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Article

Chronosequencing methanogenic archaea in ancient Longji rice Terraces in China

Youzhi Feng [a], Jan Dolfing [b], Zhiying Guo [a], Jianwei Zhang [a], Ganlin Zhang [a], Shijie Li [c,d,*], Xiangui Lin [a,*]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] School of Civil Engineering and Geosciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK
[c] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China
[d] State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China

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ABSTRACT

Chronosequences of ancient rice terraces serve as an invaluable archive for reconstructions of historical human-environment interactions. Presently, however, these reconstructions are based on traditional soil physico-chemical properties. The microorganisms in palaeosols have been unexplored. We hypothesized that microbial information can be used as an additional proxy to complement and consolidate archaeological interpretations. To test this hypothesis, the palaeoenvironmental methanogenic archaeal DNA in Longji Terraces, one of the famous ancient terraces in China, dating back to the late Yuan Dynasty (CE 1361–1406), was chronosequenced by high-throughput sequencing. It was found that the methanogenic archaeal abundance, diversity and community composition were closely associated with the 630 years of rice cultivation and in line with changes in multi-proxy data. Particularly, the centennial- and decadal-scale influences of known historical events, including social turbulences (The Taiping Rebellion, CE 1850–1865), palaeoclimate changes (the Little Ice Age) and recorded natural disasters (earthquakes and inundation), on ancient agricultural society were clearly echoed in the microbial archives as variations in alpha and beta diversity. This striking correlation suggests that the microorganisms archived in palaeosols can be quantitatively and qualitatively analyzed to provide an additional proxy, and palaeo-microbial information could be routinely incorporated in the toolkit for archaeological interpretation.

1. Introduction

Human history is intimately twined with past environmental and climatic changes [1,2]. Unraveling these interactions may shed light on possible turning points in the history of civilization and may give fresh insights for predicting its potential future trajectory [3,4]. Terraced paddy soils provide an unparalleled archive for such reconstructions [5,6]. As symbols and testimony of man's millennia-old ability to utilize and reform nature, terraces are among the most striking anthropogenic imprints on earth [6]. In parallel, rice cultivation has a long history of 8000–9000 years [7], and plays an important role in building civilizations [8] and shaping societies [9]. The pedogenic processes and transformations of paddy soils have been identified to be closely associated with anthropogenic activities [10].

Longji Terraces are one of the iconic, world-famous ancient terraces for rice cultivation, dating back to the late Yuan Dynasty (CE 1361–1406) (Fig. 1) [11]. Structurally, they are generally parallel to the contours of the hillslope, consisting of flat treads (cultivated areas), terrace walls (risers), and field ridges on the top of the risers. Their unique gravity based irrigation method continuously brings in sediment from the original slope soil and/or cultivated soils of terraces higher up on the mountain and annual heightening of field ridges ensures that sediments are preserved on terrace lands. Thus, with long-term rice cultivation, the plough layer of terraces has gradually turned into plow pan, a densified soil layer with low infiltration, making the cultivated horizon a bottom-up chronological sedimentary sequence and their soil physico-chemical properties reflect historical human-environment interactions (Fig. S1) [11,12]. Using radiocarbon dating, and physical and geochemical analyses, in combination with the analyses of *n*-alkanes and organic carbon stable isotopes ($\delta^{13}C_{org}$), these authors investigated four profiles of Longji terraced paddy soils and consistently find the intimate relations between farming activities and environmental changes over the past few hundred years. This finding strongly demonstrates terraced paddy soils as the convincing subject material for palaeoenvironmental reconstruction.

Comprehensive historical reconstruction generally requires multiple proxies and more archaeological evidence can add illumi-

* Corresponding authors.
E-mail addresses: xglin@issas.ac.cn (X. Lin), lishijie@vip.gyig.ac.cn (S. Li).

