# Supplementary Information

## Table S1

Description of soil samples from the two soil profiles at the Luojiajiao site

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Profiles | Description | depth  | Replicates | Label |
| P01 | Long-term cultivated paddy soil | 0-18 cm | 6 | P01A |
| Long-term cultivated paddy soil | 18-33 cm | 6 | P01B |
| ashpit (cultural layer) | 33-40 cm | 6 | P01C |
| P04 | ashpit (cultural layer) | 3.2-3.3 m | 3 | P04A |
| buried ancient paddy soil | 3.3-3.4 m | 6 | P04B |
| buried ancient paddy soil | 3.4-3.6 m | 6 | P04C |

## Table S2

The radiocarbon dates for soil samples from the Luojiajiao site

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Samples | Lab code | 14C age(yr BP±1σ) | Calibrated age (yr BP, 1σ) | Calibrated age (yr BP, 2σ) |
| P01A3 | BA111279 | 545±20 |

|  |
| --- |
| 1330 - 1340 (9.7%) |
| 1395 - 1425 (58.5%) |

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|  |
| --- |
| 1320 - 1350 (25.1%) |
| 1390 - 1430 (70.3%) |

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| P01B5 | BA111280 | 420±80 |

|  |
| --- |
| 1420 - 1530 (49.5%) |
| 1570 - 1630 (18.7%) |

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|  |
| --- |
| 1320 - 1350 (1.5%) |
| 1390 - 1650 (93.9%) |

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| P01C5 | BA111281 | 5740±60 |  6531- 6701 (68.2%) | 6461 - 6731 (95.4%) |
| P04A5 | BA111282 | 5635±25 |

|  |
| --- |
| 6456 - 6511 (62.8%) |
| 6411 - 6426 (5.4%) |

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|  |
| --- |
| 6451 - 6551 (77.0%) |
| 6381 - 6441 (18.4%) |

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| P04B6 | BA111283 | 5790±25 | 6616 - 6706 (68.2%) | 6561 - 6721 (95.4%) |
| P04C6 | BA111284 | 5680±30 |

|  |
| --- |
| 6496 - 6556 (59.7%) |
| 6471 - 6486 (8.5%) |

 | 6461 - 6611 (95.4%) |

## Table S3

Geochemical parameters of soil samples in this study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | P01A | P01B | P01C | P04A | P04B | P04C |
| pH | 6.44±0.07 | 6.74±0.06 | 6.76±0.06 | 6.26±0.06 | 6.34±0.05 | 6.24±0.06 |
| Moisture % | 39.05±2.47 | 30.856±3.43 | 37.98±3.74 | 34.97±1.70 | 33.06±2.98 | 38.43±4.26 |
| TOC (g Kg-1) | 26.37±1.64 | 23.43±2.84 | 24.44±1.46 | 24.44±3.52 | 22.14±1.21 | 39.21±4.00 |
| TN (g Kg-1) | 2.89±0.15 | 2.44±0.15 | 2.04±0.28 | 1.91±0.15 | 1.59±0.11 | 2.76±0.28 |
| TC (g Kg-1) | 27.43±1.34 | 24.58±1.00 | 32.60±2.17 | 29.11±1.99 | 22.83±0.88 | 42.84±4.18 |
| TS (g Kg-1) | 0.41±0.03 | 0.30±0.03 | 0.13±0.02 | 0.24±0.01 | 0.13±0.01 | 0.57±0.07 |
| DOC (mg Kg-1) | 163.55±12.20 | 137.41±25.20 | 156.00±15.34 | 93.28±15.43 | 31.56±5.14 | 56.78±16.92 |
| CEC (Cmol Kg-1) | 6.72±0.18 | 7.55±0.50 | 9.91±0.26 | 13.65±1.06 | 5.41±0.32 | 5.47±0.76 |
| NH4+ (mg Kg-1) | 7.86±0.43 | 7.74±0.75 | 5.71±0.60 | 5.46±0.49 | 9.36±0.30 | 9.27±0.98 |
| AK (mg Kg-1) | 102.59±3.14 | 100.08±4.08 | 166.25±12.34 | 204.57±10.17 | 185.53±9.85 | 178.91±14.16 |
| TP (g Kg-1) | 4.27±0.28 | 7.54±0.79 | 11.93±0.56 | 63.32±4.43 | 2.46±0.37 | 4.86±0.57 |
| AP (mg Kg-1) | 1.19±0.08 | 1.19±0.12 | 2.27±0.16 | 4.84±0.57 | 1.00±0.09 | 0.90±0.06 |
| d 13C/12C | -27.08±0.68 | -26.24±0.21 | -24.08±0.11 | -23.49±0.28 | -24.04±0.14 | -24.18±0.25 |
| d 15N/14N | 3.66±0.45 | 2.27±1.16 | 9.70±1.24 | 8.81±0.35 | 7.61±0.16 | 6.97±0.46 |
| total PLFA (mg Kg-1) | 8.91±0.47 | 5.61±0.96 | 0.65±0.11 | 0.89±0.06 | 0.94±0.20 | 0.88±0.17 |
| Phytolith (grains g-1) | 17546 | 10680 | 12063 | 13680 | 14117 | 9878 |
| TRS (cmol kg-1) | 0.98±0.12 | 0.83±0.11 | 1.01±0.19 | 1.08±0.39 | 0.91±0.19 | 0.81±0.19 |
| ARS (cmol kg-1) | 0.23±0.05 | 0.21±0.03 | 0.28±0.06 | 0.23±0.06 | 0.22±0.07 | 0.29±0.12 |
| S/G | 0.40 | 0.40 |  ND |  ND | 0.12 | 0.15 |

