly succession of soil bacterial communities occurred along with the long-term development of agroecosystems, which in turn was associated with soil physicochemical changes over time.

Keywords Pyrosequencing · T-RFLP · 16S rRNA gene · Salt marsh reclamation · Chronosequence · Yangtze Estuary

Introduction

The enormous microbial diversity in soil plays critical roles in ecosystem functioning (Chapin et al. 2002). The spatial/temporal patterns and drivers of this huge diversity in space and time have been the focus of microbial ecology but poorly understood. We are only beginning to understand the spatial patterns of bacteria (Fierer and Jackson 2006; Horner-Devine et al. 2004; Lozupone and Knight 2007; Wakelin et al. 2008), and even less is known about the temporal dynamics of bacterial diversity, particularly at long time scales (Bardgett et al. 2005). It has been hypothesized that bacterial diversity may exhibit qualitatively similar patterns to that of plants or animals (Horner-Devine et al. 2004). However, while changes in plant communities over time (i.e., succession) have been well studied, little is known about long-term bacterial succession, especially in such complex habitats as soils (Moore et al. 2010; Tarlera et al. 2008). This knowledge may provide insights into the patterns and drivers of soil bacterial diversity along with ecosystem development and have useful implications for sustainable ecosystem management. In agroecosystems, soil bacterial diversity is critical to soil and plant health because of the diverse metabolic functions of bacteria (Kennedy and Smith 1995). A better understanding of how the composition

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and diversity of soil bacteria change over time would provide important scientific bases for the development of sustainable agriculture. A recent study suggested that small-sized soil biota (e.g., bacteria, fungi, and protozoans) may be not as sensitive to agricultural practices as larger soil biota but predominantly affected by long-term changes in soil properties (Postma-Blaauw et al. 2010). However, although the short-term impacts of agriculture on soil bacterial communities have been extensively investigated (e.g., Sun et al. 2004; Yang et al. 2000), dynamics of agricultural soil bacteria at the time scale of centuries has been poorly understood. The chronosequence approach has been the most important method to study long-term soil microbial succession because it allows for space-for-time substitution (Bardgett et al. 2005; Tarlera et al. 2008). Previous studies using chronosequence have made great progress in understanding the patterns and environmental drivers of soil bacterial succession under natural conditions (Moore et al. 2010; Nemergut et al. 2007; Schütte et al. 2009; Tarlera et al. 2008; Williamson et al. 2005). However, little attention was paid to bacterial succession in disturbed soils, e.g., agricultural soils, especially at time scales longer than a century. This might be related to considerations of difficulties accompanying such studies, e.g., soils could not have developed under controlled conditions during hundreds of years and land management could have varied over time, which might obscure the patterns of bacterial succession. Recently, however, a series of explorative studies conducted on a 2,000-year rice cultivation chronosequence in the area of Cixi, Zhejiang Province, China have revealed clear temporal evolution of pedogenic properties (Cheng et al. 2009; Zou et al. 2011) and associated changes in the diversity and abundances of microbial groups (Bannert et al. 2011). These results suggested that even at a millennial scale, possible variations in management under a certain cropping system were not strong enough to obscure the overall patterns of soil physicochemical evolution or microbial succession. As almost the only tool to obtain empirical data of bacterial dynamics in agricultural soils at time scales of centuries to millennia, such cultivation chronosequence would provide valuable information for the sustainable management of agroecosystems. Chongming Island is the largest estuarine island in China (Gao and Zhao 2006). Historically, reclamation of emergent wetlands around the island has been an important way to obtain land resources (Zhou and Ji 1989). Early reclamation was performed by local landlords or farmers, which artificially accelerated the development of tidal wetlands towards agroecosystems. Since the 1950s, with an increasing demand of agricultural lands, the frequency of wetland reclamation reached nearly once every 2–3 years (Gao and Zhao 2006; Zhou and Ji 1989). Therefore, Chongming Island has chronosequences composed of soils at diverse developmental stages. Soils are all derived from sediments of the Yangtze River, which are dominated by hydromica minerals (He 1992) and have been developing under the same ecological conditions. The topography is rather flat, making the soil substrate more homogenous than that of chronosequences in other environments such as the montane glacier forelands (e.g., Sigler and Zeyer 2002). Information about the time of wetland reclamation is available in the Chongming County Annals (Zhou and Ji 1989) or in published works (Gao and Zhao 2006). In addition, cultivation rarely stopped in reclaimed soils since cropland used to be a limiting resource on the island (Zhou and Ji 1989). All those provided good opportunities for studying bacterial succession in relation to soil development under long-term agricultural activities. In this study, we examined how the diversity and structure of bacterial communities shifted in relation to soil physicochemical changes along a 500-year cultivation chronosequence on Chongming Island, China. The objectives of this study were (1) to test whether bacterial succession occurred along the soil chronosequence, and (2) to reveal the association between bacterial succession and environmental factors. For the first objective, we used 454 pyrosequencing to estimate bacterial taxonomic composition and diversity, and also determined bacterial community structure using terminal restriction fragment length polymorphisms (T-RFLP). For the second objective, we analyzed the correlation between T-RFLP profiles, and a comprehensive set of soil physicochemical variables with multivariate analysis.

Methods

Site description and soil sampling

Field sites were located in Chongming Island (31°27′–31°5l′N, 121°09′–121°54′E) of the Yangtze Estuary, China. The island has a typical north-subtropical monsoon climate, with a mean annual temperature of 15.3 °C and precipitation of 1,003.7 mm (Zhou and Ji 1989). Soil mineralogy is dominated by hydromica, with minor proportions of kaoline, chlorite, and vermiculite (He 1992).

The soil chronosequence identified in this study consisted of tidal wetland (salt marshes) soils and cropland (paddy rice and upland fields) soils that have been cultivated for about 8, 16, 40, 75, 120, 300 and 500 years since being reclaimed from wetlands (Fig. 1). Ages of the 8-, 16- and 40-year soils were checked with published literature (Gao and Zhao 2006; Zhou and Ji 1989). Ages of the 300- and 500-year soils were inferred from the earliest records available in the Chongming County Annals (Zhou and Ji 1989). However, ages of the 75- and 120-year soils were estimated by consulting at least five old local inhabitants and may be somewhat uncertain. All soils had a similar texture with dominance of silt-sized particles (Table 1).

Soils under paddy rice and upland cropping were chosen to account for the effects of agricultural land use