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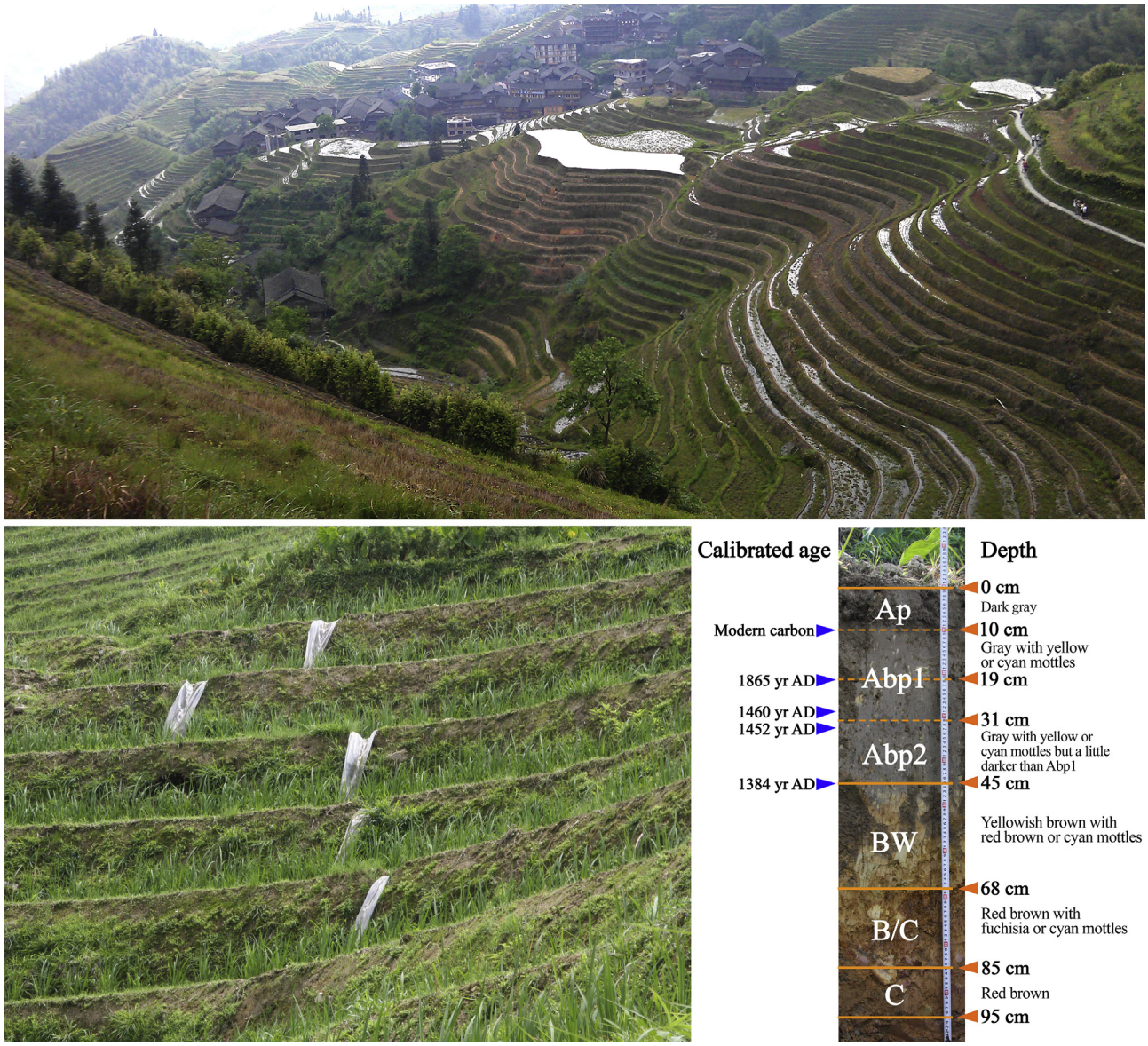

Fig. 1. Longji Terraces and the profile of LJTT-3. Longji Terraces in Guangxi province in China were built on sloping hills (upper) with a gravity based irrigation method (lower left). The chronosequence along profile LJTT-3 (lower right), dating back to CE 1384.

nating details [13]. There is another important but generally underestimated facet of archaeological interpretation, which is the associated microbial communities. Microorganisms are twined with soil physico-chemical properties influenced by extraneous interferences, such as changes in climate [14] and anthropogenic activities [15]. They can survive in palaeosols for thousands of years [13] due to a slow metabolism and a very sparse use of external energy sources [16], which is expected to preserve their initial properties [17]. Furthermore, their extracellular DNA can also be preserved in palaeosol for thousands of years [18]. However, only in the last decade, soil microorganisms have begun to attract more attention as an alternative proxy and began to fill this lacuna [19]. Palaeosol microorganisms have been evaluated based on their biomass [20], viable counts [21], tetraether lipids [22], nucleic acid [18,23] and ^{14}C fractions [24]. The authors of these studies have established for the first time that palaeosol microorganisms are associated with the history of climate changes and/or the chronosequences of soil chemical properties. More recently DNA-based high-throughput sequencing has been giving us a detailed insight into the microbial composition and increasingly into the microbial ecology of various environments. This technique is expected to be a powerful tool for providing more detailed microbial interpretation on historical reconstruction [18]. Zhu et al., [25] and Ding et al., [26] had conducted this technique to compare soil microorganisms between ancient and modern soils as well as their successions in the 2000-year chronological horizons. However, actual research on historical reconstruction using this technique to study palaeosols is still rarely reported.

The work of Sapart et al. [4] has given a strong hint that archived methanogenic archaeal information could be a proxy for historical human-environment interactions. These authors found that the atmospheric CH_4 level during the past two millennia reflects variations in anthropogenic activities. It is well known that rice cultivation is one of the major anthropogenic CH_4 sources [27] and paddy methanogenic archaea are responsible for this emission. Besides, methanogenic archaea could even better preserve their initial properties than the aerobic microorganisms did in the buried palaeosols studied by Demkina et al. [28], because the metabo-

lism of the former is relatively slow [29]. Collectively, the above characteristics suggest that methanogenic archaea could be suitable for reconstruction. Here, we tested the hypothesis, and studied methanogenic archaeal communities along the buried chronological cultivated horizons of Longji Terraces by high-throughput sequencing of their palaeoenvironmental DNA.

2. Methods and materials

2.1. Soil selection and sampling

The detailed information regarding Longji Terraces and sampling strategy has been provided by Jiang et al. [11]. Therefore, only a brief description is given here. Longji Terraces (25°35′–26°17′N, 109°32′–100°14′E) is located in Longsheng County of Guilin City in Guangxi, China. In this investigation, profile LJTT-3 (25°45.41′N, 110°6.99′E; 800 m a.s.l.) was chosen, which was probably one of the first terraces constructed on these slopes because it is in the vicinity of the inhabited area, which might have been the priority area when peasants began to build terraces. The soils with triplicates along the chronological cultivated horizons (3 cm, 5 cm, 7 cm, 9 cm... and 43 cm with 2 cm interval) of the profile LJTT-3 were sampled in July 2012 for physico-chemical analyses. Based on the changes in multi-proxy data, the chronological cultivated horizons are divided into four periods (stages) (Fig. S1). Amongst those, the subsamples of Layers of 3 cm and 5 cm in Stage D; the Layers of 13 cm, 15 cm and 17 cm in Stage C; the Layers of 19 cm, 21 cm, 23 cm, 29 cm and 31 cm in Stage B and the Layers of 35 cm, 39 cm and 43 cm in Stage A were chosen and stored in −40 °C for microbial assays.

