Science of the Total Environment 803 (2022) 150131

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Comparison of methane metabolism in the rhizomicrobiomes of wild and related cultivated rice accessions reveals a strong impact of crop domestication

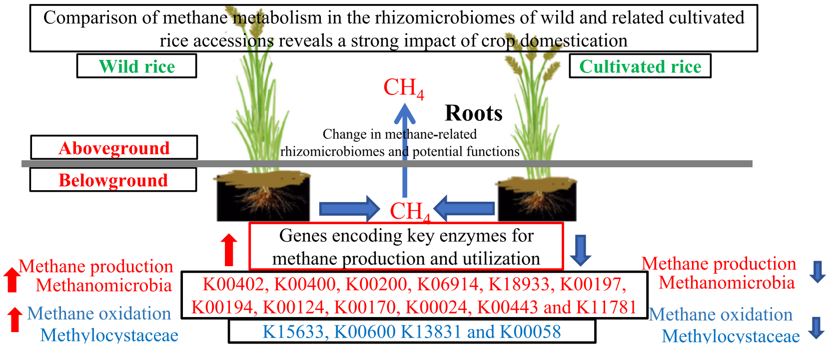
Lei Tian [a], Jingjing Chang [a,b,e,f], Shaohua Shi [a], Li Ji [a,b], Jianfeng Zhang [c], Yu Sun [a], Xiaojie Li [a], Xiujun Li [a], Hongwei Xie [d], Yaohui Cai [d], Dazhou Chen [d], Jilin Wang [d], Johannes A. van Veen [e], Eiko E. Kuramae [e,f,*], Lam-Son Phan Tran [g,h,**], Chunjie Tian [a,***]

[a] *Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, Jilin 130102, China*
[b] *University of Chinese Academy of Sciences, Beijing 100049, China*
[c] *College of Life Science, Jilin Agricultural University, Changchun, Jilin, China*
[d] *Jiangxi Super-rice Research and Development Center, National Engineering Laboratory for Rice, Nanchang, China*
[e] *Department of Microbial Ecology, Netherlands Institute of Ecology NIOO-KNAW, Wageningen, the Netherlands*
[f] *Ecology and Biodiversity, Institute of Environmental Biology, Utrecht University, Utrecht, the Netherlands*
[g] *Institute of Research and Development, Duy Tan University, Da Nang 550000, Viet Nam*
[h] *Institute of Genomics for Crop Abiotic Stress Tolerance, Department of Plant and Soil Science, Texas Tech University, TX 79409, USA*

## HIGHLIGHTS

- Methane metabolism represents a critical function of the rhizosphere microbial community of rice.
- The rhizomicrobiomes of wild and cultivated rice showed differential ecological balance for methane metabolism.
- Rice domestication has a strong impact on methane metabolism.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

*Article history:*
Received 8 September 2020
Received in revised form 28 June 2021
Accepted 31 August 2021
Available online 4 September 2021

Editor: Manuel Esteban Lucas-Borja

## ABSTRACT

Microbial communities from rhizosphere (rhizomicrobiomes) have been significantly impacted by domestication as evidenced by a comparison of the rhizomicrobiomes of wild and related cultivated rice accessions. While there have been many published studies focusing on the structure of the rhizomicrobiome, studies comparing the functional traits of the microbial communities in the rhizospheres of wild rice and cultivated rice accessions are not yet available. In this study, we used metagenomic data from experimental rice plots to analyze the potential functional traits of the microbial communities in the rhizospheres of wild rice accessions originated from Africa and Asia in comparison with their related cultivated rice accessions. The functional potential of rhizosphere microbial communities involved in alanine, aspartate and glutamate metabolism, methane metabolism, carbon fixation pathways, citrate cycle (TCA cycle), pyruvate metabolism and lipopolysaccharide biosynthesis

---

[*] Correspondence to: E.E. Kuramae, Department of Microbial Ecology, Netherlands Institute of Ecology NIOO-KNAW, Droevendaalsesteeg 10, 6708 PB Wageningen, the Netherlands.
[**] Correspondence to: L.-S. P. Tran, Institute of Genomics for Crop Abiotic Stress Tolerance, Department of Plant and Soil Science, Texas Tech University, TX 79409, USA.
[***] Correspondence to: C. Tian, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, No.4888, Shengbei Street, Changchun, Jilin 130102, China.
*E-mail addresses:* E.Kuramae@nioo.knaw.nl (E.E. Kuramae), son.tran@ttu.edu, tranplamson@duytan.edu.vn (L.-S.P. Tran), tiancj@neigae.ac.cn (C. Tian).

https://doi.org/10.1016/j.scitotenv.2021.150131

*Keywords:*
Cultivated rice
Wild rice
Microbial functional potential
Domestication
Methane production
Root-inhabited microbiomes

pathways were found to be enriched in the rhizomicrobiomes of wild rice accessions. Notably, methane metabolism in the rhizomicrobiomes of wild and cultivated rice accessions clearly differed. Key enzymes involved in methane production and utilization were overrepresented in the rhizomicrobiome samples obtained from wild rice accessions, suggesting that the rhizomicrobiomes of wild rice maintain a different ecological balance for methane production and utilization compared with those of the related cultivated rice accessions. A novel assessment of the impact of rice domestication on the primary metabolic pathways associated with microbial taxa in the rhizomicrobiomes was performed. Results indicated a strong impact of rice domestication on methane metabolism; a process that represents a critical function of the rhizosphere microbial community of rice. The findings of this study provide important information for future breeding of rice varieties with reduced methane emission during cultivation for sustainable agriculture.