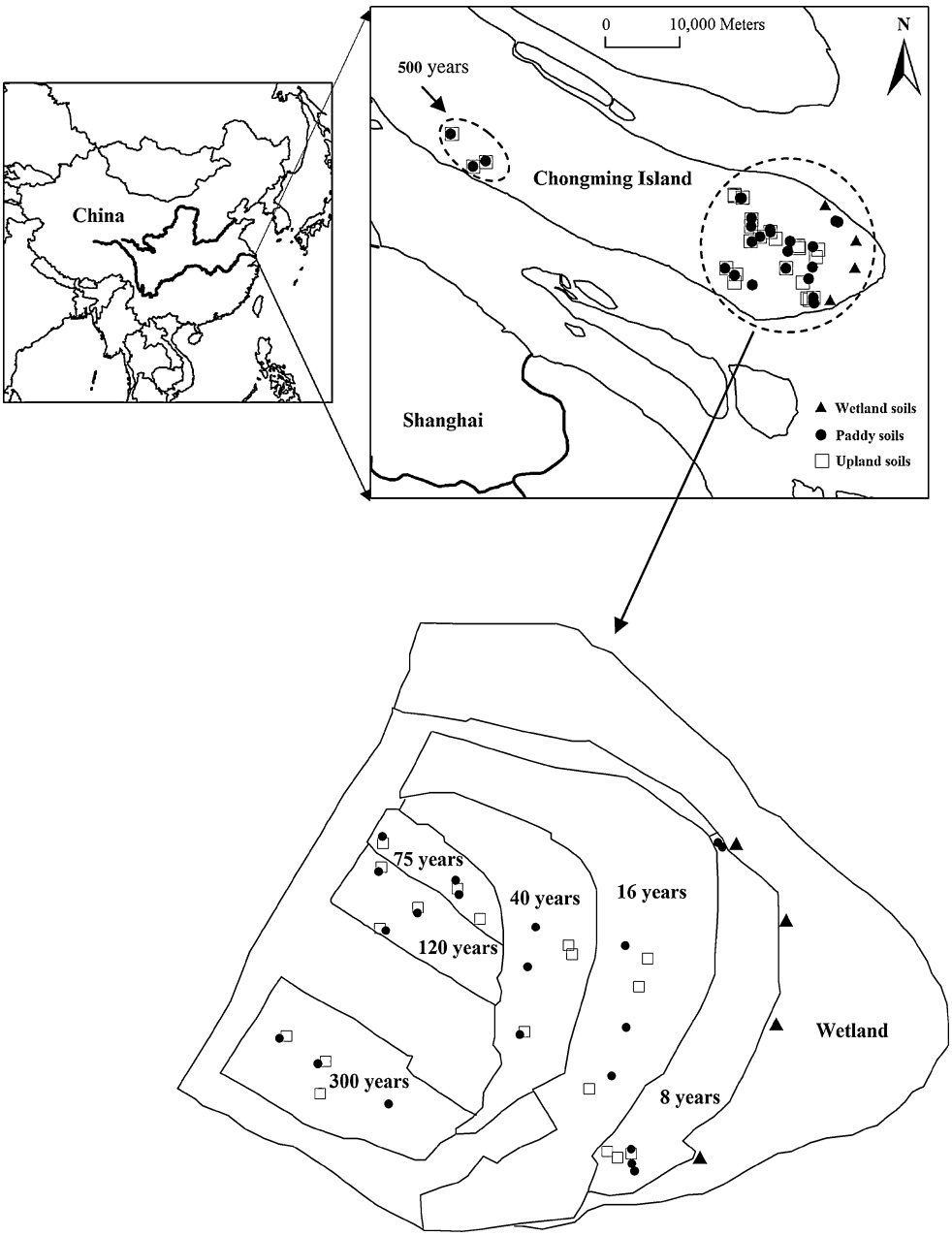

Fig. 1 Map of sampling sites. Locations of soils ≤ 300 years were amplified in the lower part of the figure, where the approximate spatial ranges of soil at different ages were indicated. Soil age is represented by years since reclamation of wetlands (salt marshes)

on bacterial communities. The cropping systems might not be continuous during the past centuries, but remained stable at least in recent decades. Agricultural management, such as the timing and rates of irrigation, fertilizer/pesticide application and the choice of crop cultivars, was comparable across the chronosequence because agricultural management has been uniformly instructed by the Chongming Agriculture Committee after the foundation of People's Republic of China in 1949. The major crops have been listed in Table 1. There was a rotation between rice cultivation and dry farming in the paddy croplands, or between different vegetables in the upland croplands (Table 1). Stubbles 15–30 cm high were commonly left in paddy fields after harvesting and about 2.35 t ha^{-1} stubble was annually returned to the soil. In contrast, there was almost no intentional return of crop residues in upland fields. Commonly used pesticides mainly included methamidophos, dimehypo, dichlorovos, and imidacloprid. Organic fertilizers were rarely used after the 1960s, and chemical fertilizers had been applied at a rate of about 400 kg ha^{-1} in recent years, of which N:P:K was estimated to be 1:0.33:0.09.

Wetland soils were sampled at four sites under the *Phragmites australis* communities in high-tide zone (Fig. 1), and at each site three 20×20 m^2 area was selected for sampling. For the paddy rice or upland soils, three to four sampling sites were selected within each soil age, and at each sampling site three replicate croplands 500–1,000 m apart were sampled. It was impossible to know the exact land use histories and hence we could

Table 1 Background information of soils along the chronosequence

Soil age (years)	Land use	Persisting years[a]	Main cover plants	Soil taxonomy[b]	Clay (%)	Silt (%)
0	Marsh	–	*Phragmites australis*	Solonchaks	13.25 ± 1.84	76.25 ± 0.24
8	Paddy	6	Rice, wheat/rape/barley	Fluvisols	11.75 ± 1.75	67.04 ± 2.86
	Upland	6	Cauliflower, rape, watermelon	Fluvisols	9.72 ± 0.76	69.93 ± 1.72
16	Paddy	14	Rice, wheat/rape/barley	Fluvisols	8.79 ± 0.94	69.90 ± 3.09
	Upland	>10	Cauliflower, rape, pakchoi	Fluvisols	6.49 ± 0.22	54.50 ± 2.05
40	Paddy	38	Rice, wheat/rape/barley	Fluvisols	10.83 ± 0.48	66.60 ± 2.15
	Upland	>30	Cauliflower, rape, pakchoi	Fluvisols	8.95 ± 0.40	69.70 ± 0.78
75	Paddy	>50	Rice, wheat/rape/barley	Fluvisols	11.17 ± 0.13	67.83 ± 0.32
	Upland	>50	Cauliflower, rape, pakchoi	Fluvisols	8.75 ± 0.11	66.80 ± 1.03
120	Paddy	>50	Rice, wheat/rape/barley	Fluvisols	9.92 ± 0.39	62.83 ± 6.00
	Upland	>100	Cauliflower, rape, pakchoi	Fluvisols	10.89 ± 0.72	75.07 ± 1.23
300	Paddy	>50	Rice, wheat/rape/barley	Fluvisols	11.67 ± 0.75	72.40 ± 0.46
	Upland	>100	Cauliflower, rape, pakchoi	Fluvisols	11.00 ± 0.35	76.13 ± 0.68
500	Paddy	>100	Rice, wheat/rape/barley	Fluvisols	14.37 ± 1.01	78.43 ± 0.56
	Upland	>100	Cauliflower, rape, pakchoi	Fluvisols	12.90 ± 1.12	79.97 ± 1.02

[a]Persisting years of land use at each site were taken as the minimal duration given by at least five local residents
[b]Based on the FAO/UNESCO taxonomy

only choose croplands known to have persisted for as long time as possible (Table 1). Six soil cores were obtained from individual croplands (or a 20 × 20 m^2 area for the wetlands) with a steel soil corer (5.4 cm inner diameter) to the depth of 8 cm and at intervals of 3–6 m along a diagonal transect. For molecular analyses, about 5 g of soil was dug from the middle of soil cores (i.e., at 4 cm depth, to represent the whole 0–8 cm soils) using an ethanol-wiped spoon and placed into a sterile plastic bag. Care was taken to avoid large plant roots in the bag. For any soil age, soils from replicate croplands were mixed, homogenized and immediately stored in a cooler packed with ice, resulting in three to four samples from each cropping system at each sampling site. The residual soils in the cores were also composited by sampling site and put into larger plastic bags for physicochemical analyses. All sampling was finished within 7 days in late March, 2009, when the paddy fields was in dry farming period after being drained, and the paddy and upland croplands were sampled simultaneously at each site.

Soil physicochemical analyses

A comprehensive set of soil physicochemical properties were measured in the laboratory and the analytical methods could be found in "Appendix". These soil properties included: soil bulk density (BD), particle size distribution (from which mean particle diameter (MPD), clay and silt contents were derived), mean weight diameter (MWD; Zhang and Horn 2001), carbonate content (IC), salinity, soluble cations (Na^+, K^+, Ca^{2+} and Mg^{2+}), free Fe and Al oxyhydrates (Fe_d and Al_d), amorphous Fe and Al oxyhydrates (Fe_o and Al_o), organic-complexed Fe and Al (Fe_p and Al_p), total P (TP; Pansu and Gautheyrou 2006), available P (P_o), cation exchange capacity (CEC), soil organic carbon (SOC), labile carbon (LC; after Blair et al. 1995), potentially mineralizable carbon (PMC) and nitrogen (PMN) (Wright et al. 2004), microbial biomass carbon (MBC), total nitrogen (TN) and inorganic nitrogen (NH_4^+ and NO_3^-). Magnetic susceptibility (MS) was also analyzed as an indirect proxy of cultivation duration.

DNA extraction and T-RFLP

For DNA extraction, three to four replicate soils at each soil age were freeze-dried after being taken to the laboratory and stored at −20 °C. DNA was extracted from 0.5 g soil with a MoBio Ultraclean™ Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. The extracted DNA was quantified by a PicoGreen assay with a ND-3300 NanoDrop Fluorospectrometer (Thermo, Wilmington, DE, USA). The 16S rRNA genes were amplified by polymerase chain reaction (PCR) with the FAM-labeled forward primer 27f (5′-[FAM]-AGAGTTTGATCCTGGCT CAG-3′) and the unlabeled reverse primer 1492r (5′-GGTTACCTTGTTACGACTT-3′) (Invitrogen, Shanghai, China). Six 50-μl reactions were prepared for each sample. The PCR program was: 5 min at 94 °C, followed by 30 cycles of 94 °C for 45 s, 56 °C for 45 s and 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The final reactions contained 0.05 U μl^{-1} Taq Plus polymerase (Tiangen, Beijing, China), 0.25 mM deoxynucleotide triphosphates (dNTPs), 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, both primers at 0.1 μM, nuclease-free water (Tiangen Biotech, Beijing) and 15 ng template DNA.

