A buried Neolithic paddy soil reveals loss of microbial functional diversity after modern rice cultivation

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Abstract

It has been documented that human activities are causing the rapid loss of taxonomic, phylogenetic, genetic and functional diversity in soils. However, it remains unclear how modern intensive rice cultivation impacts the soil microbiome and its functionality. Here we examined the microbial composition and function differences between a buried Neolithicpaddy soil and an adjacent, currently-cultivated paddy soil using high throughput metagenomics technologies. Our results showed that the currently cultivated soil contained about 10-fold more microbial biomass than the buried one. Analyses based on both 16S rRNA genes and functional gene array showed that the currently cultivated soil had significantly higher phylogenetic diversity, but less functional diversity than the buried Neolithic one. The community structures were significantly different between modern and ancient soils, with functional structure shifting towards accelerated organic carbon (C) degradation and nitrogen (N) transformation in the modern soils. This study implies that, modern intensive rice cultivation has substantially altered soil microbial functional structure, leading to functional homogenization and the promotion of soil ecological functions related to the acceleration of nutrient cycling which is necessary for high crop yields.

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Keywords

Neolithic paddy soil

Long-term rice cultivation

16S rRNA gene pyrosequencing

Bacterial community

Functional gene diversity

GeoChip

1. Introduction

Human activities are now well known to be causing rapid loss of taxonomic, phylogenetic, genetic and functional diversity [1]. Agricultural intensification has generally led to the decline in farmland biodiversity [2]. Soil is arguably the most bio-diverse habitat of the terrestrial biosphere. However, unlike plant and animal diversity, information on the impacts of human activities on soil microbial diversity is scarce, and often inconclusive. It has rarely been documented if long-term agricultural practice will lead to the loss of soil biodiversity. Some studies have demonstrated that agricultural practice has fundamental impacts on soil microbial communities and the stability of ecosystem processes catalyzed by specific groups of microbes, such as nitrite oxidizers [3]. For example, organically managed soils has been reported to significantly improve soil quality and microbial activity/diversity 4., 5., but because of the limitation of techniques used in these studies, there still lacks comprehensive assessment of the entire soil microbiota and its functionality. Using a functional gene array, Reeve et al. [4] demonstrated that management (organic vs. conventional) was the dominant determinant of soil biology, and showed that the reduced soil processes in the conventionally farmed fields were associated with decreased microbial gene abundance and diversity.

Paddy soil is one of the most ancient land use types, and is currently feeding over 50% of the world's population 6., 7., and China has a rice cultivation history of up to more than 6000 years 8., 9.. Flooded rice cultivation is considered as one of the most ancient crop production systems in human history [7], and is often cited as an example of a sustainable system [10]. Various studies have investigated the microbial communities in paddy soils, including bacterial diversity [11], methanogenic community [12], iron-reducing microorganisms [13], arsenic transformation [14], ammonium oxidizing microbes [15] and transcriptional activity [16]. Although these studies have increased our knowledge about microbial processes in paddy soils, they represented only a small fraction of microbial community. Recently, changes in abundance and diversity of the functional groups of microbial communities was observed in wetland, and paddy soils with repeated tillagemanagement for different time periods [17].

Owing to the complexity of soil microbial communities and the limitation of conventional cultivation methods, the microbial population dynamics and functionality of paddy soils after modern intensive agriculture remains poorly understood. The lack of comprehensive assessment of modern rice cultivation on soil microbial diversity is also in part due to the difficulties in getting paired comparisons. There are many archeological sites in China showing that rice cultivation can be traced back to

more than 6000 years ago, and these sites are mostly distributed in eastern China [8]. These continuously cultivated paddy soils in east China provide a unique opportunity to study the evolution of soil properties, soil processes and productivity, and to investigate the impacts of long-term agricultural practices on soil microbiota. As one of the oldest Neolithic paddy soil in the world, Luojiajiao site was identified in early 1980 and listed in the National Relics site inventory in 2001. In this study, soil samples were collected from a buried Neolithic paddy soil and a currently cultivated paddy soil from this site, by using 16S rRNA gene based pyrosequencing and function gene arrays, we aimed (1) to investigate the microbial community composition and structure and microbial functional groups in ancient paddy soils, (2) to explore the impact of long-term rice cultivation on soil microbial community structure and functionality at thousands years scale (over 6000 years), which would provide information for the cumulative effect of rice cultivation on soil microbial community.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected from paddy fields at Luojiajiao site (30°37′57″ N, 120°27′50″ E, altitude 6 m), which is listed in the National Cultural Heritages Inventory, Zhejiang, China. Two soil profiles, P01 for long-term cultivated paddy soil and P04 for buried ancient paddy soil, were excavated (Table S1, Fig. 1). Soil samples from each layer (Table S1) were collected in sterile sampling bags (about 500 g for each replicate) and transported to the laboratory on ice. Aliquots of soil samples were stored at −80°C for molecular analysis.
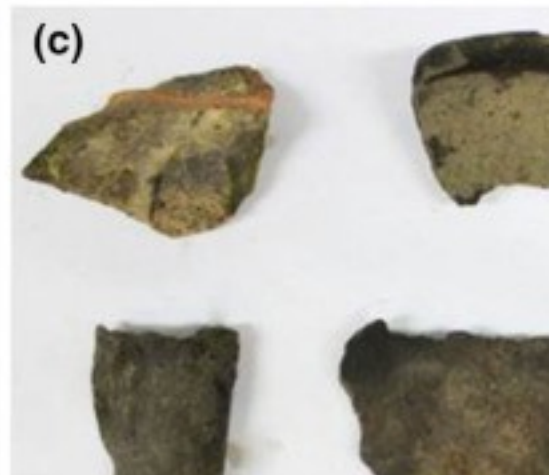
(a)