## 1. Introduction

Rice (*Oryza* spp.) is one of the major food crops produced in the world; especially *Oryza sativa* that is the major contributor to the food requirement of more than half of the world's population (Khush, 2005). There are many wild and cultivated *Oryza* species that are distributed worldwide. And genomes of *Oryza* species were classified based on the chromosomes in pairing correctly during meiosis in interspecies F1 hybrids (Kurata, 2008). All cultivated species of rice and their wild progenitors are diploid and represent species possessing the AA genome (Vaughan et al., 2008).

The development of cultivated rice from wild rice significantly increased desirable traits, such as increased yield and nutritional content (Moner et al., 2018; Zhao et al., 2018). African and Asian cultivated rice species were independently domesticated (Chang, 1976; Nayar, 2012; Waters et al., 2012). African cultivated rice, *O. glaberrima*, was domesticated from African wild rice, *O. barthii*, while Asian cultivated rice, *O. sativa*, was domesticated from common wild rice (*O. rufipogon*) or nivara wild rice (*O. nivara*) (Yamanaka et al., 2003; Li et al., 2006; Bessho-Uehara et al., 2017). *Oryza sativa* has been continuously subjected to breeding efforts, which resulted in the establishment of two main varieties indica and japonica (Cheng et al., 2019).

The long-term process of domestication and crop breeding has also led to significant changes in the composition and structure of the microbial communities surrounding the roots of many crop species, including common bean (*Phaseolus vulgaris*) (Perez-Jaramillo et al., 2019), barley (*Hordeum vulgare*) (Bulgarelli et al., 2015), lettuce (*Lactuca sativa*) (Cardinale et al., 2015) and sunflower (*Helianthus annuus*) (Leff et al., 2017). It is generally presumed that wild plant species have characteristics that support the ability of plants to survive when subjected to abiotic stresses, including drought, extreme temperatures and low-nutrient soils, and biotic stresses like soil-borne pathogens (Bin Rahman and Zhang, 2016; Cen et al., 2018; Tian et al., 2018b). The greater adaptability of wild species may be partially attributed to their association with the microbial communities in the rhizosphere (Cen et al., 2018; Shi et al., 2018; Tian et al., 2020b).

Studies comparing the rhizosphere microbiomes (rhizomicrobiomes) of wild and domesticated crops have focused on community structure, while no studies have yet explored microbiological functions at the microbiome level to better understand the basis for plant and microbiome mutual selection and co-existence (Shenton et al., 2016; Tian et al., 2017; Tian et al., 2020a). Shenton et al. (2016) studied different genotypes of rice and found that the structure of the microbial communities in the rhizospheres of wild and cultivated rice significantly differed (Shenton et al., 2016). In particular, taxa of methanotrophic *Anaerolineae* were overrepresented in the rhizocommunities of wild rice relative to cultivated rice. The authors suggested that methane emission from rice paddy fields could be lowered by introgressing genetic materials from wild rice into cultivated rice (Shenton et al. 2016). This suggestion, however, was only based on the comparative analysis of microbial community structures associated with wild and cultivated rice, but not on any assessment of the potential functional traits of the rhizosphere communities at gene level (Shenton et al., 2016), which would require a different methodology and further studies. Yet, the implications of their suggestion are highly relevant since the emission of methane from the huge areas of rice production all over the world are a major source of global greenhouse gas emission (Su et al., 2015; Bhattacharyya et al., 2016; Carlson et al., 2017; Su et al., 2018; Bhattacharyya et al., 2019), which needs to be reduced. Therefore, a comparison of the potential functional traits of the rhizomicrobiomes of wild and related cultivated rice accessions at gene level using metagenomic analysis could provide a greater understanding of the impact of crop domestication on the differential functional traits of their rhizomicrobiomes.

Thus, in the present study, microbial functional potential in the rhizospheres of wild and domesticated rice accessions originating from Asia and Africa were comparatively assessed under field conditions by conducting shotgun metagenome sequencing of rhizomicrobiomes. This approach could provide information on the impact of rice domestication on the potential functional traits in the rhizomicrobiomes of domesticated rice accessions. Additionally, results of this study may also provide information on the mechanisms responsible for potential functions of the microbial communities in the rhizospheres of wild and cultivated rice in a comparative manner.

## 2. Materials and methods

### 2.1. Site description, plant materials, and experimental design

The study area was located in the rice experimental region of the Jiangxi Agricultural Institute Station (18°19′57 N, 109°27′ E) in San'ya, Hainan Province, China. The field experiment was conducted in a tropical maritime monsoon climate with an annual average temperature of 25.7 °C, and a mean annual precipitation of 1347.5 mm, of which 85% occurs during the months between May and October.

Three wild rice species (namely African wild rice, and Asian common wild rice and nivara wild rice), and two cultivated rice species, *Oryza glaberrima* (varieties LM8 and WH20 representing African cultivated rice) and *O. sativa* (varieties 106 indica, Meitezhen indica, Jiangxi japonica and Daohuaxiang japonica representing Asian cultivated rice) were used in the study (Table 1). Thus, one African wild rice species [one variety *O. barthii* SW42 (Af_W1-5)] and one African cultivated rice species [two varieties *O. glaberrima* LM8 (Af_C1-5) and WH20 (Af_C6-10)] were used in the comparison of African wild and cultivated rice (Table 1). On the other hand, two Asian wild rice species, namely the common wild rice [two varieties *O. rufipogon* SW499 (As_W1-5) and SW502 (As_W6-9)] and the nivara wild rice [two varieties *O. nivara* SW218 (As_W11-15) and SW223 (As_W16-20)], and one Asian cultivated rice species [four varieties *O. sativa* 106 indica (As_C1-5), Meitezhen indica (As_C6-10), Jiangxi japonica (As_C11-15) and Daohuaxiang japonica (As_C16-20)] were used in the comparison of Asian wild and cultivated rice. Seeds of African wild and cultivated rice accessions were kindly provided by the International Rice Research Institute (IRRI), while those of the Asian cultivated rice accessions were obtained from the Jiangxi Academy of Agricultural Sciences (Table 1). Experimental