The six amplicons for each sample were pooled, purified using QIAquick gel extraction kits (QIAGEN, Hilden, Germany) and digested with *Hha*I and *Hae*III

(New England Biolabs, Beijing, China) in 30 μl reactions. For *Hha*I digestion, the mixture contained 3 μl of buffer, 0.75 μl of restriction enzyme (20 U μl^{-1}), 0.3 μl of bovine serum albumin (BSA, 0.1 μg μl^{-1}), 30–50 ng of amplicons and ddH_2O to adjust the final volume to 30 μl. For *Hae*III digestion, the mixture contained 3 μl of buffer, 0.75 μl of restriction enzyme (10 U μl^{-1}), 30–50 ng of amplicons and ddH_2O to adjust the final volume to 30 μl. Digests of both enzymes were incubated for 4 h at 37 °C followed by 20 min at 80 °C to deactivate the enzymes. Terminal fragment-size analysis was performed using a 3730 ABI electrophoretic capillary sequencer (Applied Biosystems) by GeneCore BioTechnologies Co. Ltd, Shanghai, China.

Barcoded 454 pyrosequencing

For soils at the same ages and under the same land use, equal amounts of DNA extracted from replicate soils were pooled and then 16S rRNA genes were amplified using the bacterial primer set 338f and 907r. The forward primer (5′-NNNNNNNN-TC-ACTCCTACGGG WGGCWGCAG-3′) contained an 8-bp barcode (designated by NNNNNNNN; Hamady et al. 2008), a "TC" linker and the bacterial 338f primer. The reverse primer contained an 8-bp barcode, a "TC" linker and the bacterial 907r primer (5′-NNNNNNNN-TC-CCCCGT CAATTYMTTTGAGTTT-3′). The PCR reactions contained 0.05 U μl^{-1} Taq Plus polymerase (Tiangen Biotech, Beijing, China), 0.25 mM dNTPs, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, both primers at 0.2 μM, nuclease-free water (Tiangen Biotech, Beijing) and 2 μl template DNA. Samples were initially denatured for 5 min at 95 °C, followed by 25 cycles of 1 min at 94 °C, 45 s at 54 °C and 45 s at 72 °C and 10 min at 72 °C for the final elongation. Four replicate PCR reactions were prepared for each sample, and the products were pooled to make a composite sample. The PCR products were then purified using QIAquick gel extraction kits (QIAGEN, Hilden, Germany), and sent to the Chinese National Human Genome Center (Shanghai) for pyrosequencing on a Genome Sequencer FLX System (Roche).

Processing of pyrosequencing data

Sequences were assigned to individual samples according to the 8-bp barcode. Then the barcodes were trimmed and sequences attached to the reverse primer were flipped. Only those sequences ≥450 bp in length, with no homopolymers longer than 8 bp and no ambiguous bases were included in subsequent analysis. The sequences were checked for chimeras with the Bellerophon tool (http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cgi) (Huber et al. 2004) and aligned using the NAST tool (http://greengenes.lbl.gov/cgi-bin/nph-NAST_align.cgi) (DeSantis et al. 2006). The chimera-checked high-quality sequences were degapped and polygenetically affiliated using the RDP Classifier (http://rdp.cme.msu.edu/classifier/classifier.jsp) at 80 % confidence threshold (sequences have been deposited in GenBank). The aligned sequences were then filtered with mothur (Schloss et al. 2009) to remove common gaps and trimmed to keep the region between positions 487 and 792 (*Escherichia coli* numbering), which covered the whole V4 region. For OTU-based analysis, sequences were clustered into operational taxonomic units (OTUs) at dissimilarities of 3 and 10 % with mothur, and then three diversity measures (Shannon, Chao1 and Simpson indices) were calculated. We fixed the number of sequences to be 425 in the calculation of diversity measures, which was close to the minimum number of reads (428) of all samples. This was conducted with mothur by randomly selecting 425 sequences from each soil for 1,000 times.

A quantitative polygenetic method of comparing microbial communities, the weighted UniFrac, was applied to the pyrosequencing data to reveal changes in bacterial community structure with time. UniFrac integrated taxonomic information at all levels of resolution and thus could avoid disadvantages of OTU-based methods (Lozupone and Knight 2008). An approximately maximum-likelihood tree was built with FastTree 2 (with options -gtr and -nt; Price et al. 2010) and inputted into Fast UniFrac (http://128.138.212.43/fastunifrac//) for UniFrac analysis.

Statistical analysis

To reveal patterns of soil development, principal component analysis (PCA) of soil physicochemical variables (except MS) was performed using PC-ORD 5.0 (MjM Software, Gleneden Beach, OR, USA), with the matrix of correlation coefficients used. Data of replicate soils were averaged prior to PCA. Profiles of T-RFLP were treated with the IBEST tool developed by Abdo et al. (2006). Only peaks in the fragment size range of 50–500 bp and of height >50 fluorescence units (FU) were included in subsequent analyses. The cutoff level of 50 FU was used in Culman et al. (2008) and we also used this threshold to filter noises because our pre-test confirmed that presence of peaks <50 FU had rather poor reproducibility between replicate runs. Peaks of different samples were aligned using the binning function in IBEST, with the clustering threshold set to be 1. Data from the digests of *Hha*I and *Hae*III for each sample were combined. Non-metric multidimensional scaling (NMDS; Fierer and Jackson 2006) was conducted with PC-ORD based on Jaccard distances calculated from T-RFLP profiles. For the pyrosequencing data, principal coordinate analysis (PCoA) based on weighted UniFrac distances (Lozupone and Knight 2007) was performed using the function provided by Fast UniFrac. The environmental fit function in the R package Vegan (Oksanen et al. 2007) was used to

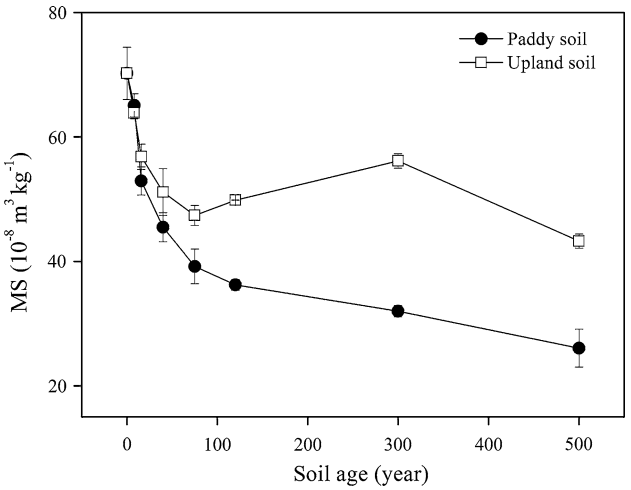

Fig. 2 Changes in soil magnetic susceptibility (MS) with time under paddy rice and upland cropping. *Error bars* represent the standard error

correlate PCoA scores to the relative abundances of each phylum. We also used Mantel's test in the R package Ecodist (Goslee and Urban 2007) to test whether physicochemical properties, T-RFLP profiles or UniFrac distances were significantly correlated with soil ages (log-transformed). ANOSIM was performed with Primer 5 (Primer-E Ltd., UK) to test whether between-age differences in soil properties or T-RFLP profiles were larger than the within-age differences. To find potential environmental factors influencing bacterial succession, canonical correspondence analysis (CCA) was performed between T-RFLP profiles and soil physicochemical variables (Fig. 7). For this aim, data of replicate samples were not pooled. We used the forward selection procedure in Canoco 4.5 (Plant Research International, The Netherlands) to find the most important soil variables influencing bacterial community structure at the 0.05 significance level with 1,000 permutation tests. Soil age was also included as a variable in CCA to identify the direction towards which T-RFLP profiles temporally changed, which helped to find soil variables best related to bacterial succession.

Results

Magnetic susceptibility

Soil MS was highest in the tidal wetlands (70.23×10^{-8} m^3 kg^{-1}) and showed a clear decreasing trend with time in the paddy fields, where the 500-year soils had a mean MS of 26.07×10^{-8} m^3 kg^{-1}. MS in upland fields also slightly decreased after reclamation of salt marshes, but did not change evidently between year 40 and 500 (Fig. 2). Land use had a significant influence on MS, with paddy fields having lower MS than upland fields ($F = 35.35$, $p < 0.001$).