P04

3.7m

Box-field

~130

P01

~6300 a BP

P04A
P04B
P04C

(b)

(c)
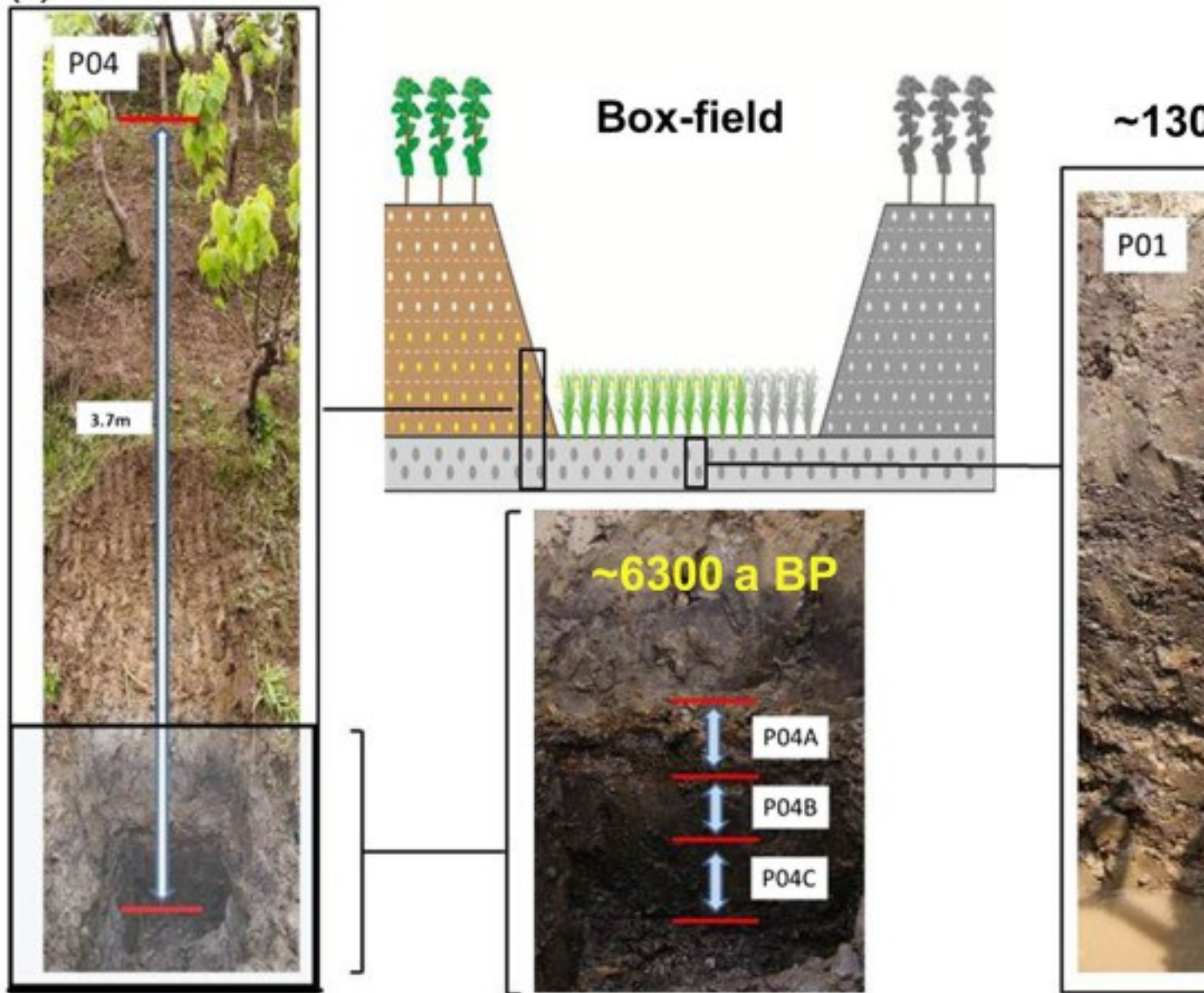
Fig. 1. Two soil profiles (P01 and P04) at the Luojiajiao site were sampled in this study. **a** Soil profiles, P01 for modern paddy soils and P04 for buried ancient paddy soils, **b** animal remains, **c** pottery shards