**Table 1**
Wild rice species and their cultivated relatives used in this study.

| Origin | Cultivar name | Species | International code | Sample name | Distribution |
|---|---|---|---|---|---|
| Africa | African wild rice SW42 | *Oryza barthii* | 106,238 | Af-W1-5 | Western, eastern, southern Africa |
| | African cultivated rice LM8 | *O. glaberrima* | LM8 | Af-C1-5 | Western Africa |
| | African cultivated rice WH20 | *O. glaberrima* | WH20 | Af-C6-10 | Western Africa |
| Asia | Common wild rice SW499 | *O. rufipogon* | 106,286 | As-W1-5 | Asia |
| | Common wild rice SW502 | *O. rufipogon* | 106,452 | As-W6-9 | |
| | Indian wild rice SW218 | *O. nivara* | 86,655 | As-W11-15 | Asia |
| | Indian wild rice SW223 | *O. nivara* | 88,949 | As-W16-20 | |
| | 106 indica | *O. sativa* subsp. indica | – | As-C1-5 | China |
| | Meitezhen indica | *O. sativa* subsp. indica | – | As-C6-10 | China |
| | Jiangxi japonica | *O. sativa* subsp. japonica | – | As-C11-15 | China |
| | Daohuaxiang japonica | *O. sativa* subsp. japonica | – | As-C16-20 | China |

field plots were designed in the same size (2-m width and 2-m length) for rice planting. The experiment was arranged in a randomized block design with a distance of 0.5 m between two plots. Under such conditions, the plots used to grow the replicates could be considered to have neglectable differences in soil properties. Each rice accession had 5 replicates, except the common wild rice accession SW502 (As_W6-9) that had 4 replicates.

### *2.2. DNA extraction, sequencing and data analysis*

Four plants were harvested for each collected sample during the flowering stage. The rhizosphere soil was carefully collected by gentle brushing of the whole root system (Schlemper et al., 2017b). A total of 0.5 g soil from each sample were used for the extraction of DNA from the rhizomicrobiome following the protocol provided by the manufacturer of the Fast DNA SPIN Kit (Catalog No. 6560-220, USA). The concentration and quality of the extracted DNA were determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Germany). The DNA from each sample was fragmented using an ultrasonic probe, resulting in an average fragment length of 300-bp length. An adapter was added to each of the DNA fragments by PCR for the construction of the DNA libraries. The DNA libraries were submitted for a paired-end shotgun sequencing using an Illumina HiSeq X TEN (San Diego, CA, USA) platform. The raw metagenomic sequencing data were quality-controlled using fastp (https://github.com/OpenGene/fastp). Clean data were assembled into contigs using megahit (Li et al., 2015; Yan et al., 2016). The contigs ≥ 500 bp were used for gene prediction with MetaGeneMark (Zhu et al., 2010). Cd-hit was used to remove the redundancy of any predicted genes with 90% coverage and 90% similarity (Li and Godzik, 2006). For quantification of the abundance of the predicted genes, Bowtie 2 was used to blast the contigs of the predicted genes against the raw metagenomic sequencing reads, and SAMtool was used to convert SAM files into BAM files (Li et al., 2009; Langmead and Salzberg, 2012). Subsequently, transcripts per million reads (TPM) representing the gene abundances were calculated by eXpress software (https://github.com/adarob/eXpress). To analyze the bacterial functional potential and annotated genes, the sequences of the microbial genes were queried in KAAS (KEGG Automatic Annotation Server) (https://www.genome.jp/tools/kaas/) to obtain the KEGG orthology (KO) number (Moriya et al., 2007). Differentially abundant genes in the rhizomicrobiomes of Asian wild rice versus the rhizomicrobiomes of Asian cultivated rice accessions were determined by using the TPM data [one-way ANOVA and Student's *t*-tests ($P < 0.01$) in R v.3.3.1 (https://www.r-project.org/)]. Then, KEGG pathway enrichment analysis was conducted to identify microbial genes more abundant in the rhizomicrobiomes of African wild rice versus rhizomicrobiomes of African cultivated rice accessions by the package GOstats in the package of Bioconductor in R v.3.3.1. To analyze the microbial compositions in the rhizospheres of wild and cultivated rice, the metagenomic data was explored to annotate the taxonomies of the related archaea and bacteria using Metaphlan2 software (https://bitbucket.org/biobakery/biobakery/wiki/metaphlan2) (Truong et al., 2015).

### *2.3. Statistical analysis*

Principal component analysis (PCA) based on the TPM data of the genes or TPM data of methane metabolism-related genes was conducted using the PCA function in the FactoMineR package in R v.3.3.1. A bubble diagram was constructed from the enriched KEGG pathways of microbial genes in the rhizospheres of wild versus the rhizospheres of cultivated rice samples using the ggplot2 package in R v.3.3.1. One-way ANOVA and Tukey's tests were used to determine the statistical significance of differences ($P < 0.05$) between rhizomicrobiomes of African wild rice and African cultivated rice, and between rhizomicrobiomes of Asian wild rice and Asian cultivated rice accessions, in terms of the functional KOs of the methane metabolism-related microbial genes. The correlation between rhizosphere functional KOs were assessed by SparCC analysis (SparCC's rho cut-off = 0.8, $P < 0.01$).