Changes in soil physicochemical properties

Some important physicochemical properties were listed in Table 2. A rapid drop of NH_4OAc-extractable Na^+ was observed within 8 years after reclamation, but thereafter Na^+ decreased slowly to 24.33 mg kg^{-1} in

Table 2 Main soil properties mentioned in the text

Age (years)	Land use	Na^+ (mg kg^{-1})	IC (g 100 g^{-1})	pH	SOC (g kg^{-1})	MBC (mg kg^{-1})	TN (g kg^{-1})	PMN (mg N kg^{-1} day^{-1})	TP (P_2O_5%)	Fe_o (g Fe kg^{-1})	Fe_o/Fe_d (%)
0	Marsh	2761.00	7.89	8.17	15.68	86.41	1.30	0.83	0.22	8.12	85.68
8	Paddy	107.66	8.66	8.16	8.21	64.94	0.80	0.37	0.19	6.09	83.54
8	Upland	69.66	8.88	8.02	7.43	31.48	0.93	0.86	0.26	3.66	50.24
16	Paddy	208.00	9.19	8.24	5.83	68.81	0.59	0.62	0.21	4.19	71.89
16	Upland	93.00	8.82	8.28	5.56	68.31	0.67	0.71	0.25	2.66	48.33
40	Paddy	89.00	7.96	8.01	18.14	128.97	1.44	1.11	0.28	4.54	53.26
40	Upland	318.33	8.92	8.41	14.59	120.38	1.63	1.74	0.37	4.40	36.76
75	Paddy	120.33	6.66	8.11	14.47	103.35	1.24	0.92	0.30	4.74	44.47
75	Upland	142.66	7.56	8.31	9.88	91.70	1.20	1.24	0.34	2.68	36.83
120	Paddy	62.00	5.30	7.96	17.58	95.82	1.48	1.02	0.30	4.07	39.92
120	Upland	29.00	6.48	8.02	9.97	89.04	1.31	1.55	0.37	2.58	30.64
300	Paddy	41.33	5.30	7.96	19.76	159.41	1.56	1.32	0.32	2.83	39.69
300	Upland	57.00	5.43	7.87	16.41	145.88	1.37	2.00	0.35	2.15	42.57
500	Paddy	33.33	3.45	7.71	29.98	412.27	2.30	1.85	0.30	3.04	30.64
500	Upland	15.33	2.49	7.12	18.72	111.79	1.77	2.12	0.28	3.01	25.51

All values were averaged over replicate soils with the same ages. Standard error (see Table S1) was not shown here for clarity. *Age* years since reclamation of salt marshes; Na^+ was extracted by 1 M NH_4OAc; *IC* soil carbonates; *SOC* soil organic carbon; *MBC* microbial biomass carbon; *TN* total nitrogen; *PMN* potentially mineralizable nitrogen; *TP* total phosphorus; Fe_o amorphous Fe oxyhydroxides; Fe_o/Fe_d ratio of Fe_o to total free Fe oxyhydrates (Fe_d)

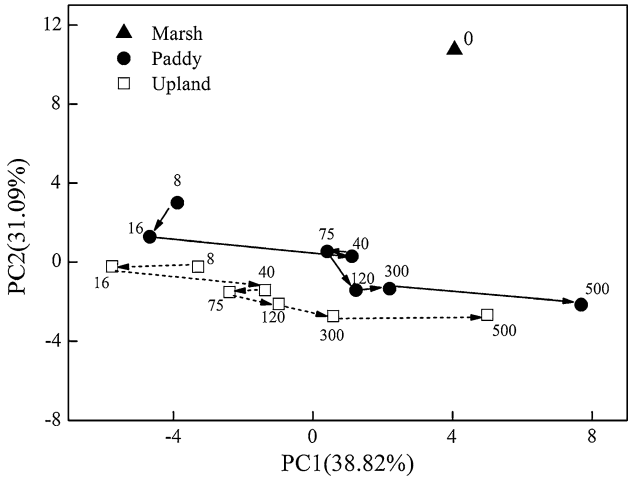

Fig. 3 Patterns of soil development as revealed by principal component analysis (PCA). PCA was based on 34 soil variables listed in Table 3. The percentages of total variance explained by the first two PCA axes (PC1 and PC2) are given in *parentheses*. Soil ages are labeled beside each *symbol*. *Arrows* indicate the direction of soil development under paddy and upland cropping

year 500. There was also evident loss of soil carbonates, which lowered soil pH from >8 before year 75 to 7.12 in the 500-year upland soils. SOC dramatically decreased within 16 years after reclamation, from 15.68 to 5.69 g kg^{-1}, then increased rapidly to 16.36 g kg^{-1} in year 40 and changed slowly thereafter. MBC declined slightly after reclamation but showed an overall increase from 48.21 mg kg^{-1} in year 8 to 262.03 mg kg^{-1} in year 500. The temporal dynamics of TN and PMN were similar to that of SOC. TP obviously accumulated in the first 40–75 years and fluctuated around 0.3 % in following years. Both Fe_o and Fe_o/Fe_d dropped greatly in the first 120 years (especially before year 16) and then remained relatively constant.

A total of 34 soil variables (Table S1) were used in multivariate analysis to depict patterns of soil development. ANOSIM revealed that differences in soil variables between different ages were significantly greater than that within ages (paddy soils: $r = 0.417$, $p < 0.01$; upland soils: $r = 0.407$, $p < 0.01$). Mantel's test suggested a significant correlation between soil physicochemical properties and soil age (paddy soils: $r = 0.66$, $p < 0.01$; upland soils: $r = 0.59$, $p < 0.01$). PCA revealed overall clear patterns of soil development (Fig. 3). The first two principal components (PCs) together accounted for 69.9 % of total variance, of which 38.8 and 31.1 % were explained by PC1 and PC2, respectively. Compared to PC2, PC1 more clearly separated soils by age, i.e., soil ages increased with PC1. It was notable that PC1 changed more dramatically within the first 40 years than in the following centuries, indicating that soil development proceeded at much higher rates before year 40 (Fig. 3). PC2 mainly differentiated

Table 3 Loadings of 34 soil variables on the first two principal components (PC1 and PC2) derived from principal component analysis (PCA)

Soil properties	Variable loadings		Soil properties	Variable loadings	
	PC1	PC2		PC1	PC2
SOC	0.95	−0.07	IC	−0.80	0.46
MBC	0.71	−0.28	TN	0.89	−0.32
PolyS	0.98	−0.01	NH_4^+	−0.28	0.76
LC	0.91	0.04	NO_3^-	0.09	−0.52
PMC	0.21	0.86	NH_4^+/NO_3^-	0.01	0.94
LC/SOC	−0.82	0.12	PMN	0.61	−0.59
PMC/SOC	−0.58	0.76	PMN/TN	−0.14	−0.52
MPD	−0.78	−0.21	P_o	−0.48	−0.33
BD	−0.64	−0.52	TP	0.22	−0.64
MWD	0.47	−0.62	P_o/TP	−0.65	−0.13
Salinity	0.21	0.91	Al_d	0.90	0.26
Na^+	0.25	0.89	Fe_d	0.87	0.27
K^+	0.22	0.87	Al_o	0.82	0.50
Ca^{2+}	−0.06	−0.07	Fe_o	0.15	0.95
Mg^{2+}	0.37	0.85	Al_p	0.87	−0.23
CEC	0.94	0.16	Fe_p	0.53	0.25
pH	−0.67	0.34	Fe_o/Fe_d	−0.40	0.77

PC1 and PC2 explained 38.82 and 31.09 % of total variance, respectively. Na^+, K^+, Ca^{2+} and Mg^{2+} were extracted by 1 M NH_4OAc. NH_4^+ and NO_3^- were the inorganic N extracted by 2 M KCl

SOC soil organic carbon, *MBC* microbial biomass carbon, *PolyS* polysaccharide, *LC* $KMnO_4$-oxidizable C, *PMC* potentially mineralizable C, *LC*/*SOC* ratio of LC to SOC, *MPD* mean particle diameter, *BD* bulk density, *MWD* mean weight diameter of aggregates, *CEC* cation exchange capacity, *IC* soil carbonates, *TN* total nitrogen, *PMN* potentially mineralizable nitrogen, P_o available phosphorus, *TP* total phosphorus, Fe_d (Al_d) free Fe(Al) oxyhydroxides, Fe_o (Al_o) amorphous Fe(Al) oxyhydroxides, Fe_p (Al_p) organic-Fe(Al) complexes

paddy from upland soils. Land use did not modify the direction but only caused displacement in the trajectories of soil development. Overall, soil age explained more variation of soil properties than land use, as indicated by Fig. 3 as well as ANOVA of individual soil variables (Table S2). Variable loadings on the two PCs were listed in Table 3, from which main temporal changes in soil properties can be inferred in conjunction with Fig. 3 (see Table S1 for detailed physicochemical data). Positive loadings on PC1 indicated increases in corresponding soil properties.