2.2. Soil DNA extraction

For each soil sample, genomic DNA was extracted from 0.5 g of soil using the FastDNA® SPIN Kit for soil (MP Biomedicals, Santa Ana, CA) according to the manufacturer's instructions. The extracted DNA was dissolved in 50 μL of TE (10 mmol/L Tris-HCl (pH 7.6) and 1 mmol/L EDTA (pH 8.0)) buffer, quantified by Nanodrop 2000 (Thermo, USA) at 260 nm wavelength and stored at −20 °C until further use.

2.3. Real-time quantitative PCR

The copy numbers of methanogenic archaea along the chronological cultivated horizons of the profile LJTT-3 were quantified by real-time quantitative PCR (qPCR), targeting the methyl coenzyme-M reductase (*mcr*A) gene [30]. The protocol of qPCR is briefly described here. Standard curves were obtained using 10-fold serial dilutions of the linear *Escherichia coli*-derived vector plasmid pMD18-T (TaKaRa) containing a cloned target gene, using 10^2–10^7 gene copies/μL. The reactions were performed in a C1000™ Thermal Cycler equipped with a CFX96™ Real-Time system (Bio-Rad, USA). The 25-μL reaction mixture contained 12.5 μL of SYBR® *Premix Ex Taq*™ (TaKaRa), primer set (0.5 μmol/L each), 200 ng BSA/μL, 1.0 μL template containing approximately 2–9 ng DNA. Negative control was always run with water as the template instead of soil DNA extract. The qPCR program used was: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s, and extension and signal reading. The specificity of the amplification products was confirmed by melting curve analysis, and the expected size of the amplified fragments were checked in a 1.5% agarose gel. qPCR was performed in triplicate and amplification efficiencies of 97.4%–104% were obtained with R^2 values of 0.966–0.977.

2.4. The preparation of the amplicon libraries for high-throughput sequencing

The methanogenic archaeal community was assayed by high-throughput sequencing for the chronological cultivated horizons of profile LJTT-3. For each DNA, the primer set, 1106F and 1378R, was used to amplify approximately 280 bp of methanogenic archaeal 16S rRNA gene fragments [14]. Briefly, the oligonucleotide sequences included a 5-bp barcode fused to the forward primer as follows: barcode + forward primer. PCR was carried out in 50-μL reaction mixtures with the following components: 4 μL (initial 2.5 mmol/L each) of deoxynucleoside triphosphates, 2 μL (initial 10 mmol/L each) of forward and reverse primers, 2 U of Taq DNA polymerase with 0.4 μL(TaKaRa, Japan), and 1 μL of template containing approximately 50 ng of genomic community DNA as a template. Thirty-five cycles (95 °C for 45 s, 56 °C for 45 s, and 72 °C for 60 s) were performed with a final extension at 72 °C for 7 min. The purified bar-coded PCR products from all of the samples were normalized in equimolar amounts, then prepared using TruSeq™ DNA Sample Prep LT Kit and sequenced using MiSeq Reagent Kit (600-cycles-PE) following the manufacturer's protocols. The sequences were deposited in DDBJ database (accession no. DRA005073).

2.5. Processing of the high-throughput sequencing data

The high-throughput sequencing data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) 1.9.0-dev pipeline ([31]; http://www.qiime.org) using default parameters unless otherwise noted. In brief, the sequences were binned into OTUs using a 97% identity threshold, and the most abundant sequence from each OTU was selected as a representative sequence for that OTU. Taxonomy was assigned to methanogenic archaeal OTUs against a subset of the Silva 119 database (www.arb-silva.de/). OTU representative sequences were aligned using PyNAST. A phylogenetic tree was then constructed using FastTree2 [32] to support the phylogenetic diversity calculations.

We obtained total 717,556 sequences of methanogenic archaeal 16S rRNA gene, and between 8018 and 36,503 sequences per sample. Because an even depth of sampling is required for alpha and beta (β) diversity calculations, we reduced the datasets to the equal number available to correct for differences in survey effort between the samples. Namely, we conducted both diversity measurements using a randomly selected subset of 5000 sequences per soil sample. This approach allows us to compare general diversity patterns among layers even though it is highly unlikely that we surveyed the full extent of diversity in each community [33]. Richness and phylogenetic diversity (PD) using Faith's index [34] of phylotypes were calculated to compare community-level alpha diversity. For beta diversity, the Bray-Curtis distance and weighted UniFrac distance [35] were respectively calculated for taxonomical and phylogenetic community composition comparisons and were respectively visualized using non-metric multidimensional scaling (NMDS) plots and principal coordinate analysis (PCoA) plots. The variability of alpha diversity index within period stages was quantified by the coefficient of variation in diversity index: $CV = \sigma/\mu$, where σ is the s.d. of the diversity index within staged groups and μ is the mean value of the diversity index within staged groups [36]. β diversities (the extent of variation in microbial community composition [37]) within period stages and among the specific different layers were respectively calculated by Bray-Curtis dissimilarity distance and weighted UniFrac dissimilarity distance.