2.2. Soil chemical analyses

Soil samples were freeze-dried and sieved to 2 and 0.15 mm mesh before chemical analyses, respectively. Concentrations of total carbon, nitrogen and sulfur were assessed with an Elementar Vario Max Analyzer (Elementar Americas, Mt Laurel, NJ). Dissolved organic carbon (DOC) was extracted with 0.5 mol/L potassium sulfate and the extract was filtered through 0.45 μm membrane before analysis. Extracted DOC and total organic carbon were determined using a TOC analyzer (Shimadzu, TOC—Vcph, Japan) with a SSM-5000A solid module. Soil pH, available phosphorus (AP), available potassium (AK), available nitrogen (nitrate and nitrite, Nit), ammonium (NH_4^+), total reducing substances (TRS) and active reducing substances (ARS) were assessed according to recommended soil analyses [18]. Total phosphorus (TP) was determined after strong acid digestion in a programmable digestion system (Aim600, Aim Lab Pty Ltd., Australia). Nit and AMM were measured with a Flow Injection Analyzer (Lachat Instrument QC8500, USA). Cation exchange capacity (CEC) was assessed after extraction with barium chloride [19]. AP and TP were measured by a colorimetric procedure [20], CEC and AK were measured using an ICP-OES (Optima 7000DV, PerkinElmer, Inc., USA). The phytolith density in soil samples was analyzed microscopically as described previously [21]. Soil organic matter was extracted using NaOH and analyzed using pyrolysis–gas chromatography/mass spectrometry (pyrolysis-GC/MS) [22]. $\delta^{13}C$ and $\delta^{15}N$ were analyzed by isotope ratio mass spectrometry (Delta V advantage, Thermo Fisher, Scientific, USA) after pretreatment with 1 N HCl for 24 h at room temperature [23] and combustion in a flash 2000 HT elemental analyzer.

2.3. PLFA analysis of microbial biomass

Phospholipid fatty acid (PLFA) analysis was performed on extracts from 5 g of freeze-dried soil [24], followed by analysis using the Sherlock Microbial Identification System (MIDI Inc.). The amount of each identified fatty acid methyl ester (FAME) was calculated based on the amount of an internal standard (19:0, 5 μg) added before the analysis and expressed as mg kg^{-1} soil.

2.4. DNA extraction

High molecular weight community DNA was extracted by the freeze-grinding, SDS-based methods [25] and was purified using a low melting agarose gel followed by phenolextraction. DNA concentration and quality was determined with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE).

3. 16S rRNA gene amplification and pyrosequencing

DNA extracted from the soil samples served as template in triplicate PCR reactionsperformed using the Roche High Fidelity PCR system (Roche Diagnostics Gmbh, Mannheim, Germany). The V4RF-V5R primers [26] were used for amplification of 16S rRNAgenes as described before [27]. PCR amplicon libraries were purified using a QIA-quick gel extraction kit (QIAGEN, Maryland, USA) per the manufacturer's directions. Equal DNA masses of each sample were combined and was sent to the Research Technology Support Facility (RTSF) at Michigan State University (East Lansing) for emulsion PCR (emPCR), GS amplicon library preparation, and pyrosequencing on a 454 Life Sciences GS-FLX machine (Roche) [27]. Analysis of pyrosequencing data were conducted as described previously [28]. In brief, acquired pyrosequencing data were processed using RDP's Pyrosequencing Pipeline (http://pyro.cme.msu.edu/) to filter low quality sequences and trim off the barcode and primers. Chimeric sequences were detected using UCHIME [29] and removed. The chimerafree sequences were clustered into OTUs at the 97% similarity level. Representative sequences were retrieved and classified using RDP classifier [30], after which a phylogenetic tree of the representative sequences was created with FastTree [31]. Further analysis was performed in R 3.0.0 (The R Foundation for Statistical Computing, Vienna, Austria). All sequencing data have been deposited in the NCBI Short Read Archive under accession number SRP049535.

3.1. Functional gene array (GeoChip) analysis

GeoChip 4.0 hybridization was performed 32., 33. and the arrays were scanned using a MS 200 Microarray Scanner (Nimblegen, Madison, WI) and the signal intensities were quantified and processed using the data analysis pipeline as previously described 32., 34.. Molecular Ecological Networks (MENs) were constructed as previously described [35].