## **3. Results**

### *3.1. Summary of the metagenomic data set*

The metagenomic sequencing data representing the microbiota in rhizosphere soil samples associated with wild and cultivated rice genotypes were used to evaluate and compare the potential functional traits and community structures of their rhizomicrobiomes. The raw sequencing data for the rhizosphere soil samples each contained reads between 40.7 and 153.0 million. Quality trimming of raw metagenomic data resulted in clean data sets of 39.2–147.7 million raw reads per sample (Table S1). De novo co-assembly generated high-quality scaffolds for each sample after discarding scaffolds shorter than 500 bp and contaminant scaffolds (Table S1). The N50 value ranged from 583 to 690 bp of the scaffolds (Table S1).

### *3.2. Functional profiles of the rhizomicrobiomes of different wild and related cultivated rice accessions*

PCA analysis of the microbial functional structure in the rhizospheres of wild and cultivated rice varieties based on the KO assignment was conducted (Fig. 1). A clear separation in the potential functional profiles of the rhizomicrobiomes of wild and related cultivated rice samples was evident for both the African and Asian rice accessions. Notably, the overall functional profiles of the rhizomicrobiomes of wild rice and domesticated rice only explained 9.04% of the variation on PC1 (Fig. 1a). Clustering of the KEGG functional profiles of the rhizomicrobiomes of African rice species showed that African wild rice was well separated from the African cultivated rice; and interestingly, the two African cultivated rice varieties were also clearly separated from each other (Fig. 1b). On the other hand, based on the clustering data, the two Asian wild rice species displayed a clear separation from each other

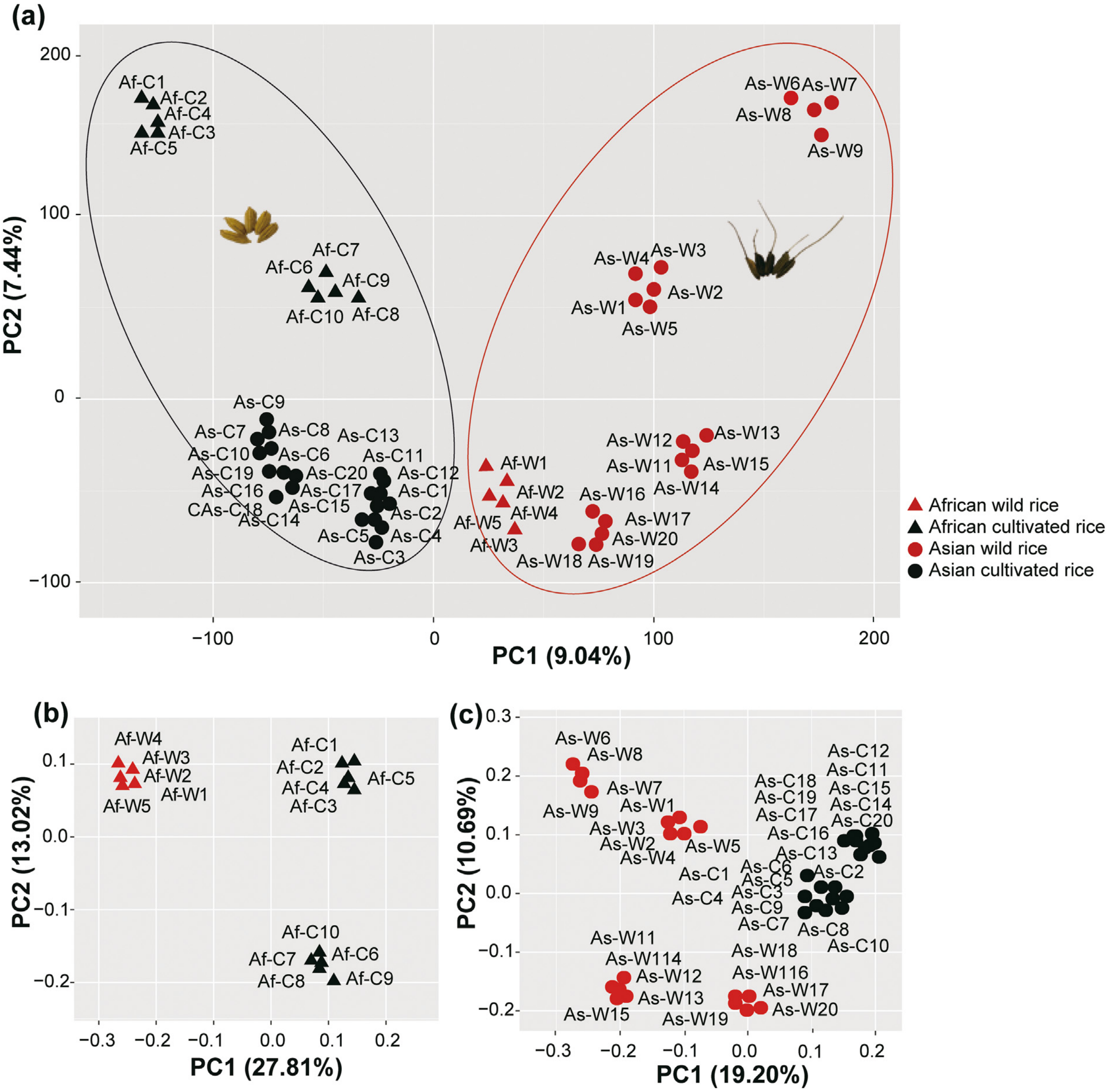


**Fig. 1.** Principal component (PC) analysis of samples based on the KEGG functional profiles of the different rhizomicrobiomes. (a) Functional profiles of the rhizomicrobiomes of all samples. (b) Functional profiles of the rhizomicrobiomes of African wild and cultivated rice. (c) Functional profiles of the rhizomicrobiomes of Asian wild and cultivated rice. The four groups were African wild rice *Oryza barthii* (Af_W1-5), African cultivated rice *O. glaberrima* (Af_C1-10), Asian wild rice (As_W1-9 and As_W11-20), and Asian cultivated rice varieties (As_C1-20). Af_W1-5, African wild rice; Af_C1-5, African cultivated rice LM8; Af_C6-10, African cultivated rice WH20; As_W1-5, Asian common wild rice SW499; As_W6-9, Asian common wild rice SW502; As_W11-15, nivara wild rice SW218; As_W16-20, nivara wild rice SW223; As_C1-5, 106 indica; As_C6-10, Meitezhen indica; As_C11-15, Jiangxi japonica; As_C16-20, Daohuaxiang japonica. Each rice accession has 5 replicates, except the common wild rice accession SW502 that has 4 replicates (As_W6-9).