2.6. Statistical analysis

Statistical procedures were calculated using the IBM Statistical Product and Service Solutions (SPSS) Statistics for windows (Ver-

sion 13). The data were expressed as the means with standard deviation (SD), and different letters indicate significant differences between different samples. Mean separation was conducted based on Tukey's multiple range test, following the tests of assumptions of normal distribution, homogeneity of variance and ANOVA. The significant shifts of methanogenic archaeal community composition along the chronological cultivated horizons were tested by PERMAONVA (Permutational Multivariate Analysis of Variance). Pearson's correlation was used to relate methanogenic archaeal abundance to the measured soil parameters, which were further tested by linear regression analysis. Mantel tests were conducted to test the statistical significance between the Bray-Curtis composition and weighted Unifrac composition of the methanogenic archaeal communities and soil depth as well as soil chemical properties, using *R* software (the vegan package, Version 3.1.2). Besides, the scores of the first principal coordinate of the principal coordinate analysis were used to test for significant correlations between Bray-Curtis distances and soil depth as well as between weighted Unifrac distances and soil depth. A difference of $P < 0.05$ was considered statistically significant.

3. Results

3.1. The variations in the methanogenic archaeal abundances along the chronological cultivated horizons of profile LJTT-3 during 630-year rice cultivation

qPCR was conducted to measure the variations in methanogenic archaeal abundances along the cultivated horizons of profile LJTT-3 (Fig. 2a). Copy numbers per gram of dry weight soil (d.w.s) ranged between 1.09×10^6 and 1.23×10^7. Generally, the copy number decreased with increasing depth. The abundance of the methanogens was significantly ($P < 0.0001$) correlated with the depth of the chronologically cultivated horizons (Fig. 2b). Furthermore, positive correlations with depth were observed for SOC, TN and P contents ($P < 0.05$), while Al and Fe showed negative correlations with depth ($P < 0.05$) (Table S1).

3.2. The variations in the methanogenic archaeal alpha diversity

The rarefaction curves of observed OTUs and PD were used to estimate sequencing depth (Fig. S2). All amplified rarefaction curves almost reached the plateau when sequences were over 5000, which means that the sequences deriving richness and diversity in this study are sufficient to characterize methanogenic archaeal species in each sample. The variation in the methanogenic archaeal alpha diversity along the chronological cultivated horizons of profile LJTT-3 during 630-year rice cultivation was evaluated by using two different indices: OTU richness (Fig. 2c) and phylogenetic diversity (Fig. S3A). Generally, these indices showed a similar and decreasing pattern along chronological cultivated horizons of profile LJTT-3. Significant correlations were observed for two diversity indices against depth (Figs. 2d and S3B). Specifically, Layers 19 and 23 had the highest alpha diversity ($P < 0.05$). Layer 35 had the lowest diversity with both indices.

3.3. The variations in the methanogenic archaeal community composition

Non-metric multidimensional scaling plots (NMDS) based on either the Bray-Curtis distance (Fig. 2e) or the weighted UniFrac distance (Fig. S3C) ordinations consistently demonstrated overall variations in methanogenic archaeal community along the chronological cultivated horizons of profile LJTT-3 in both taxonomic and phylogenetic composition. Visually, the succession of four staged groups could be observed in the NMDS plots, regardless of Bray-Curtis distance (Fig. 2e) or weighted Unifrac distance (Fig. S3C).