There were two turning points in the trajectories of soil development between year 8 and 16 as well as between year 40 and 75, both corresponding to decreases in PC1 (Fig. 3). Examination of variable loadings on this axis suggested that both turning points were associated with declines in SOC-associated properties (MBC, PolyS, LC, TN, PMN, etc.). Other temporarily decreased soil properties including Fe/Al oxyhydrates or complexes (Fe_d, Al_d, Al_o and Al_p) were also correlated with SOC (Table S3). It may thus be considered that the two temporary turning points were mainly caused by reductions in SOC. However, this should not influence the overall direction of soil development.

Phylogenetic affiliation of 16S rRNA gene sequences

We obtained 9892 high-quality 16S rRNA gene sequences with an average read length of 503 bp. There were 1638 (16.6 %) sequences unclassified at the phylum level. Of the classified sequences, 22 phyla were identified. The five most dominant phyla across all soils were *Proteobacteria* (29.2 %), *Actinobacteria* (13.1 %), *Acidobacteria* (9.6 %), *Chloroflexi* (9.3 %) and *Firmicutes* (8.1 %). The five most abundant families were *Anaerolineaceae* (5.6 %), *Sphingomonadaceae* (4.4 %), *Bacillaceae 1* (4.2 %), *Planctomycetaceae* (2.2 %) and *Rhodospirillaceae* (2.0 %). The five most abundant genus were *Bacillus* (4.1 %), *Sphingomonas* (3.9 %), *Gp4*

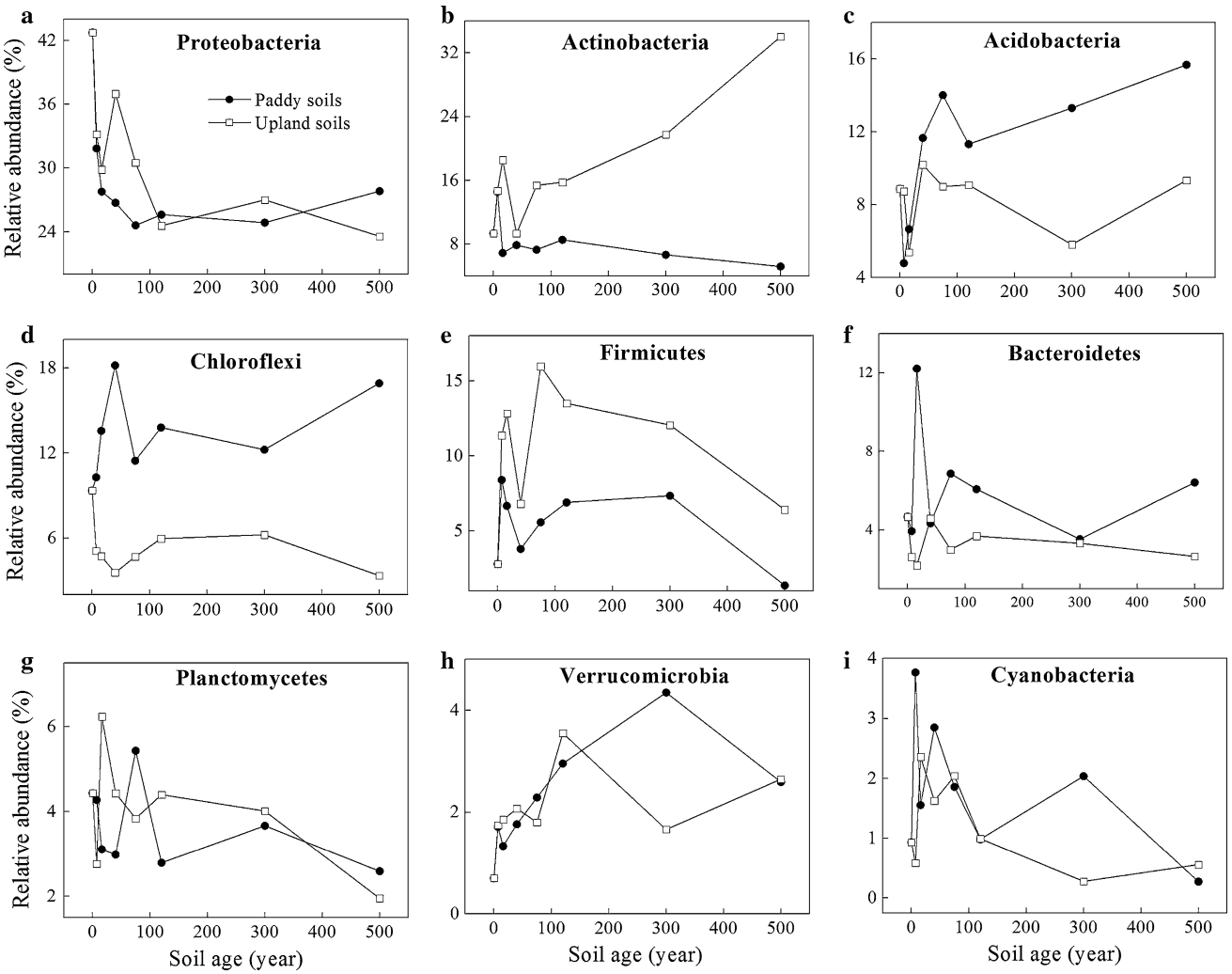

Fig. 4 Changes in the relative abundances of the nine most abundant bacterial phyla with soil development. The average relative abundances are in decreasing order from **a** to **i**. The 0-year soil represents the marsh soil

(1.4 %), *Gp16* (1.3 %) and *Ilumatobacter* (1.0 %). Detailed phylogenetic information of dominant bacteria in each soil has been provided in Table S4.

There were significant changes in the distribution of major bacterial groups, among which *Proteobacteria* changed most evidently. Its abundance was highest in the salt marsh (42.8 %), but decreased to 25.1 % within 120 years and remained around that level thereafter (Fig. 4a). In contrast, *Actinobacteria* increased with time in upland soils and peaked in year 500 (34.2 %; Fig. 4b), but showed an overall decrease in paddy soils. The temporal trends of *Acidobacteria*, *Chloroflexi*, *Fircumates*, *Bacteroidetes* and *Planctomycetes* were not clear (Fig. 4c–g). *Verrucomicrobia* increased with time during the first 120 years (Fig. 4h), while *Cyanobacteria* was more abundant in soils before year 75 (~2.0 %; Fig. 4i).

Obviously, paddy soils had higher abundances of *Acidobacteria*, *Chloroflexi* and *Bacteroidetes* than upland soils (Fig. 4c, d, f), while upland soils had more *Actinobacteria* and *Firmicutes* (Fig. 4b, e). In addition, more sequences in the paddy soils were unclassified than in the upland soils, especially after year 40 (data not shown).

Changes in bacterial diversity over time

In order to calculate bacterial diversity indices, the 16S rRNA gene sequences were clustered into OTUs. At the 3 % dissimilarity level, there was a total of 3609 OTUs, of which only 3.9 % were represented by ≥10 sequences and 54.4 % by one single sequence. No asymptote was seen for the rarefaction curves at the 3 % dissimilarity level (Fig. S1a, b). This was the case even when we defined OTU at the 10 % dissimilarity level, although the curves became flatter (Fig. S1c, d). Therefore, our sequencing depth was still far from enough for capturing the real extent of soil bacteria diversity.

The most evident improvement in bacterial diversity occurred within the first 40 years after reclamation of tidal wetlands, as clearly demonstrated by the increasing Shannon index (Fig. 5a, d). Consistent with this, bacterial richness (indicated by Chao1 estimator, Fig. 5b, e) and evenness (Simpson index, Fig. 5c, f) both increased during the same period. After year 40, however, all three indices changed slowly, although Shannon index seemed to decrease slightly and Simpson index increased at the 10 % dissimilarity level (Fig. 5b, f). Paddy soils had higher Shannon but lower Simpson index than upland soils, which was particularly evident after year 40.

Succession of bacterial communities

ANOSIM indicated that between-age differences in T-RFLP profiles were significantly larger than within-age differences (paddy soils: $r = 0.657$, $p < 0.01$; upland soils: $r = 0.553$, $p < 0.01$). Mantel's test indicated a significant correlation between T-RFLP profiles and soil age (paddy soils: $r = 0.623$, $p < 0.001$; upland soils: $r = 0.554$, $p < 0.001$). Orderly succession of bacterial community structure over time could be seen from the results of NMDS (Fig. 6a). The first axis (NMDS1) well separated soils by their ages, with older soils having higher scores, while the second axis (NMDS2) discriminated paddy from upland soils. The marsh soil was distantly separated from cropland soils. More dramatic changes in bacterial community structure were observed before year 40 than in the following centuries.