TOC, total organic carbon; TC, total carbon; DOC, dissolved organic carbon; TN, total nitrogen; TS, total sulfur; CEC, cation exchange capacity; TP, total phosphorus; AP, available phosphorous; TRS, total reducing substances; ARS, active reducing substances; S/G, syringyl to guaiacyl ratio of organic matter. ND: data were not detected

## Table S4

Significance tests based on the dissimilarities of overall functional gene diversity among samples with three statistical approaches

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | MRPP |  | ANOSIM |  | Adonis |  |
|  | δ | P | R | P | F | P |
| Long-term cultivated soil vs ancient buried soil |
| GeoChip | 0.202 | <0.001 | 0.901 | 0.001 | 0.484 | 0.001 |
| 16S rDNA | 0.6649 | 0.001 | 0.9647 | 0.001 | 9.6894 | 0.001 |
| PLFA | 0.1528 | 0.002 | 0.47 | 0.001 | 0.2404 | 0.01 |
|  |  |  |  |  |  |  |
| Paddy soil vs mulberry soil (GeoChip) |
| P01 - P05 | 0.142 | <0.001 | 0.519 | 0.003 | 0.23 | 0.001 |
| P04 - P05 | 0.1 | <0.001 | 0.976 | 0.001 | 0.3 | 0.001 |

MRPP, multi-response permutation procedure; ANOSIM, analysis of similarity; Adonis, non-parametric multivariate analysis of variance.

## Table S5

Blast results of 16 most abundant unclassifiable OTUs

| **OTU ID** | **Length (bp)** | **The most closest sequence****in GenBank** | **Accession No.** | **Coverage %** | **Identity %** |
| --- | --- | --- | --- | --- | --- |
| 00005 | 329 | Uncultured bacterium clone 1020B13 16S ribosomal RNA gene | HM641676.1 | 100 | 98 |
| 00084 | 329 | Uncultured bacterium clone GA21498d09 16S ribosomal RNA gene | JN607009.1 | 100 | 94 |
| 00003 | 330 | Uncultured bacterium clone: B0610D002\_E05 gene for 16S rRNA | AB660631.1 | 100 | 99 |
| 00154 | 331 | Uncultured bacterium clone Out22 16S ribosomal RNA gene | HQ677574.1 | 100 | 99 |
| 00015 | 331 | Uncultured bacterium clone HDB\_SIOH1020 16S ribosomal RNA gene | HM185832.1 | 100 | 99 |
| 07170 | 332 | Uncultured bacterium clone GB4XUSJ08JD6VI 16S ribosomal RNA gene | JQ504537.1 | 99 | 99 |
| 00180 | 332 | Uncultured bacterium clone GB4XUSJ08JOCML 16S ribosomal RNA gene | JQ505595.1 | 100 | 97 |
| 00018 | 332 | Uncultured bacterium clone GB4XUSJ08JPSD7 16S ribosomal RNA gene | JQ493420.1 | 100 | 99 |
| 00019 | 332 | Uncultured bacterium clone 16S-T6-1-F11 16S ribosomal RNA gene | KC664071.1 | 100 | 99 |
| 00002 | 333 | Uncultured bacterium clone B1\_58 16S ribosomal RNA gene | HM228610.1 | 100 | 99 |
| 00111 | 333 | Uncultured bacterium clone HDB\_SIPI600 16S ribosomal RNA gene | HM187031.1 | 100 | 95 |
| 00190 | 333 | Uncultured bacterium clone 4040RB13 16S ribosomal RNA gene | JQ976562.1 | 100 | 100 |
| 00132 | 333 | Uncultured bacterium clone 1020B13 16S ribosomal RNA gene | HM641676.1 | 100 | 99 |
| 00009 | 333 | Uncultured bacterium clone HDB\_SIOI1125 16S ribosomal RNA gene | HM185962.1 | 100 | 99 |
| 00119 | 333 | Uncultured bacterium clone PH1-25 16S ribosomal RNA gene | DQ444026.1 | 100 | 100 |
| 00282 | 335 | Uncultured bacterium clone T10.165 16S ribosomal RNA gene | GQ124291.1 | 99 | 98 |