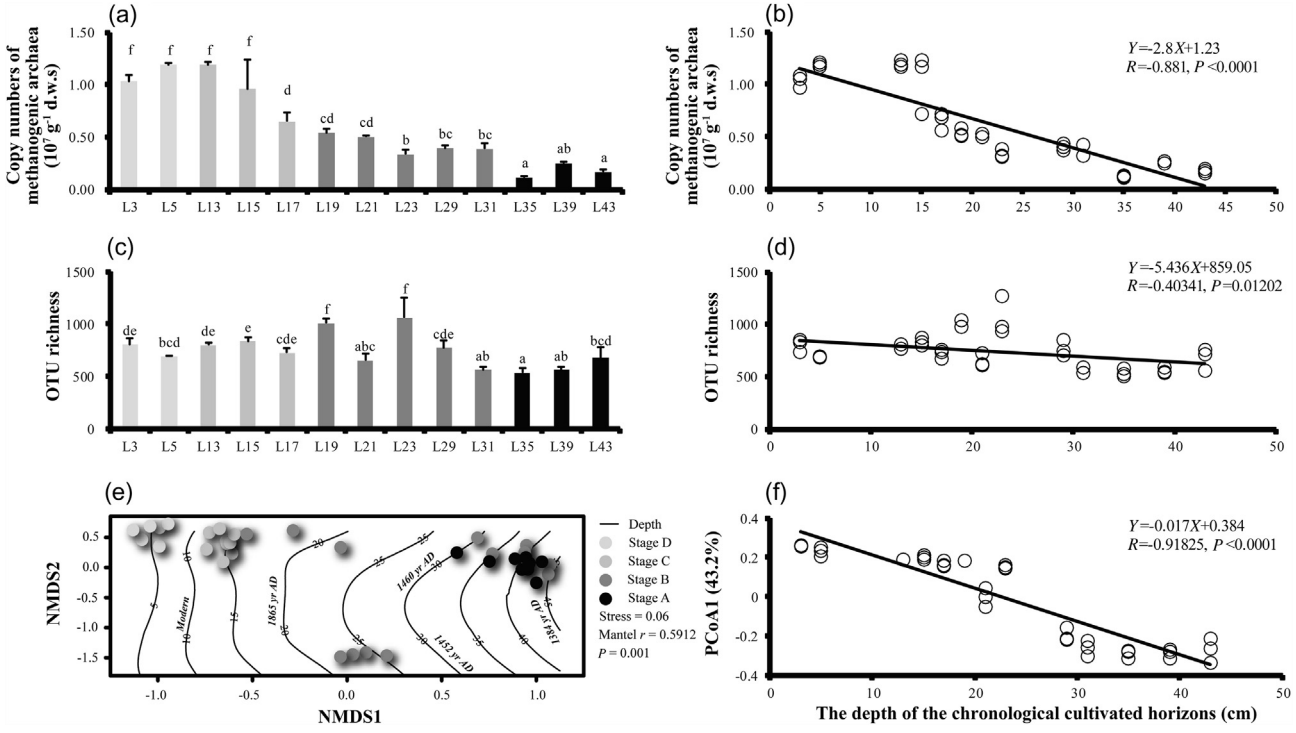

Fig. 2. The variations in methanogenic archaeal community and their correlations against cultivated horizons. The successions of the abundance (a), richness (c) and community composition (nonmetric multidimensional scaling plot of taxonomic similarity (Bray-Curtis distance)) (e) of the methanogenic archaeal community derived from 5000 sequences per sample in profile LJTT-3 are significantly correlated with cultivated horizons (b, d and f), which indicates that methanogenic archaea are closely associated with 630-year rice cultivation and could be used as an additional proxy for historical reconstruction. Lettering over error bar denotes significant differences.

Further statistical analysis confirmed that the depths of the chronological cultivated horizons negatively correlated with variations in methanogenic archaeal community composition, as indicated by the Mantel test (Figs. 2e and S3C) and the significant correlation between PCoA1 axle and depth (Figs. 2f and S3D). All measured soil chemical properties were found to be significantly correlated with the succession of methanogenic archaeal community composition (Table S2). With respect to specific groups, Fig. S4 revealed that the soil samples of the Layers at 3, 5, 13, 15 and 17 cm depth were grouped together, while the Layers of 29, 31, 35, 39 and 43 cm grouped separately. In contrast, the Layers of 19, 21 and 23 cm depth were dispersed in the middle of the plots and were distant from the above two groups. This implies that the methanogenic archaeal community composition was changing "dramatically", or at least more strongly than during other periods. Similarly but to a lesser extent, Layers 29 and 43 also had distinct community compositions, different from those of Layers 31, 35 and 39 (Fig. S5). All abovementioned shifts in methanogenic community along chronological cultivated horizons were confirmed by PERMAONVA tests (Tables S3–S5).

3.4. The variability in methanogenic archaeal community composition within four period stages

The horizon of profile LJTT-3 has undergone four soil development stages: Stage A (46–31 cm), Stage B (31–19 cm), Stage C (19–10 cm) and Stage D (10–0 cm) (Fig. S1) [11]. Following this assigning, we divided and analyzed methanogenic archaeal communities along 630-year rice cultivation into four groups. Among these staged groups, Stage A had the lowest ($P < 0.05$), whilst Stage B had the highest alpha diversity indices, however its significant differences were not observed, compared to Stages C and D (Figs. 3a and S6A). The variability (the coefficient of variation) of the alpha diversity indices within four period stages revealed that Stage B had the highest values (Figs. 3b and S6B). The second highest values were observed in Stage A. Stages C and D had the lowest values of two diversity indices. Community dissimilarities derived from both Bray-Curtis dissimilarity distance (Fig. 3c) and weighted UniFrac dissimilarity distance (Fig. S6C) were significantly higher in Stage B, compared to those of other stages. These phenomena are consistent with the result of Figs. 2e and S4 and imply that the extent of change in methanogenic archaeal community composition is greater in Stage B than those of other stages.

3.5. Taxonomic distributions of methanogenic archaeal communities

High-throughput sequencing revealed that the methanogenic archaeal community in Longji Terraces was dominated by two classes, Methanomicrobia (91.9%) and Methanobacteria (8.1%). With higher resolution, it was found that Methanocellales were most abundant (77.9%), followed by Methanosarcinales (9.0%), Methanobacteriales (8.1%) and Methanomicrobiales (4.9%), at the order level. At the family level, the dominant methanogenic archaea were found to be hydrogenotrophic Methanocellaceae (77.9%) and Methanobacteriaceae (8.1%), followed by the aceticlastic Methanosaetaceae (3.1%) and Methanosarcinaceae (4.6%) (Fig. 3d).

We also observed that the methanogenic archaeal community composition from the different staged groups was different. The largest decrease among the four staged groups was in the Methanocellaceae, which decreased from an average of 80.0% to 85.2% in Stages A, C and D to 66.5% in Stage B. Meanwhile, the largest increases among four staged groups were in the Methanobacteriaceae and Methanosarcinaceae, which increased from 5.2% to 8.7% in Stage A, C and D to 13.5% in Stage B and from 2.1% to 5.0% in Stage A, B and C to 8.3% in Stage D.

3.6. The dissimilarity distances in methanogenic archaeal community composition among Layers 15–23 or Layers 29–43

To unravel the extent of the variations in the methanogenic archaeal community composition around 1865 yr AD and 1452–1460 yr AD (Fig. S1), the dissimilarity distances were respectively calculated among Layers 15–23 (Figs. 4a and S7A) and among Layers 29–43 (Figs. 4b and S7B). As shown, Layers 19 and 23 had the higher dissimilarity distances from any Layer in Figs. 4a and S7A, compared to the other layers, except Layer 21. This indicates that methanogenic archaeal community in Layers 19 and 23 had