For the pyrosequencing data, PCoA based on the UniFrac distances also suggested orderly bacterial succession (Fig. 6b). The first axis (PCo1) separated the two land uses and the second axis (PCo2) separated soils at different ages. However, the successive order of soils was less clear than that revealed by T-RFLP analysis. This was especially true for upland soils, where the soils were largely in two clusters, soils ≤ 40 years and soils >40 years. Nevertheless, the overall trend that bacterial community structure changed over time largely resembled the pattern indicated by T-RFLP data. Mantel's test confirmed a significant relationship between UniFrac distances and soil age (for paddy soils: $r = 0.68$, $p < 0.01$; for upland soils: $r = 0.71$, $p < 0.01$), suggesting that soils with larger differences in age were more divergent in their bacterial communities.

Fitting of PCoA axes to the relative abundances of dominant bacterial phyla (Fig. 6b; Table S5) suggested that bacterial succession was mainly linked to the decreasing abundance of *Proteobacteria* (mainly the class α-*Proteobacteria*), and the increasing abundance of *Verrucomicrobia*. The divergence of bacterial communities between land uses was mainly related to *Actinobacteria, Firmicutes, Chloroflexi and Bacteroidetes*, and modestly to *Acidobacteria* and the unclassified bacteria (Fig. 6b).

Relationship between bacterial community structure and soil variables

The results of CCA indicated that 13 soil variables were significantly correlated with T-RFLP profiles. They together explained 60.9 % of the total variance in T-RFLP profiles, 28.1 % of which was explained by the first two axes ($p < 0.001$). The first axis mainly separated marsh soils from cropland soils (Fig. 7) and was best correlated to Na^+ (Table S6). The second axis mainly separated cropland soils by their ages, as indicated by the distinct clusters of soils ≤ 40 years and that >40 years, and was mainly related to Fe_o/Fe_d, PMN, TP, CEC, NH_4^+, SOC and soil age (Table S6). The biplot of CCA clearly showed that the vectors of TP, PMN and Fe_o/Fe_d were more closely correlated to soil age after reclamation and thus influenced bacterial succession more strongly than other soil variables (Fig. 7).

Discussion

Possible uncertainties in durations of cropping systems

Although there were reliable records on soil ages, the exact land use history was impossible to know, especially for soils older than 100 years. It was likely that the present cropping systems had once been interrupted during the last 500 years. However, our results would still provide useful information if the duration of paddy (or upland) cropping had been longer on older soil substrates. This was confirmed by measurements of MS.

It was well known that rice cultivation tended to reduce high-MS magnetic materials (mainly Fe minerals) into low-MS minerals (Chen and Zhang 2009; Lu 2003; Yu et al. 1981). This would result in that (1) soils under longer rice cultivation would have lower MS, which was absent in upland fields, and (2) upland soils usually had higher MS than paddy soils (Lu 2003; Yu et al. 1981). Data in Fig. 2 supported that duration of paddy rice cropping were indeed longer on older soil substrates. Similar observations have been reported by Chen and Zhang (2009) along a 1,000-year cultivation chronosequence of paddy rice. The slight declines in MS within 16 years under both paddy and upland cropping systems might be due to drops in Fe_o after reclamation (Table 2) and associated with leaching of dissolvable Fe oxyhydrates from original marshes (Luther et al. 1992), which contained most of magnetic materials and were subject to leaching after reclamation (Chi et al. 2009; Dong and Xu 1991; Yu et al. 1981). Hence, upland fields should not have undergone rice cultivation for long time because otherwise their MS would be close to that of paddy fields. The evidence by MS was only indirect and

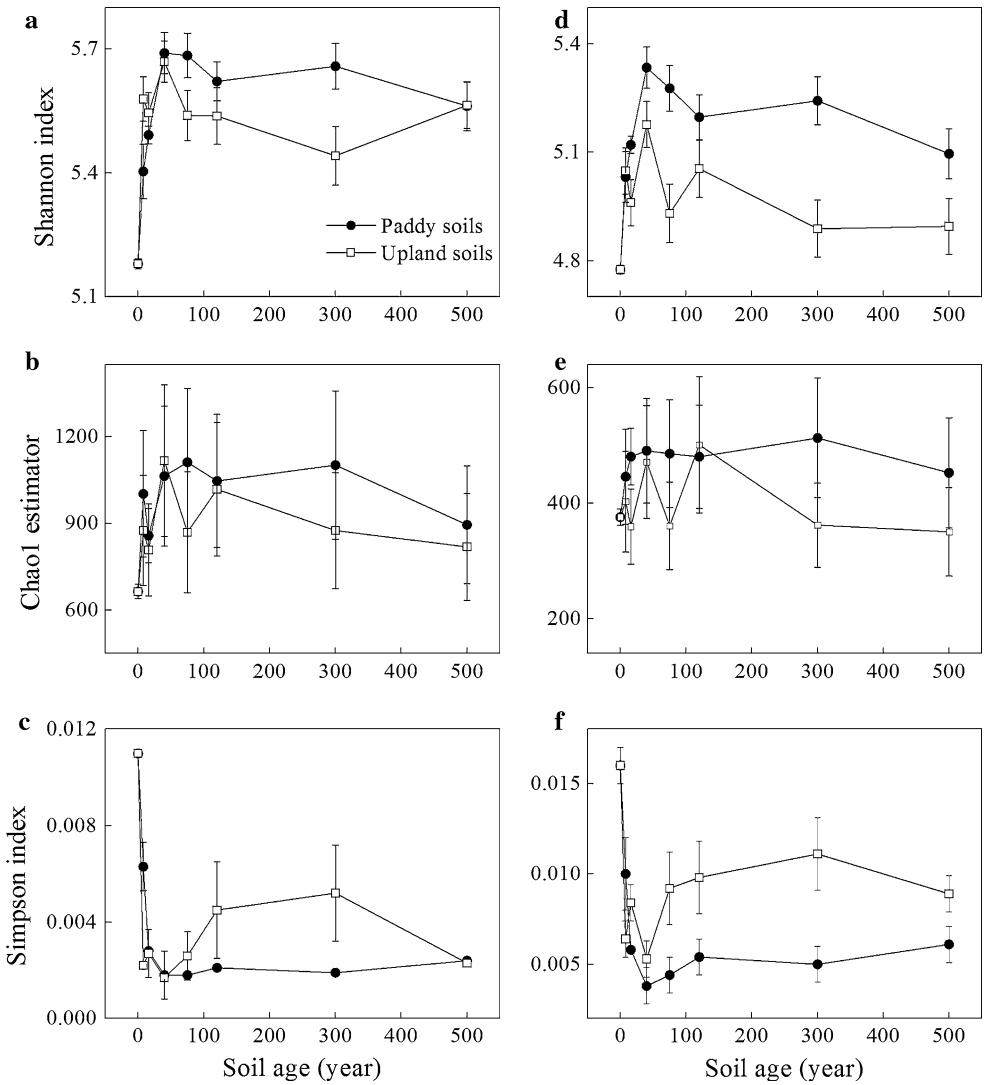

Fig. 5 Diversity indices calculated for OTUs defined at 3 % (**a**–**c**) and 10 % (**d**–**f**) dissimilarities. Chao1 estimator (**b**, **e**) is a measure of bacterial richness. Simpson index (**c**, **f**) measures bacterial evenness and the Shannon index (**a**, **d**) measures overall bacterial diversity. The 0-year soil refers to the marsh soil. *Error bars* represent 95 % confidence intervals

qualitative, but there was no better way to compare cultivation duration among soils.

Overall, it could be roughly concluded that the present cropping systems had not been changed for much time in history. Short-term interruptions of cropping systems might have occurred occasionally, but would not fundamentally modify previous soil properties and obscure the long-term bacterial succession patterns.