3.2. Statistical analysis

Statistical analysis was performed as described previously 28., 32., 34.. Briefly, diversity estimation, principal component analysis (PCA), correlation with environmental factors, detrended correspondence analysis (DCA), three non-parametric tests (multiple

response permutation procedure (MRPP); permutational [multivariate analysis](#) of variance, Adonis; analysis of similarity, anosim), canonical correlation analysis (CCA), Mantel test and analysis of variance (ANOVA) were performed by R version 3.0.0 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org/) with the phyloseq [36], vegan [37], [Biodiversity](#) R [38], and packfor [39] packages. Differences between treatments were compared by *Post hoc* Fisher's least significant difference (LSD) test with Holm–Bonferroni adjustment. The significant differences were defined as $P<0.05$, or with listed P values.

4. Results

4.1. Sampling site

This site is a typical agricultural landscape in the core area of Yangtze River delta-"Box-field", with soil taken from the paddy to build a raised upland (terrace) for mulberry trees (therefore silk production) and the lowland kept for paddy rice. Historical records indicate that this type of landscape started at least 2000 years ago when silk production in this region was flourishing. Thus the [Neolithic](#) [paddy soil](#) was exposed, and has been used for paddy rice production since, while the paddy soil underneath the mulberry upland has been buried for 2000 years.

Under this "box-field" landscape, paired [soil profiles](#) (at the same level) were taken allowing the comparison of paddy soils between continuous cultivation and those buried underneath the raised upland ([Fig. 1](#)). Soil samples were taken from both the currently cultivated field (P01) and the ancient (buried) paddy soil (P04) ([Table S1](#)). Excavation of these two soil profiles recovered assemblages of [animal remains](#) and [pottery](#) shards ([Fig. 1](#)). The [buried soil](#) samples and ashpit layer samples were dated to between 6300 and 6750 cal. a BP, while the currently cultivated soils were dated to between 1300 and 1650 cal. a BP ([Table S2](#)). The [phytolith](#) concentration of soil samples were all beyond 9000 grains g^{-1} ([Table S3](#)), and the highest phytolith concentration were detected in the surface layer. The natural ^{15}N abundance of buried ancient soils was markedly higher than that of the currently cultivated soils ([Table S3](#)). Soil moisture, total [reducing substances](#) and active reducing substances were similar in both currently cultivated soil and buried soil, indicating that they were under similaranaerobic conditions ([Table S3](#)). Analysis of microbial [phospholipids](#) [fatty acid](#) (PLFA) showed that the currently cultivated paddy soil contains much higher [microbial biomass](#) than the buried one ([Table S3](#)).