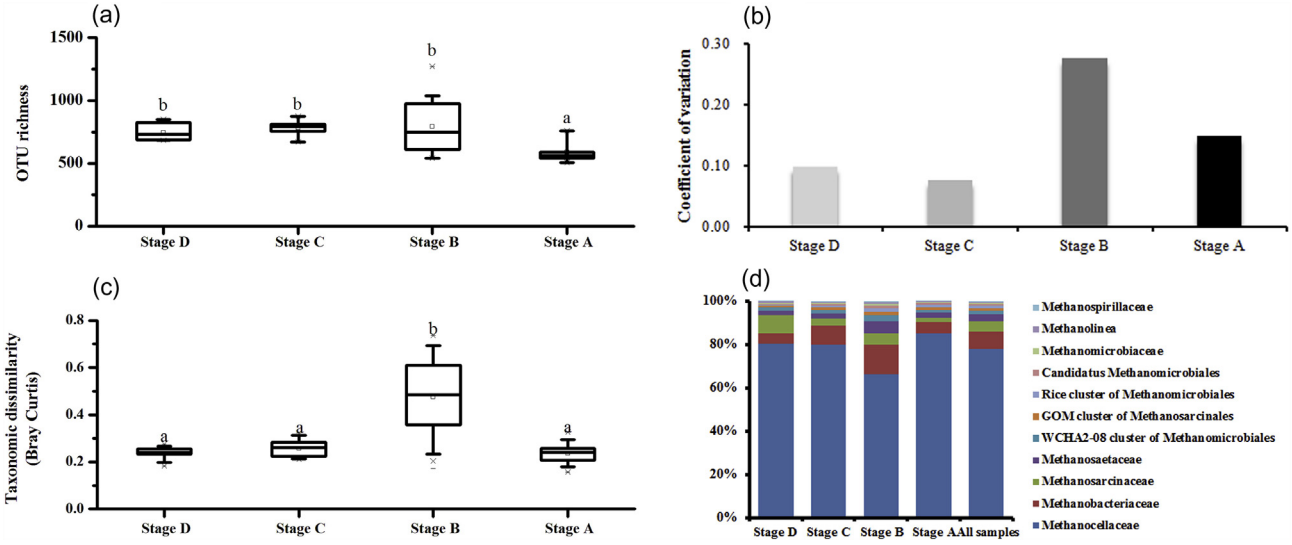

Fig. 3. The variations in methanogenic archaea within four stages during 630-year rice cultivation. The variations of methanogenic archaeal richness within four staged groups (a), their coefficients of richness variation (b) and Bray-Curtis based pairwise dissimilarity distances among layers within four staged groups (c) indicate that the methanogenic archaea in Stage B have been exposed to more external influences in Stage B than those in other stages. The stack plot of the methanogenic archaeal community composition in the four staged groups indicates that Methanocellaceae are the overwhelmingly dominant group in Longji Terraces (d). The minimum number per sequence of the OTUs used to generate (d) is 0.2%. Lettering denotes significant differences among staged groups. Lettering over error bar denotes significant differences.