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 S6

Summary of key parameters of network topology

Ancient: Buried paddy soils; Modern: Currently cultivated paddy soils;

avgK, Average connectivity; GD, Average path; avgCC, Average clustering coefficient.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Classification | Samples | Size (n) | Links | avgK | GD | avgCC |
| Carbon cycling | Ancient | 3066 | 57787 | 37.695 | 3.613 | 0.612 |
| Modern | 2890 | 68622 | 47.489 | 2.967 | 0.715 |
| Nitrogen cycling | Ancient | 2172 | 25699 | 23.664 | 3.362 | 0.571 |
| Modern | 2058 | 33042 | 32.111 | 3.854 | 0.693 |

Buried ancient

samples

Long-term cultivated samples

Ash pit

## Fig. S1

Jackknifing Support unweighted unifrac UPGMA clustering of 16S rRNA gene 454 pyrosequencing data.



## Fig. S2

Phylum distribution of bacterial communities of all the samples as revealed by 16S rRNA gene based pyrosequencing. “Others” includes Actinobacteria, Cyanobacteria, Armatimonadetes, Spirochaetes, Chlamydiae, Deferribacteres, Chlorobi, Bacteroidetes, BRC1, Aquificae, Verrucomicrobia, TM7, OD1, OP11, Deinococcus-Thermus, Gemmatimonadetes, and Planctomycetes, each less than 1 % of the total sequences per group.



## Fig. S3

Differential distribution of Proteobacteria and Acidobacteria among treatments. OTUs that contained at least 1% of the total sequences in at least one treatment were selected and agglomerated to order. The orders depicted here are significantly different (p<= 0.05) among treatments.



## Fig. S4

Canonical correspondence analysis (CCA) compares the 16S rRNA gene pyrosequencing data (symbols) and environmental variables (arrows). Environmental variables were chosen based on significance calculated from individual CCA results and variance inflation factors (VIFs) calculated during CCA. The percentage of variation explained by each axis is shown, and the relationship is significant (P < 0.005)

Ancientt

Modern

## Fig. S5

UPGMA clustering of samples based on normalized signal intensity data for detected functional genes.



## Fig. S6

Canonical correspondence analysis (CCA) compares the GeoChip hybridization signal intensities (symbols) and environmental variables (arrows). Environmental variables were chosen based on significance calculated from individual CCA results and variance inflation factors (VIFs) calculated during CCA. The percentage of variation explained by each axis is shown, and the relationship is significant (p < 0.005).



## Fig. S7

The normalized average signal intensity of the detected functional genes involved in carbon cycling. The complexity of carbon is presented in order from labile to recalcitrant. All data are presented as the mean ± SE. \*\*p < 0.01, \*p < 0.05.



## Fig. S8

The normalized average signal intensity of the detected functional genes involved in methane metabolism. mcrA encoding alpha subunit of methyl coenzyme M reductase; mmoX encoding particulate methane monooxygenase; pmoA encoding methane monooxygenase. All data are presented as the mean ± SE. \*\*p < 0.01.