(Fig. 1c), and the four Asian cultivated rice varieties were closer to each other than the two Asian wild rice species (Fig. 1c).

### *3.3. Comparative KEGG pathway enrichment analysis in the rhizomicrobiomes of wild rice and cultivated rice accessions*

The KEGG pathway enrichment analysis was then used to identify the genes more abundant in the rhizomicrobiomes of wild rice vs. relative cultivated rice accessions revealed that there were a wide variety of pathways that differed between African wild and related cultivated rice accessions, as well as between Asian wild and related cultivated rice accessions (Fig. 2). Pathways associated with methane metabolism, alanine, aspartate and glutamate metabolism, carbon fixation, lipopolysaccharide biosynthesis, nitrogen metabolism, pyruvate metabolism, etc. were more highly enriched in the rhizomicrobiome of African wild rice (Af_W1-5) than those of African cultivated rice (Af_C1-10) (Fig. 2a). Pathways associated with methane metabolism, pyruvate metabolism, glycolysis/gluconeogenesis, pyrimidine metabolism, purine metabolism, etc., were found to be more enriched in the rhizomicrobiomes of Asian wild rice (As_W1-9 and As_W11-20) than those of Asian cultivated rice varieties (As_C1-20) (Fig. 2b).

Among the differentially enriched pathways, alanine, aspartate and glutamate metabolism, methane metabolism, carbon fixation, citrate cycle (TCA cycle), pyruvate metabolism and lipopolysaccharide biosynthesis were found to be enriched most consistently and pronounced in the rhizomicrobiomes of wild rice species originated from both Africa or Asia (Fig. 2). From the aspect of methane metabolism, 30 methane metabolism-related genes were more abundant in the rhizomicrobiome of African wild rice relative to those of African cultivated rice (Table S2), while 181 methane metabolism-related genes were more abundant in the rhizomicrobiomes of Asian wild rice compared with those of Asian cultivated rice accessions (Table S3). In addition, we also carried out