Evident soil development during 500 years

Our results indicated evident desalinization, decalcification of soil and accumulation of SOC (and SOC-associated properties such as TN, PMN and MBC) along the chronosequence, which were all typical pedogenic processes in reclaimed tidal wetland soils as reported by previous studies (Bannert et al. 2011; Cheng et al. 2009; Iost et al. 2007; Zou et al. 2011). After an initial depletion induced by wetland reclamation (Mitra et al. 2005), SOC rapidly increased within several decades and then accumulated slowly with cultivation time. This was consistent with Li et al. (2005), who found that SOC of paddy soils mainly increased during the first 30 years of rice cultivation on previous C-depleted soils. The buildup of soil P should be a result of P fertilization, as widely observed in the Yangtze River delta region (Darilek et al. 2010). Decreases in amorphous Fe oxyhydrates and increases in the crystallinity of Fe (indicated by decreasing ratio of Fe_o to Fe_d; Chi et al. 2009) were common pedogenic processes accompanying soil maturation (Bockheim et al. 1996; Tsai et al. 2007). All these observations, together with the clear soil development patterns (Fig. 3), confirmed that soils were developing in a chronosequential order. The two temporary turning points in soil developmental trajectories were both correlated with declines in SOC, which might be due to short-term management effects or some uncertainties in soil ages, but this did not affect the overall trends of soil development.

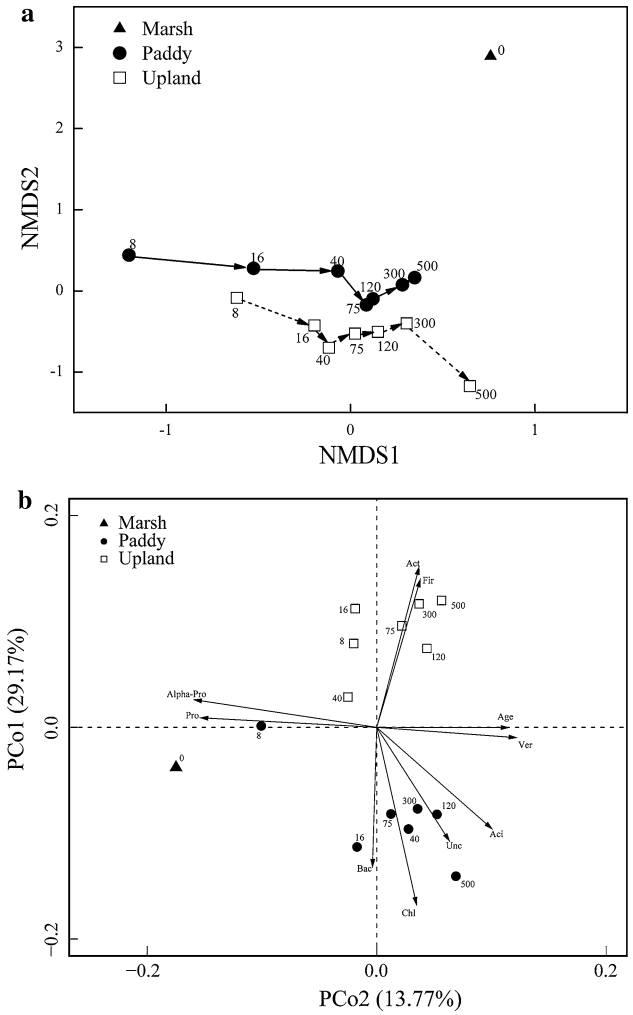

Fig. 6 Ordination of soil bacterial communities by nonmetric multidimensional scaling (NMDS) (**a**) and principal coordinate analysis (PCoA) (**b**). NMDS was based on T-RFLP profiles and PCoA based on UniFrac distances. *Numbers beside each symbol* indicate soil ages. *Arrows* in **a** indicate the direction of bacterial succession, and that in **b** represents bacteria phyla whose relative abundances were significantly correlated with bacterial succession. Soil age was also included in **b** as a vector to indicate the direction of bacterial succession. (Alpha-)Pro, (α-)*Proteobacteria*; Act, *Actinobacteria*; Fir, *Fircumates*; Ver, *Verrucomicrobia*; Aci, *Acidobacteria*; Chl, *Chloroflexi*; Bac, *Bacteroidetes*; Unc, unclassified bacteria; Age, soil age

Consistent with previous studies (Cheng et al. 2009; Zou et al. 2011), our results suggested that soil developmental patterns at long time scales could not be masked by short-term variations in agricultural practices. This might be because that agricultural management of the selected cropping systems did not fundamentally change in the past centuries (Zhou and Ji 1989), despite possible alterations in some aspects such as the use of fertilizers and tillage methods.

Orderly bacterial succession with soil development

Orderly soil bacterial succession was clearly seen from changes in their taxonomical composition with time (Fig. 4). Of the dominant phyla, decreases in *Proteobacteria* (especially α-*Proteobacteria*) and increases in *Verrucomicrobia* were mainly responsible for changes in bacterial community structure (Fig. 6b). Decreases in *Proteobacteria* with time were also reported in young deglaciated soils (Nemergut et al. 2007). It was unclear why cultivation led to declines of *Proteobacteria*, the most abundant soil bacteria (Janssen 2006). A possible speculation was that some groups of *Proteobacteria*, presumably inherited from the seawater, were less competitive than other bacteria in the agricultural environment. For example, the genus *Sphingomonas*, which had high abundances in all soils (1.0–14.2 %), evidently decreased with time after reclamation (Table S4). These bacteria were tolerant of the energy-poor environment in deeply buried sediments (>200 m) of coastal Atlantic plain (Fredrickson et al. 1999), but might not grow as well as other bacteria in well-managed

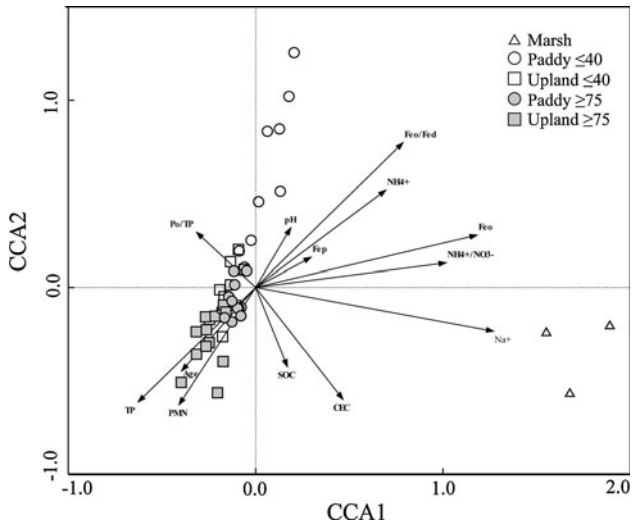

Fig. 7 Canonical correspondence analysis (CCA) showing the relationship between T-RFLP profiles and soil variables. *Vectors* represent soil variables that were significantly correlated to T-RFLP profiles. Each vector points to the maximum increasing direction of corresponding soil variable and its length indicates the strength of the correlation. Soils ≤ 40 years are differentiated from older soils. Abbreviations of soil variables are the same as in those in Table 3. Soil age was also included in the analysis to indicate the direction of bacterial succession

agricultural soils that had high carbon availability. *Actinobacteria*, another common bacterial phylum in agricultural soils (Kennedy 1999), seemed to be favored by upland cropping as it was distinctively abundant in the 300- and 500-year old upland soils (Fig. 4b).

Like soil development, temporal changes in bacterial community structure showed a common direction in both paddy and upland soils (Fig. 6). This might be because that the most abundant phylum, *Proteobacteria*, declined similarly under both cropping systems (Fig. 4a). However, the two type of soils still differed significantly in some bacterial groups, e.g., paddy soils had much lower abundances of the genus *Bacillus* (in the phylum of *Firmicutes*) than upland soils (Table S4), which were mostly aerobic endospore-forming bacteria favored by the upland environment (Gardener 2004). In contrast, the family *Anaerolineaceae* (in *Chloroflexi*) was more abundant in paddy soils, probably because that they usually grew under strictly anaerobic conditions (Yamada et al. 2006).

The different successional patterns of community structure revealed by T-RFLP and pyrosequencing data might be due to their different coverage of bacterial communities. Theoretically, T-RFLP covered all the extracted 16S rRNA genes of soil bacteria, while pyrosequencing only sampled a proportion of these sequences (659 sequences per sample). In addition, pyrosequencing targeted only a small region (305 bp) of the 16S rRNA gene, while T-RFLP used nearly the full-length gene (1465 bp). Therefore, we consider the community structure derived from T-RFLP to be more reliable than that from pyrosequencing.

Stages of soil bacterial succession after wetland reclamation

Changes in bacterial diversity (Fig. 5) and community structure (Fig. 6) both suggested that there were two stages of bacterial succession: a short dramatic-succession stage within the first 40 years when bacterial diversity increased and community structure greatly shifted, and a long gradual-succession stage that lasted for centuries when bacterial diversity and community structure changed slowly. Interestingly, we also noticed higher rates of soil development in the first 40 years than in the following centuries (Fig. 3). These were understandable since reclamation of tidal wetlands was a dramatic transformation of ecosystem states, which would lead to rapid changes in soil physiochemical properties and soil biota at the initial stage.