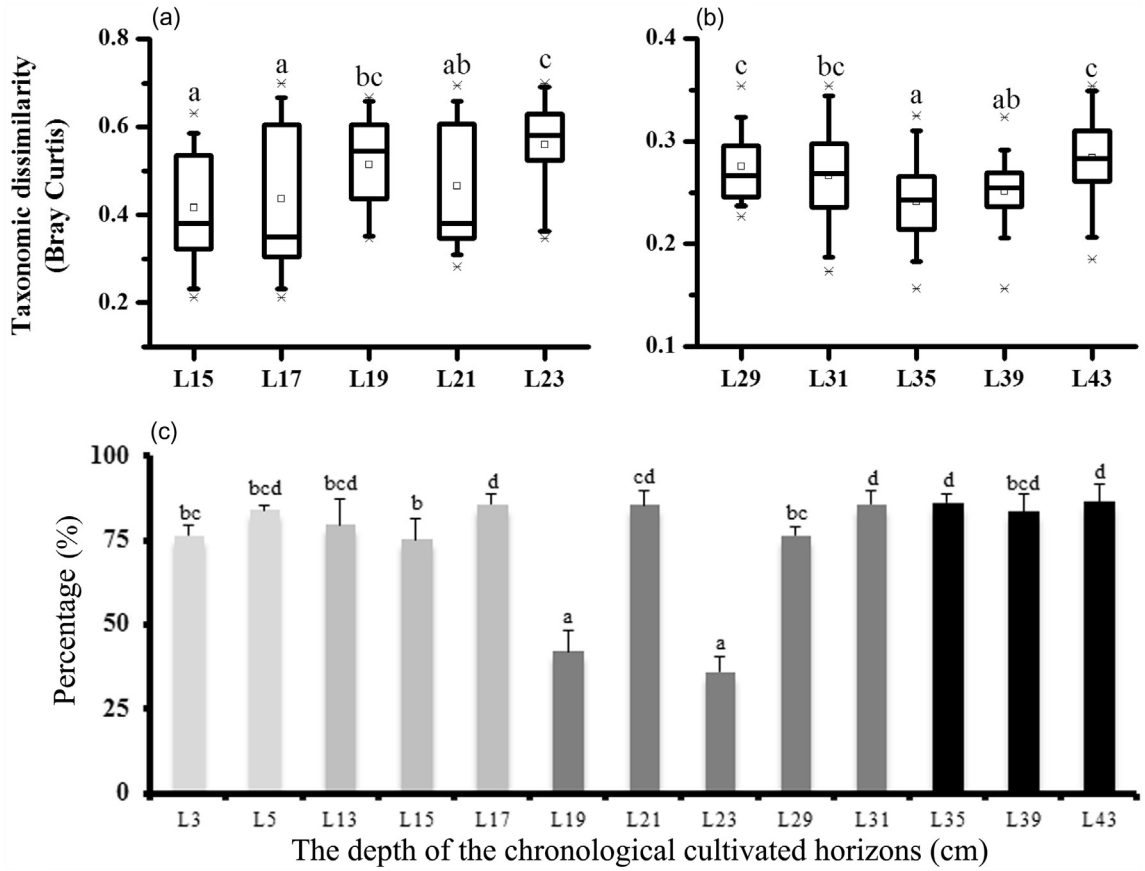

Fig. 4. The dissimilarities of methanogenic archaeal community composition among the several specific chronological cultivated horizons. Bray-Curtis based pairwise dissimilarity distances of the methanogenic archaeal community composition among Layers 15–23 (a) or Layers 29–43 (b) indicate that the community composition in the years when Layers 19, 23 and 29 were formed was distinct from those of others. Concomitantly, the relative abundance of the Methanocellaceae scaled to the abundance of the total methanogenic archaea suddenly decreased in Layers 19 and 23 (c). Lettering over error bar denotes significant differences.

the distinct community structure from the others. A similar phenomenon was observed for Layers 29 and 43 in Figs. 4b and S7B. Layer 29 also had the highest dissimilarity distances, indicating that the methanogenic archaeal community of that period had a distinct composition. Radiocarbon dating indicates that layer 43 dates back to the beginning of the rice cultivation in profile LJTT-3 (Fig. S1). Therefore, its methanogenic archaeal community composition is expected to be distinct.

With respect to specific groups, the variations in the relative abundance of Methanocellaceae to total methanogenic archaea are shown in Fig. 4c. The lowest percentages of Methanocellaceae were found in Layers 19 and 23. Furthermore, this percentage was lower in Layer 29 than in Layers 31–43, except that of Layer 39.

4. Discussion

It is now 40 years ago that Seaward et al. [38] first suggested that it may be possible to utilize qualitative and quantitative analyses, based on the incidence/abundances of microbial taxa with specific environmental requirements, for archaeological interpretation. Nevertheless, Seaward's prognosis has still not become a reality for now, and the study of soil microorganisms has not been routinely incorporated into the archaeologist's toolkit for the reconstruction of human-environment in historical periods [13]. In the current investigation, using DNA-based high-throughput sequencing, we present here the first example of the qualitative and quantitative microbial analyses of a palaeosol for historical reconstruction. This information is of help towards building a bridge between the disciplines of archaeology, microbiology and ecology.

4.1. The ecology of the methanogenic archaeal community is closely associated with anthropogenic activity

As a possible proxy for archaeological interpretation, it is required that the selected microbial group should be characteristic for the ecosystem studied and sensitively respond to perturbations of the systems. For this reason, methanogenic archaea were targeted in this investigation. Methanogenic archaea are the model anaerobic microorganisms in paddy soil, living at and off the terminal stage of carbon degradation as derived from rice cultivation [39]. Any factor influencing element cycling and redox conditions in the paddy ecosystem inevitably influences the ecology and ecophysiology of methanogenic archaea. For example, continuous no-tillage increases methanogenic archaeal abundance and CH_4 emission [40]. Different straw returning modes [41], water management strategies [42] and fertilization [43] can influence paddy methanogenic archaeal diversity and ecological function. Not only agricultural activities, but also fluctuations in climate influence the methanogenic archaeal community. It has been reported that elevated atmospheric CO_2 or temperature can increase methanogenic archaeal abundance [44] or influence their community composition [45]. Our own previous research has shown that elevated ground-level O_3 decreases their abundance [14].

Accordingly, close correlations between the depth of the chronological cultivated horizons and methanogenic archaeal abundance, diversity and community composition as well as soil chemical parameters were observed (Figs. 2, S3 and Tables S1 and S2). The intensity of rice cultivation, an anthropogenic activity, influences paddy soil. For example, rice cultivation helps the accumulation of soil organic carbon [46], due to the high input of exogenous organic material derived from crop residues and litter [47] coupled to low decomposition rates under anaerobic conditions [48]. With respect to soil N and P contents, in ancient China people used urine and feces as fertilizers, which contain abundant nitrogen and phosphorus and anthropogenic fertilization can increase their contents. These variations are echoed in the methanogenic archaeal community. A high SOC content promotes the metabolism of carbon-degrading microbial communities, and provides more reducing equivalents and carbon sources for methanogenic archaea [49]. Similar responses have been observed for increases in soil N, P and K content [50].

Fe and Mn contents can characterize paddy soil redox status [10,51] and reflect rice cultivation or its periodic termination. Due to sensitivity to variations in soil redox status [52], methanogenic archaea are closely correlated with Fe and Mn contents. Although some viable methanogenic archaea could still have faint metabolism in buried palaeosols, the correlations against multiproxy data indicate that their community most likely reflects the conditions at the time the layer was formed. Collectively, methanogenic archaeal community in Longji Terraces is found to be associated with 630-year rice cultivation.