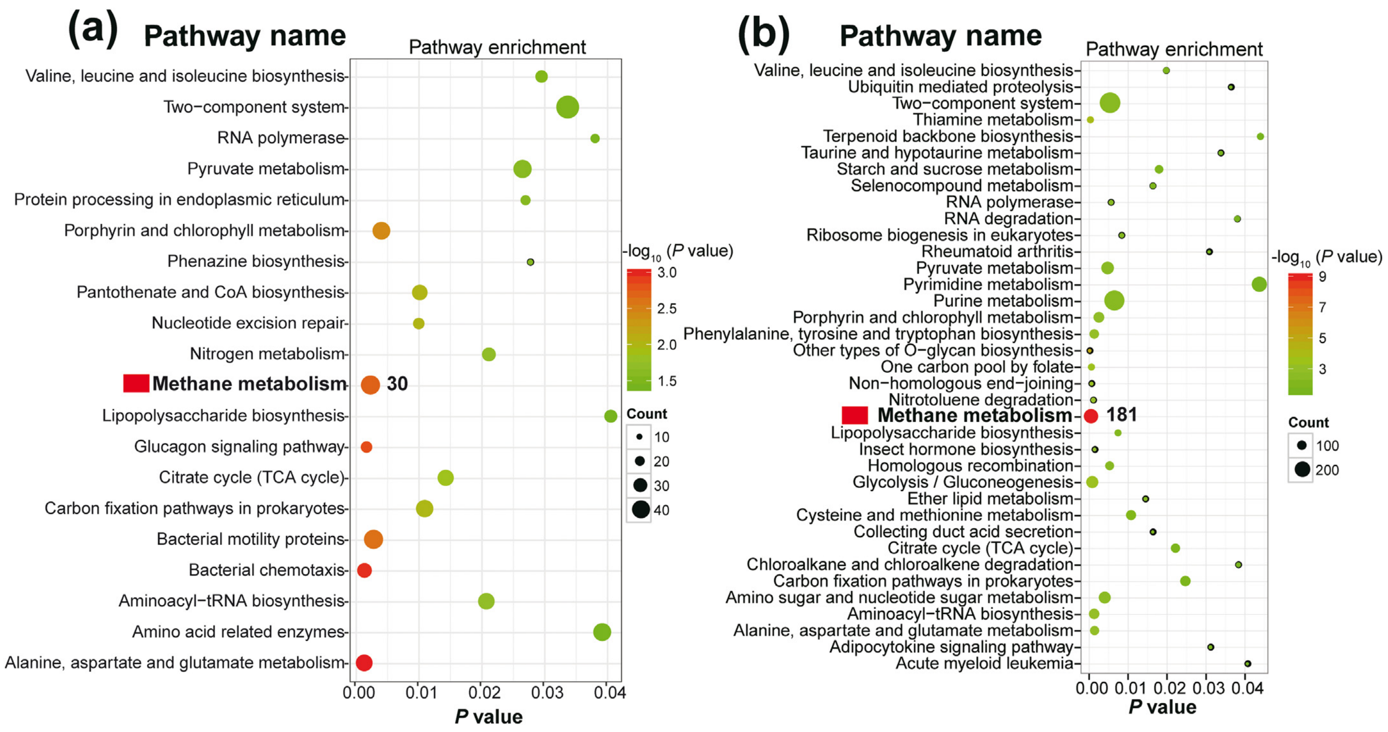


**Fig. 2.** KEGG pathway enrichment analysis of the rhizomicrobiomes of wild rice versus cultivated rice. (a) Comparison of the rhizomicrobiomes of African wild rice *Oryza barthii* (Af_W1-5) and African cultivated rice *O. glaberrima* (Af_C1-10). (b) Comparison of the rhizomicrobiomes of Asian wild rice (As_W1-9 and As_W11-20) and Asian cultivated rice varieties (As_C1-20). KEGG pathway enrichment with a *P* value < 0.01 was considered statistically significant. The count indicates the number of genes related to the KEGG pathway enrichment analysis. $-\log_{10}$ (*P* value) data are used to represent significantly enriched KEGG pathways, and color intensity indicates the significant levels as designated by the colored bar.

the KEGG analysis of the less abundant genes obtained from the comparison of the rhizomicrobiomes of wild rice vs. relative cultivated rice accessions, and the result revealed that methane metabolism-related less abundant genes were not present in such comparison (Fig. S1).

### 3.4. Profiles of methane metabolism-related genes that are more enriched in the rhizomicrobiomes of wild versus cultivated rice accessions

As illustrated in Fig. 3a, the profiles of genes involved in methane metabolism and more enriched in the rhizospheres of wild and cultivated rice accessions clearly clustered separately (PC1 23.16%). Notably, the separation between wild and cultivated accessions was even more distinct, when genetically-related accessions (African wild vs. cultivated rice and Asian wild vs. cultivated rice) were compared (Fig. 3b and c). A total of 18 common genes involved in methane metabolism were more significantly enriched in wild versus cultivated rice accessions originating from both Africa and Asia (Table S4). Among these common genes, 12 genes encoding K00402, K00400, K00200, K06914, K18933, K00197, K00194, K00124, K00170, K00024, K00443 and K11781 are involved in methane production, while 6 genes encoding K15633, K00600 K13831 and K00058 are involved in methane utilization (Table S4).

### 3.5. The relative abundance of methanogenic archaea and methane oxidation-related bacteria

Next, methane metabolism-related reads were annotated to taxonomic status using Metaphlan2 software. The annotated taxa were mainly affiliated with the Methylocystaceae (methane consumers) and Methanomicrobia (methane producers). The relative abundance of Methanomicrobia was significantly enriched in the rhizomicrobiomes of both African and Asian wild rice relative to their related cultivated rice accessions (Fig. S2). The relative abundance of Methanomicrobia in the rhizosphere of African wild accession (46%) was remarkably higher than that in the rhizosphere of related cultivated rice varieties (21%) (Fig. S2a and b). The relative abundances of Methanomicrobia in the rhizospheres of Asian rice accessions were 34% and 24% for wild and cultivated accessions, respectively (Fig. S2c and d). On the other hand, the relative abundances of Methylocystaceae in the rhizospheres of all four groups were low, ranging between 1.03 and 2.08%. More specifically, the relative abundance of Methylocystaceae in the rhizospheres of African cultivated rice (1.19%) was lower than in the rhizosphere of African wild rice (1.93%), and the relative abundance of Methylocystaceae in the rhizospheres of Asian cultivated rice (1.03%) was lower than in the rhizospheres of Asian wild rice accessions (2.81%).

### 3.6. Co-occurrence of methane metabolism-related KOs and functional KOs associated with other pathways in the rhizomicrobiomes of wild and cultivated rice accessions

Functional interactions play an important role in pathway assembly (Yan et al., 2016). Therefore, the interactions between methane metabolism-related KOs and other pathway-related functional KOs enriched in the rhizomicrobiomes in African wild rice, Asian wild rice, African cultivated rice and Asian cultivated rice were illustrated in a network analysis based on the obtained SparCC correlation coefficients (Fig. 4). Visualization of the interactions between methane metabolism-related KOs and functional KOs representing other pathways revealed that the interaction network was the most complex in the rhizosphere of African wild rice, which had the largest numbers of nodes and correlations (Fig. 4a). The network in the rhizomicrobiomes of African cultivated rice accessions was less complex, although a relatively large number of interactions were still observed (Fig. 4b). The co-occurrence networks in Asian rice accessions were rather simpler compared with those of the African rice accessions (Fig. 4c–d). The complexity of the network parameters, including nodes, correlations, modularity and betweenness centrality distribution, followed (from greatest to lowest complexity) the order: African wild rice (Af_W) > African cultivated rice (Af_C) > Asian wild rice (As_W) > Asian cultivated rice (As_C) (Table S5). Furthermore, the numbers of negative correlations in African wild rice and Asian wild rice were