4.2. Microbial community composition

Analysis of microbial PLFA showed that the currently cultivated paddy soil contains different composition and much higher microbial biomass than the buried one (Table S3, Fig. 2e). DCA of PLFA showed that currently cultivated samples were well separated from buried ones and ashpit layer samples (Fig. 2e). The currently cultivated paddy soil harbored not only higher microbial biomass, but also diverse microbial community. The phylogeneticcomposition of bacterial communities were investigated using 16S rRNA gene based pyrosequencing. A total of 175,754 classifiable sequences were obtained from 30 samples, with a mean of 5858 sequences per sample (ranging between 3518–8738). Higher richness ($P<0.05$) was observed in currently cultivated paddy soils, where the highest bacterial diversity was detected in the upper layer of currently cultivated paddy soil (P01A, $P<0.01$) and the lowest bacterial diversity was observed in second layer of buried paddy soils (P04C, $P<0.01$, Fig. 2a, b). PCA revealed that the bacterial community structure was markedly different between currently cultivated paddy soils and the buried Neolithic paddy soils, as currently cultivated samples were separated from buried ones by the first axis, while samples from the ashpit layer were grouped together (Fig. 2c). The differences in bacterial community structure were also demonstrated by unweighted unifrac UPGMA cluster (Fig. S1). Significant differences ($P<0.01$) were observed between currently cultivated and buried samples on the basis of pyrosequencing data and PLFA data using ANOSIM, adonis and MRPP test (Table S4).
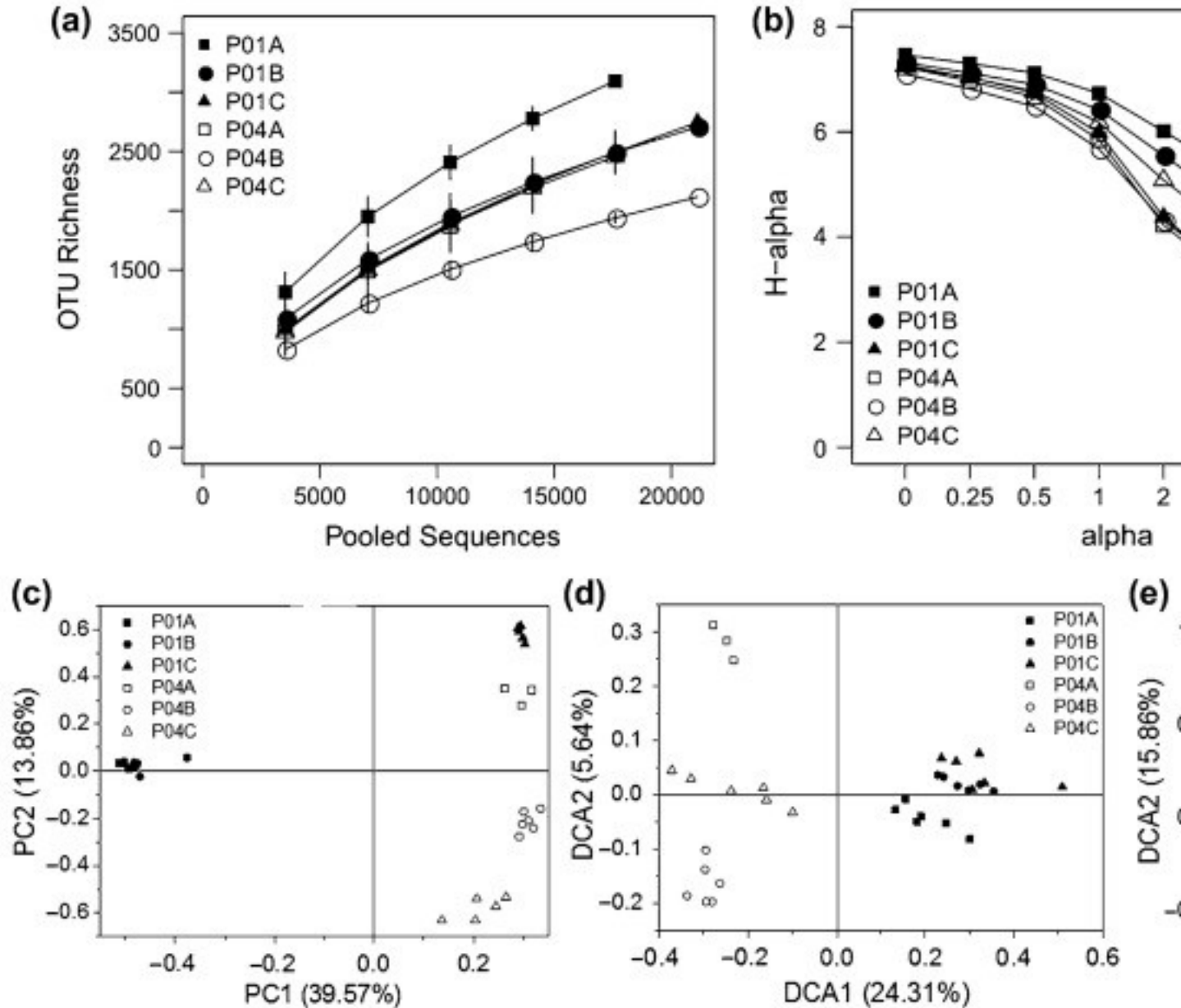
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Fig. 2. **a** OTU accumulation curves and **b** Rényi diversity profiles for partial sequences of bacterial 16S rRNA genes from the buried ancient paddy soils and long-term cultivated paddy soils. **c** PCA of 16S rRNA genes showed that currently cultivated soil samples (P01A and P01B) were well separated from buried Neolithic soil samples (P04B and P04C), while ashpit (P01C and P04A) samples group together. **d**DCA of GeoChip 4.0 data showing that modern rice cultivation significantly affected the functional structure of soil microbial community. **e** DCA of PLFA data