Improved carbon availability sometimes accounted for enhancements in bacterial diversity during soil development (Nemergut et al. 2007; Tarlera et al. 2008), but could not explain increases in bacterial diversity at the dramatic-succession stage of this study, when SOC and microbial biomass decreased with time in the first 16 years (Table 2). An alternative explanation might be the greater spatial isolation in soil matrix after drainage of wetlands, as indicated by higher extent of soil aggregation (Table S1). Zhou et al. (2002) suggested that water could disrupt spatial isolation of soil bacteria and increase chance of bacterial competition, leading to the dominance of certain species and decreasing microbial diversity. In agreement with this, Hartman et al. (2008) observed decreased bacterial diversity after restoration of agricultural lands to wetlands.

Mechanisms responsible for the long gradual-succession stage were unclear. Changes in Shannon and Simpson indices (Fig. 5) indicated that the overall diversity and evenness of bacteria slightly declined with time after year 40. Hence, we hypothesized that the intensive agricultural disturbances (e.g., fertilization, flooding) had selected for certain bacterial groups (e.g., *Actinobacteria*) best adapted to the cropland environment. This might explain why microbial biomass significantly increased after 500 years of cultivation (Table 2) while bacterial diversity did not. In support of this, Yang et al. (2000) reported that application of chemical fertilizers increased soil bacterial biomass but reduced diversity. The relatively higher bacterial diversity and evenness in paddy croplands (Fig. 5a, b, e, f) should be due to the frequent wet-dry cycles, which could create more niches and lessen the selective pressure on bacteria than under pure dry cropping. However, our results suggested that such selection occurred rather slowly and would not be obvious even after 500 years of cultivation.

Primary importance of soil development in driving bacterial succession

We found that soil development explained 60 % of variability in bacterial community structure. The

similarity between temporal patterns of bacterial communities and that of soil properties (Figs. 3, 4) strongly suggested that bacterial succession had been primarily driven by soil development. This was consistent with Moore et al. (2010), who proposed that changes in soil chemistry over time led to shifts in subsurface microbial community structure across a marin terrace.

One might suspect that both soil bacterial succession and soil development merely resulted from the evolution of agricultural management over time. This was unlikely because the most dramatic bacterial succession and soil development occurred before year 40, i.e., about 20 years after the foundation of People's Republic of China. Since that time, land management has uniformly followed the central instructions of local government and changed little (Zhou and Ji 1989). Obviously, the dramatic succession was mainly a function of rapid soil physicochemical changes induced by wetland reclamation. Although management of older soils (especially those after year 120) might have varied with time, bacterial succession in these soils proceeded very slowly in several centuries (Figs. 5, 6).

However, we have to acknowledge some effects of land management on bacterial communities, as reflected by variations in bacterial community structure within each soil age as revealed by T-RFLP data (Fig. 7). This might be somewhat due to inter-site variations in the rates or methods of pesticide and fertilizer application (Sun et al. 2004; Yang et al. 2000), which was unavoidable for field observations. However, both ANOSIM and Mantel's test indicated that variation within soil ages was significantly smaller than between different ages. A similar pattern was observed for soil physicochemical properties, implying that even within-age variations in bacterial communities were to some extent affected by edaphic variables. Therefore, soil properties rather than management should be the primary controller of bacterial community structure in this study.

Soil environmental factors influencing bacterial succession

Relationships between soil microbial diversity and environmental factors are of primary interests in microbial ecology but not well understood (Fierer and Jackson 2006; Lozupone and Knight 2007; Wakelin et al. 2008). In this study, drop of Na^+ (an indicator of soil salinity) was primarily responsible for changes in bacterial communities induced by wetland reclamation (Fig. 7). This agreed with Lozupone and Knight (2007), who considered salinity to be the most important determinant of global bacterial distribution. The reason why bacterial succession was strongly correlated with Fe_o/Fe_d (an indicator of Fe crystallinity; Chi et al. 2009) in cropland soils might be due to the importance of amorphous minerals to soil biogeochemical processes. These minerals had high surface areas to adsorb soil organic matter. Torn et al. (1997) suggested that Fe crystallinity could affect the turnover of soil carbon, which would further affect energy availability to soil microbes. Soil P (TP) and N availability (PMN) also exerted important influences on bacterial succession in croplands (Fig. 7; Table S6). P and N have been identified as two most important limiting nutrients for plants during long-term soil and ecosystem development (Vitousek and Farrington 1997). Developmentally young soils were generally limited by N availability while old soils were limited by P (Chadwick et al. 1999; Vitousek and Farrington 1997). It was thus understandable that changes in TP and PMN influenced soil microbial succession (Williamson et al. 2005).

Conclusions

This study provided insights into how the bacterial diversity and community structure might change in relation to soil development under long-term agricultural cultivation of soils reclaimed from tidal wetlands. The anthropogenic disturbances in croplands did not mask the clear temporal trends of soil development and bacterial succession. Along with the duration of cultivation, the relative abundances of dominant bacterial phyla (particularly *Proteobacteria* and *Actinobacteria*) evidently changed. Different cropping systems differed significantly in their bacterial composition, but likely had common effects on some bacterial groups, mainly the *Proteobacteria*. At a temporal scale of several centuries, bacterial succession was primarily driven by soil physicochemical development. It was notable that subsequent to the initial rapid bacterial succession after reclamation, soil bacterial diversity and community structure changed slowly during several centuries of cultivation, the mechanisms and implications of which deserve further research.

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Appendix: Methods of soil physicochemical analyses

Soil bulk density (BD) was determined by oven-drying soil cores of a fixed volume at 105 °C to constant weight. Soil particle size distribution was analyzed with a LS 230 laser particle size analyzer (Beckman, USA) and the mean particle diameter (MPD) was calculated using the software equipped with LS 230. Aggregate distribution analysis was performed by wet-sieving through a series of sieves with a vibratory sieve-shaker (Analysette 3,

Fritsch, Germany), and the obtained data were used to calculate mean weight diameter (MWD). Soil pH was measured on soil slurry at 2.5:1 water: soil ratio using a glass electrode. Carbonate content (IC) was determined by back-titrating soils neutralized with excessive 1 M HCl. Soil salinity was measured with a platinum electrode in the supernatant of soil slurries at 5:1 water: soil ratio and expressed as the percentage of total water-soluble salts on the dry weight base. Soluble cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) were extracted with 1 M NH_4OAc at pH 7.0. Free Fe and Al oxyhydrates (Fe_d and Al_d, respectively) were extracted with citrate–dithionite–bicarbonate (DCB), and amorphous Fe and Al oxyhydrates (Fe_o and Al_o) with oxalic acid–ammonium oxalate. Complexed Fe and Al (Fe_p and Al_p) were extracted with sodium pyrophosphate at pH 8.5. The extracted Na, K, Ca, Mg, Fe and Al were then measured with a P-4010 inductively coupled plasma (ICP) spectrometer (Hitachi Ltd., Japan). Total P (TP) was also measured with ICP after fusion with lithium metaborate at 1,000 °C. Available P (P_o) was extracted with 0.5 M $NaHCO_3$ at pH 8.5 and measured by colorimetry. Cation exchange capacity (CEC) was measured with the ammonium acetate method. Soil organic carbon (SOC) was measured with a TOC analyzer (Analytikjena HT1300, Germany) after removing soil carbonates with 1 M HCl. Labile carbon (LC) was estimated as the SOC fraction oxidizable by 333 mmol $KMnO_4$. Potentially mineralizable carbon (PMC) was determined by a 28-day incubation of 45 g field moist soils at 25 °C. The soil was placed in a 50-ml beaker, together with a plastic vial containing 20 ml of 1 M NaOH. The amount of CO_2–C trapped in NaOH (i.e., PMC) was quantified by titration with 0.5 M HCl after precipitation of Na_2CO_3 with $BaCl_2$. Potential mineralizable nitrogen (PMN) was determined as changes in the sum of NH_4^+ and NO_3^- after incubation at 25 °C for 28 days. Microbial biomass carbon (MBC) was determined by the $CHCl_3$ fumigation-extraction method. Total nitrogen (TN) was determined with a C/N elemental analyzer (FlashEA 1112 NC analyzer, Thermo, Italy). Inorganic nitrogen (NH_4^+, NO_3^-) was extracted by 2 M KCl and measured with a discrete auto analyzer (Smartchem 200, Westco, France). Magnetic susceptibility (MS) was determined with a magnetization meter (MS-2B, Bartington, UK) at the frequency of 0.47 kHz.

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