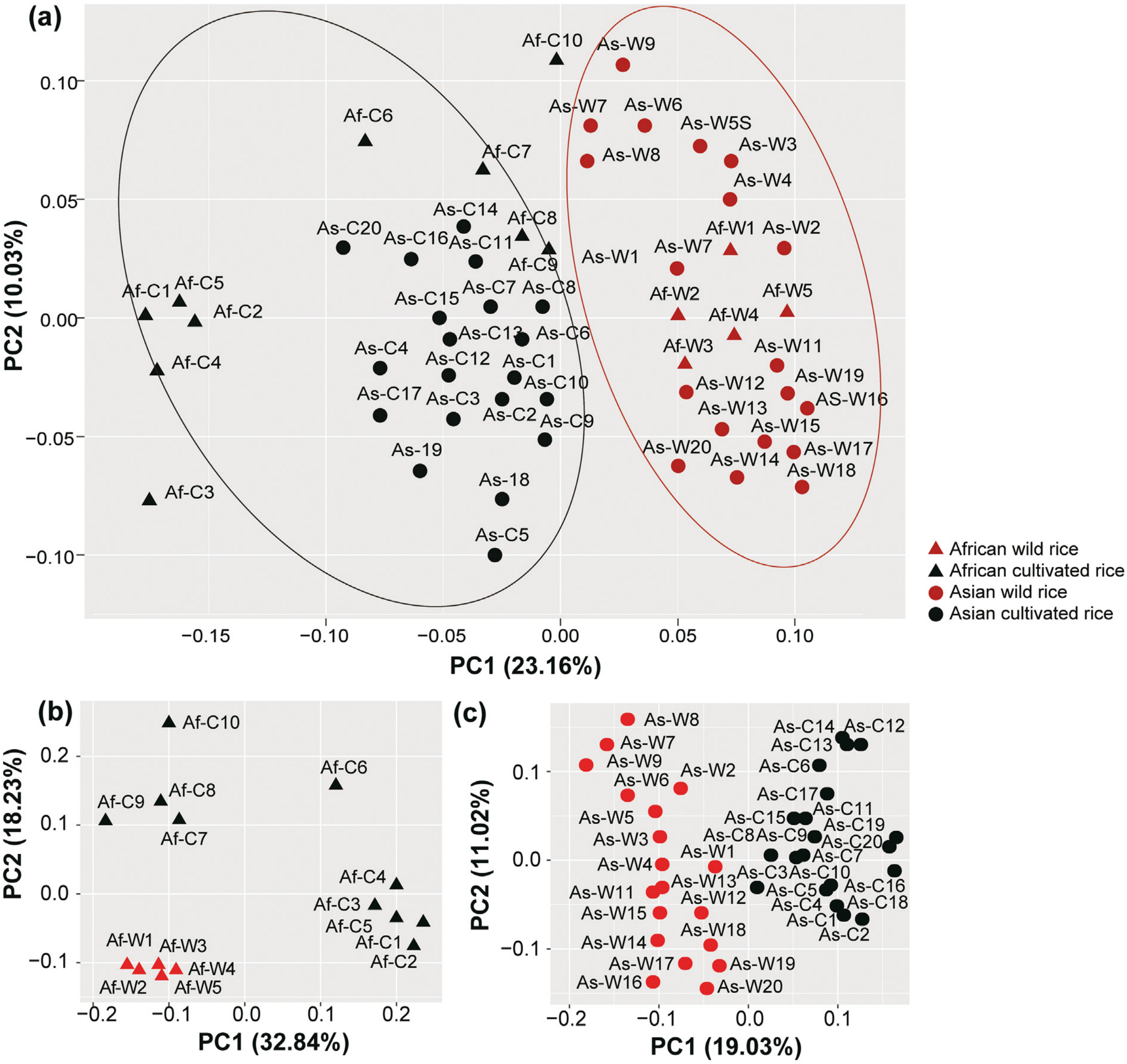


**Fig. 3.** Principal component (PC) analysis of KOs (KEGG orthologs) functioning in the methane metabolism in the rhizomicrobiomes of various rice samples. (a) Distribution of methane metabolism-related KOs in all samples. (b) Distribution of methane metabolism-related KOs in the rhizomicrobiomes of African wild and cultivated rice samples. (c) Distribution of methane metabolism-related KOs in the rhizomicrobiomes of Asian wild and cultivated rice samples. The four groups were African wild rice *Oryza barthii* (Af_W1-5), African cultivated rice *O. glaberrima* (Af_C1-10), Asian wild rice (As_W1-9 and As_W11-20), and Asian cultivated rice varieties (As_C1-20). Af_W1-5, African wild rice; Af_C1-5, African cultivated rice LM8; Af_C6-10, African cultivated rice WH20; As_W1-5, Asian common wild rice SW499; As_W6-9, Asian common wild rice SW502; As_W11-15, nivara wild rice SW218; As_W16-20, nivara wild rice SW223; As_C1-5, 106 indica; As_C6-10, Meitezhen indica; As_C11-15, Jiangxi japonica; As_C16-20, Daohuaxiang japonica. Each rice accession has 5 replicates, except the common wild rice accession SW502 that has 4 replicates (As_W6-9).

higher than in their related African cultivated rice and Asian cultivated rice (Table S5).

### *3.7. Correlation analysis of methane metabolism-related genes with the relative abundances of Methanomicrobia and Methylocystaceae*

Results revealed significant correlations between the relative abundance of methane-producing Methanomicrobia and the overrepresentation of methyl-coenzyme M reductase gamma subunit (K00402), 5-amino-6-(D-ribitylamino) uracil L-tyrosine 4-hydroxyphenyl transferase (K11781) and formylmethanofuran dehydrogenase subunit A (K00200) (Fig. 5). A significant linear relationship was also observed between the TPM of K00402, K11781 and K00200 and the relative abundance of Methanomicrobia (Fig. 5a, b and c). Additionally, a significant correlation was observed between the TPM of K13812, which encodes the bifunctional enzyme Fae (formaldehyde lyase)/Hps (3-hexulose-6-phosphate synthase) that functions in the assimilation of formaldehyde in the ribulose monophosphate pathway (Grochowski et al., 2005), and the relative abundance of methane-consuming Methylocystaceae (Fig. 5d).