The most abundant phyla were Proteobacteria > Acidobacteria > Chloroflexi > WS3 > Nitrospira > Firmicutes (Fig. S2). Chloroflexi and WS3 were more abundant in currently cultivated soil than that in buried soils and ashpit samples (Fig. S2). A large proportion of unclassified bacteria were detected in these samples, especially buried paddy soil and ashpit samples, with approximately 40%– 50% of total sequences that could not be assigned to phylum level. Representative sequences for the 16 most abundant unclassifiable OTUs (at least 500 sequences per cluster) were blasted against NCBI nr database. The results showed thirteen matched uncultured bacteria with 99% or better coverage with at least 98% identity (Table S5). An obvious shift in distribution of Proteobacteria and Acidobacteria was observed between currently cultivated paddy soils and the buried Neolithic paddy soils (Fig. S3).
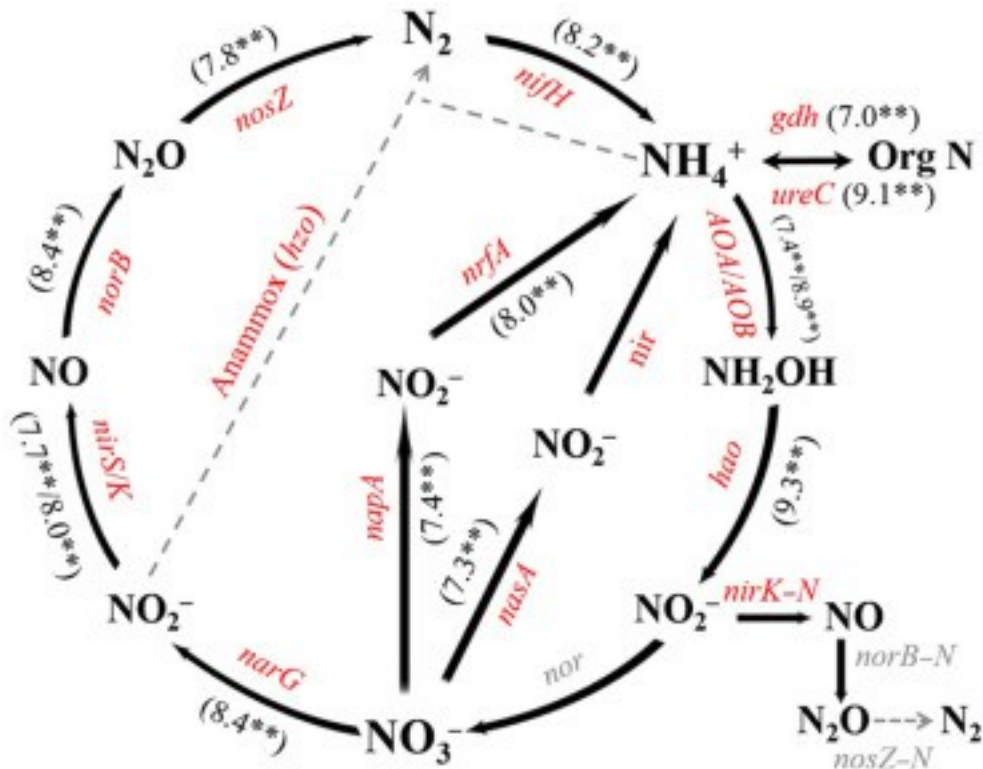
CCA was used to further examine the linkages between bacterial phylogenetic composition and soil environmental variables. Based on the significance calculated from individual CCA results and variance inflation factors (VIFs), nine environmental variables were selected in the CCA biplot (Fig. S4), including pH, total organic carbon (TOC), total nitrogen (TN), total carbon (TC), total sulfur (TS), cation exchange capacity (CEC), ammonium concentration (AMM), available potassium (AK) and dissolved organic carbon (DOC). A total of 55% of the variation was significantly explained ($P<0.005$) by the selected variables. Long-term cultivated samples were separated from buried ancient samples and ashpit samples by the first axis. The first axis was positively correlated with AK but negatively correlated with TN, DOC and pH. The second axis was positively correlated with TS, AMM, TOC and TC, but negatively correlated with CEC. AK was the most important variable, while the others had less but comparative effect on the bacterial phylogenetic composition shifts. The currently cultivated samples were positively correlated to TN, DOC and pH but negatively correlated to AK. However, the buried ancient samples were positively correlated to TOC, TC and AK, but negatively correlated to DOC and pH.

4.3. Responses of soil microbial functional genes to long-term cultivation

DCA of GeoChip 4.0 data showed the structure of microbial functional genes was different between currently cultivated and buried samples, as they appeared to be well separated by the first axis (Fig. 2d) and Simple hierarchical clustering analysis also showed that all buried samples clustered together and were well separated from currently cultivated samples (Fig. S5). In addition, significant differences ($P<0.01$) were observed between currently cultivated and buried samples when three non-parametric multivariate statistical tests, ANOSIM, adonis and MRPP were analyzed (Table S4).

The relationships between microbial community structure and soil geochemical parameters were assessed with CCA. Seven parameters were selected based on VIFs and significant test, including pH, TOC, TN, AMM, AK, total phosphorus (TP) and DOC. The specified CCA model was significant ($P<0.005$) with more than 44% of the microbial community functional variation was explained by the selected parameters based on GeoChip data. The buried and currently cultivated paddy soils were separated along the first axis. The currently cultivated samples were positively correlated with DOC, pH and TN, but negatively correlated with AK, TOC and AMM. pH, AMM, AK, TP and DOC appeared to play major roles in shaping the microbial functional community structures (Fig. S6).