4.2. The methanogenic archaeal community reflects centennial-scale anthropogenic activities

Based on physico-chemical proxies, the chronosequencing horizon of profile LJTT-3 has undergone four soil development stages (periods): Stage A (46–31 cm), Stage B (31–19 cm), Stage C (19–10 cm) and Stage D (10–0 cm) (Fig. S1) [11], which correspond to CE 1384–1452, CE 1460–1865, CE 1865-modern era and the modern era respectively. The variations in the methanogenic archaeal communities within the four stages can well reflect the centennial-scale variations in local anthropogenic activity. In Stage B, Jiang et al. [12] found a general enhancement of farming activity, compared with Stage A. Correspondingly methanogenic archaeal abundances in Stage B were generally higher than those in Stage A (Fig. 2a). Besides, South China entered the Little Ice Age after 1480s [1]. Climate deterioration decreased rice yield and aggravated class and ethnic conflicts [1]. It is recorded that both standard rice price index in China [1] and the number of wars in South China [53] were highest in Stage B during the past 1000 years. Such social turbulences and climate change are expected to be concomitant with weakening farming activity, which is supported by the evidence of decreased exogenous carbon input, characterized by the decrease in the values of the average chain length of odd-carbon-number *n*-alkanes, ranging from *n*-C_{25} to *n*-C_{33} (ACL_{25-33}) [12]. Consistently, the SOC content gradually decreased in Stage B (Fig. S1). Due to climate deterioration, the alternation of hydroponic and dry farming activity in Stage B occurred [12]. Furthermore, there are indications that several events have occurred in Longsheng village nearby the Terraces in Stage B, because there are two obvious peaks of soil Mn content, around CE 1865 (Layers 19–23) and in CE 1452–1460 (Layers 29–33) (Fig. S1). This information indicates that during these periods the paddy soils of Longji Terraces were temporarily turned into an oxidizing state. Correspondingly, the variability of alpha diversity and the value of β diversity were highest in Stage B (Figs. 3a–c and S6), indicating that the methanogenic archaeal community compositions in Stage B are distinct from those of other stages in profile LJTT-3 (Fig. S4).

In contrast, during Stages C and D, the climate [1] and the moisture condition in south China [54] was favorable for rice cultivation, as supported by for example the increased SOC (Fig. S1) and dramatically increased rice cultivation in Stage C [12]. As a result, methanogenic archaeal abundances were also steeply increased between Layers 19 and 13 (Fig. 2a). Meanwhile, the variations in the alpha and beta diversities of methanogenic archaea are small but clear (Figs. 3 and S6). During the early period of Stage A the temperature and the precipitation were low; the climate improved in the late period of Stage A [1]. This led to an intermediate variability in alpha diversity of the methanogenic community in Stage A relative to the corresponding variabilities in stages B, C and D, when conditions were respectively more (Stages C and D) and less (Stage B) favorable for anthropogenic activity.

4.3. The methanogenic archaeal community preserves the archives of social unrest and natural events

At a decadal scale, the influences of historical events on anthropogenic activity are also recorded in methanogenic archaeal community composition. The dissimilarity distances reveal that the community composition of Layers 19 and 23 is more distinct than those of Layers 15 and 17 (Figs. 4a and S7A), while Layer 29 is more distinct than Layers 35 and 39 (Figs. 4b and S7B). These sudden variations in methanogenic archaeal community composition are temporally in line with historical events. At the end of Stage B, the infamous national Taiping Rebellion (CE 1850–1864) broke out in Guangxi Region. During this catastrophe, 40 million people directly died and the population of China decreased from 400 million in CE 1850 to 240 million in CE 1864. The local people in Guangxi were forced to participate in this rebellion. The local chronicle records that on February 10th in CE 1850, there was a battle (Yuan Li rebels (800 persons) fought Qing dynasty) in Longsheng village, which could correspond to Layer 23. Besides, an inundation in Longsheng village was recorded in 1878, which can correspond to Layer 19. Such societal turbulence and natural events could have resulted in a temporary abandonment of Longji Terraces or a shift into upland agricultural fields as these require less labor [9]. The microbial evidence for these contentions includes that the most distinct response to these extraneous events was by Methanocellaceae (Figs. 3d and 4c). Their relative abundances decreased from over 70% to less than 40% in Layers 19 and 23. The growth of Methanocellaceae is closely associated with rice growth [55]. Thus, the sudden decrease of this group in Layers 19 and 23 supports our speculation that rice cultivation could have been periodically interrupted at the end of Stage B.

Similarly but differently, the combined information in the peaks in Mn and Fe content around CE 1452–1460 (around Layer 29) suggests that the paddies were exposed to the introduction of extraneous dry land soils. Unlike around CE 1865 (Layers 19–23), the Fe content suddenly increased around CE 1452–1460, concomitant with an increase in Mn content (Fig. S1). This combined information points to the introduction of extraneous soil. The local chronicle records that there were eight earthquakes in Guangxi in the years CE 1478–1524. Earthquakes are expected to have brought dried and/or oxidized soil from higher up the slope of the hill into profile LJTT-3. As a consequence, Layer 29 had a relatively distinct methanogenic archaeal community composition, compared to the composition in Layers 35 and 39 (Figs. 4b, S5 and S7B); meanwhile, the relative abundance of Methanocellaceae was lower in Layer 29 than in Layers 31–43 (except Layer 39) (Fig. 4c).

There is an important but generally underestimated facet when people think of the reconstruction of human-environment interaction in historical periods, that is the associated microbial commu-

nities. The microbial perspective can enrich and extend our understanding of the trajectory of the development of human civilization. In the present investigation, we found that the succession and saltation in the microbial community reflect variations in local anthropogenic activities as affected by social unrest, natural events and climate change. Although our investigation on the palaeobiology of the ancient terraces is in its infancy, the data presented here indicate that the methanogenic archaea in ancient paddy terraces could serve as a microbial proxy for historical reconstructions. More importantly, this information could lead to a dialog between archaeologists, microbiologists and ecologists and build a bridge between the disciplines. Given that there are more numerically abundant and taxonomically diverse microorganisms archived in palaeosol, not just methanogens, more intensive and extensive microbial ecological research should be conducted to further explore and build up this field.

Conflict of interest

The authors declare that they have no conflict of interest.

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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.scib.2017.05.024.

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