Microbial functional genes responsible for the degradation of starch, hemicellulose, cellulose, pectin, chitin and lignin were detected in all samples. All carbon degradation genes had significantly higher signal intensities in currently cultivated paddy soil as compared to the buried one (Fig. S7), indicating that modern rice cultivation has accelerated the turnover of organic carbon. Three genes encoding enzymes involved in methanemetabolism were included in GeoChip 4.0: *mcrA* encoding the alpha subunit of methyl coenzyme M reductase for methane production, *pmoA* encoding particulate methane monoxygenase and *mmoX* encoding methane monoxygenase for methane oxidation. For all these genes, significantly ($P<0.01$) higher signal intensities were detected in currently cultivated soils, suggesting that modern rice cultivation may have promoted both methane production and oxidation (Fig. S8). Nitrogen cycling consists of various functional processes. All N cycling associated genes targeted by GeoChip were detected with higher signal intensity in currently cultivated soil than in the buried ones, suggesting that modern rice cultivation have stimulated microbe-mediated N biogeochemical cycling in paddy soils (Fig. 3). Network analysis of C- and N-related genes showed that the currently cultivated soil had higher connectivity and links than the buried ones.

Fig. 3. The relative changes of the detected genes involved in the N cycle. The value of a functional gene in a bracket was the fold change of the signal intensity of this gene. For each functional gene, red means that this gene had a higher signal intensity in currently cultivated soil than that in buried ancient soil and their significance was indicated with two stars (**) at $P<0.05$, while blue means that this gene had a lower signal intensity. Gray-colored genes were not targeted by this GeoChip, or not detected in those samples

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5. Discussion

Both 16S rRNA gene based pyrosequencing and functional gene arrays were integrated to investigate the shift in microbial community structure and functional genes for specific biogeochemical cycles after long-term cultivation of rice by comparing a pair of currently cultivated paddy soil and buried ones. The ^{14}C data showed that the buried soil could be dated to ca. 6000 cal. a BP, providing the evidence that it is ancient. To further verify if the buried soil was paddy soil, phytolith from both buried and currently cultivated paddy soils were extracted and examined. The phytolith concentration of soil samples were all beyond 5000 grains g^{-1} (Table S3), providing evidence of rice cultivation in the sampling field [40], which is also supported by earlier archeological

data on this Neolithic paddy soil [41]. The natural ^{15}N abundance of buried ancient soils was markedly higher than that of the currently cultivated soils (Table S3), indicating the use of chemical N fertilizers in modern rice production, as chemical fertilizers have considerably lower ^{15}N abundance [42].

The currently cultivated paddy soil harbored not only higher microbial biomass, but also diverse microbial community. The higher microbial biomass detected by PLFA analysis in currently cultivated paddy soils suggested that the longer cultivation and the resulting primary productivity inputs led to the richer microbial community under rice cropping [43]. By using 16S rRNA gene based pyrosequencing, the cultivated paddy soil was detected with more diverse microbial community and significant different microbial community structures when compared to the buried paddy soil, indicating that long-term cultivation has increased the bacterial species diversity and has shifted the microbial community structure in paddy soils. A large proportion of unclassified bacteria were identified in both soil profiles, suggested that the microbial community in this site, especially in buried paddy soils, remain largely unknown. Representative sequences for the 16 most abundant unclassifiable OTUs (at least 500 sequences per OTU) were blasted against NCBI nr database. The results showed thirteen matched uncultured bacteria with 99% or better coverage with at least 98% identity (Table S5), which indicated that these unclassified sequences were not PCRartifacts.

To evaluate the response of soil microbial functional community to modern rice cultivation, the microbial functional genes were determined with functional gene arrays (GeoChip 4.0). Long-term cultivation of rice significantly changed the functional gene structures of paddy soil and further stimulated those genes involved in carbon and nitrogen cycling. All carbon degradation genes had significantly high signal intensities in currently cultivated paddy soil as compared to the buried one (Fig. S7), indicating that modern rice cultivation has accelerated the turnover of organic carbon, and the adaptation of soil microbiome to continuous organic C input in modern agriculture. This is supported by the data on soil organic matter contents and ^{14}C dating (Fig. 1 and Table S3). Both currently cultivated and buried paddy soils contained comparable organic matter but with much different age (1300 a BP vs. 6300 a BP), showing significant C turnover in currently cultivated soil, which is reflected by the elevation of functional genes (Fig. S7). For example, the currently cultivated soils were detected with significantly higher signal intensity of those genes involved in methane production and oxidation, indicating that long-term rice cultivation may stimulate methane metabolism in paddy soils. This finding is supported by studies on a chronosequence of paddy soils ranging between 50 and 2000 years, which showed

that potential methanotrophic activity increased substantially with the age of paddy soil, possibly through the selection of adapted populations by continuous rice cultivation [44]. A comparable variation of bacterial community structure (55%) and microbial functional genes (44%) could be explained by selected chemical factors, respectively. Among these factors, TN, DOC, pH, AMM, AK and TOC play important roles in shaping both bacterial phylogenetic and functional structures (Figs. S4, S6). pH is an important factor for soil microbial community composition across different ecosystems and soil types [45]. The currently cultivated samples were positively correlated to DOC, supporting our hypothesis that modern rice cultivation has accelerated the turnover of organic carbon. Continuous input of organic matter by root exudate from rice could be another reason for elevated DOC in currently cultivated soil. Although large amounts of chemical N fertilizers have been applied in modern rice cultivation, there was no dramatic difference in total N contents in both soil profiles, and the ammonium concentration in currently cultivated soil is significant lower than that in buried ones. This implied that much rapid N cycling is occurring in the currently cultivated paddy soil, as indicated by the much lower ^{15}N abundance in currently cultivated soil as compared to the Neolithic one (Table S3). These results support our hypothesis that modern rice culture is stimulating microbial N cycling, largely driven by the large input chemical N fertilizers. These results corresponded well to the elevated functional genes involved in N biotransformation, as revealed by functional gene array (Fig. 3). Although, real-time PCR has been used to investigate the impact of rice cultivation on N-related genes, and most of these functional genes were elevated (per gram soil) in aged paddy soils [17]; while in the present study, we were able to compare currently cultivated soil with the Neolithic one (thus with minimal input of chemical N fertilizers) using a more comprehensive assay which provided a higher resolution picture of N functional genes. All these results demonstrated that the potential functional activity of microbial communities in currently cultivated paddy soils were significantly different from the buried one.

In contrast to the phylogenetic diversity, functional diversity revealed by functional gene array showed that the currently cultivated soil had lower functional diversity than the Neolithic one (Table 1), suggesting that under modern rice cultivation the soil microbial community has been homogenized to perform key functions towards intensive nutrient cycling in continuous modern rice cultivation. Furthermore, the currently cultivated soil had a more dense and complex interactions among these functional genes revealed by network analysis, indicating that the pathway preference was different between

ancient and currently cultivated paddy soils (Table S6), and that microbial community was more active after long-term rice cultivation.

Table 1. Overall microbial functional diversity detected by GeoChip 4.0

Diversity indices	Buried ancient soil	Currently cultivated soil
Shannon–Weaver H′	10.69 ± 0.031	10.44 ± 0.049[*]
Simpson's(1/D)	43686.36 ± 1426.93	34194.64 ± 1695.48[*]
Detected gene number (total)	44234 ± 1465	34381 ± 1735[*]
Detected gene number (C-cycling)	5810 ± 197	4495 ± 215[*]
Detected gene number (N-cycling)	4113 ± 150	3148 ± 160[*]
Detected gene number (P-cycling)	429 ± 15	323 ± 14[*]

[*]

$P<0.01$

One of the challenges in soil biodiversity research using "omics" approach is to link the changes in microbial community to soil biogeochemistry [46]. In the current study, since the relics site is listed in China's national cultural heritage inventory, in situ measurement of biogeochemical processes was not allowed, therefore it is not possible to investigate the direct linkage between biogeochemical fluxes and functional gene abundances/diversity. When possible, ancient soils should be employed to conduct time-course incubation studies in the future. In such studies, changes in biogeochemical fluxes, metagenome and metatranscriptome should be characterized to establish the linkage between microbial potential and soil functions, such as nutrient cycling under different scenarios.

It is important to note that analysis of microbial community in original ancient paddy soil is improbable. The ancient soil is buried under natural condition and the soil microbiota may have changed during the burial years, which could add in the uncertainty of this study. However, the soil moisture, total reducing substances and active reducing substances were similar in both currently cultivated soil and buried soil, indicating that they were under similaranaerobic conditions (Table S3), which would reduce the impact of oxygen level on the soil microbial community. The Neolithic paddy soil were deeply buried under the mulberry field and were separated from current cultivated paddy soil, suggesting that it was barely affected by farming activities, which is supported by ^{14}C dating and ^{15}N abundance measurement (Table S3). In addition, over 40% of 16S rRNA gene sequences in buried paddy soil remains unclassified (Fig. S2), suggesting that the Neolithic soil microbes are largely un-explored and may still be ancient. Rice cultivation is characterized by repeatedly flooding, tillage, application of

chemical fertilizers and the input of organic matter into soil, the shift in microbial composition and functional genes should be largely attributed to the composite effect of these activities.

In conclusion, using this unique pair of soil profiles and comprehensive high throughput techniques, we have demonstrated that modern rice cultivation has significantly increased soil microbial phylogenetic diversity, but modern rice cultivation has significantly reduced the microbial functional diversity, leading to more focused functionality, thus to support the acceleration of nutrient cycling in currently cultivated paddy soil, particularly C and N cycling. Our study suggests that at community level, soil microbiome has evolved to cope with the modern intensive rice cultivation, which is symbolized by high input (particularly N input) and high yield.

Conflict of interest

The authors declare that they have no conflict of interests.

Acknowledgments

Electronic supplementary material

The online version of this article (doi:10.1007/s11434-016-1112-0) contains supplementary material, which is available to authorized users.

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