## 4. Discussion

Whether domestication of crops from wild ancestor accessions had a specific impact on rhizosphere microbial communities is still a topic of debate, and the information reported in different studies provides a variable picture. Most studies have indicated that crop domestication resulted in directed selection of the rhizosphere microbiome, but that the characteristics of the selection will vary depending on the environment in which the selection has occurred (Nallanchakravarthula et al., 2014; Bulgarelli et al., 2015; Schlemper et al., 2017a). Notably, observations indicate that crop domestication has had a lesser selective effect on bacteria than on fungi (Leff et al., 2016; Carvalhais et al., 2019). This premise is consistent with results reported in our previous study

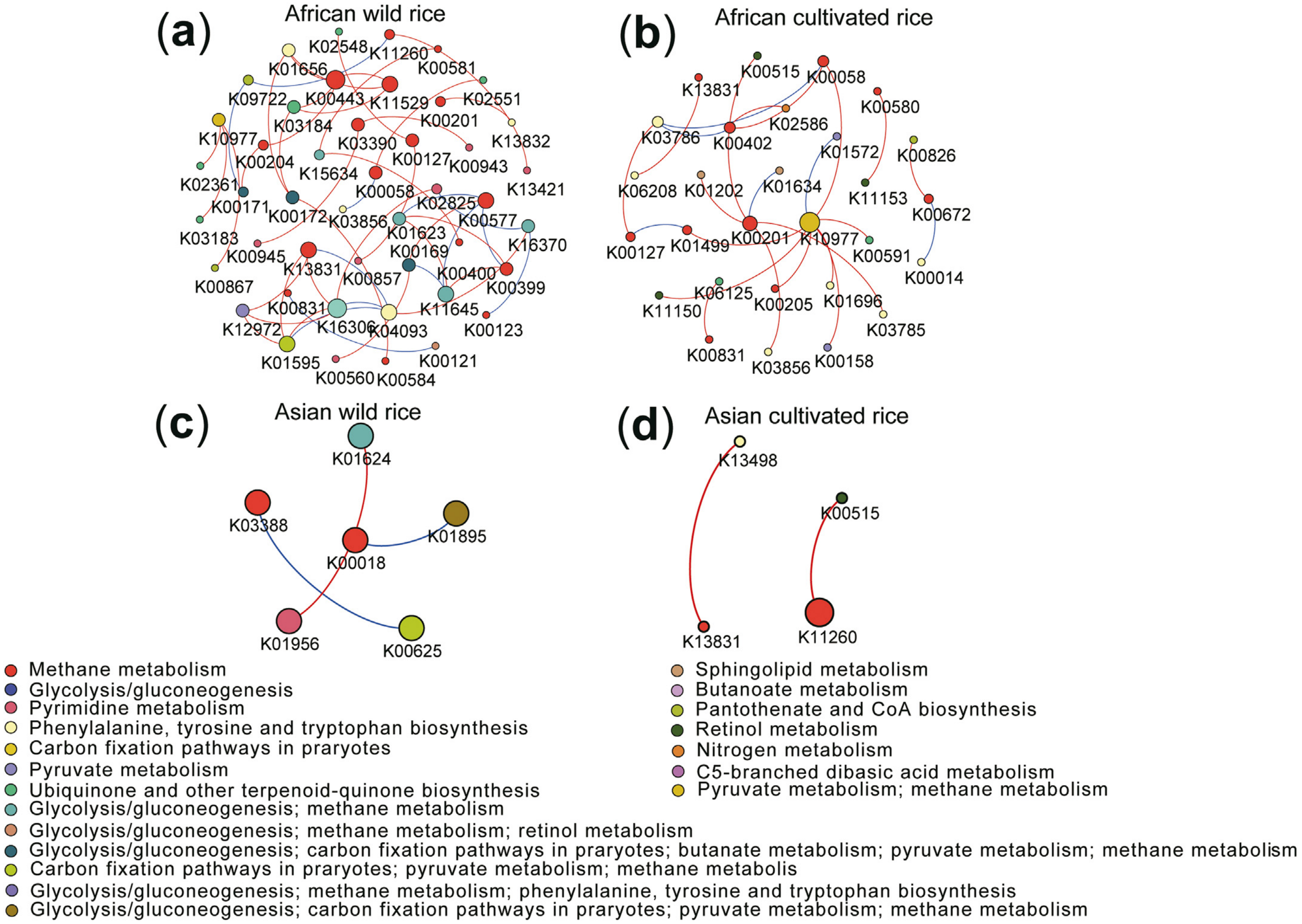


**Fig. 4.** Network based on SparCC correlation coefficients (SparCC's rho cut-off = 0.8, $P$ value $< 0.01$) illustrates how functional KEGG orthologs (KOs) of other pathways are associated with the methane metabolism-related KOs in the rhizomicrobiomes of wild rice (a and c) and cultivated rice (b and d). Red lines represent significant positive ($P < 0.01$) linear relationships, and blue lines represent significant negative ($P < 0.01$) linear relationships. Different colors represent different metabolic pathways. The four groups were African wild rice *Oryza barthii* (Af_W1-5), African cultivated rice *O. glaberrima* (Af_C1-10), Asian wild rice (As_W1-9 and As_W11-20), and Asian cultivated rice varieties (As_C1-20).

comparing the structures of the rhizomicrobiomes of wild and cultivated rice in natural environments (Xu et al., 2019).

Studies addressing the question of the impact of domestication on rhizosphere microbial selection have utilized next generation sequencing of amplicons generated by the PCR-amplification of extracted DNA with universal primer sets to assess microbial diversity (Pérez-Jaramillo et al., 2016; Tian et al., 2018a; Brisson et al., 2019; Tian et al., 2020a). It would be highly relevant for future crop development, however, to increase our knowledge on the intrinsic traits of wild relatives and better understand how domestication has impacted the functioning of the rhizomicrobiome. Therefore, DNA shotgun metagenomic sequencing was used in the current study to identify the major functions potentially carried out in the rhizomicrobiomes of wild and domesticated accessions of rice at their flowering stage, which is sensitive to available nutrients, associated rhizomicrobiome and surrounding environment (Edwards et al., 2018; Yang et al., 2019), to better understand how their functional potential has been impacted by crop domestication. Results from our study, in which all rice varieties were treated in identical experimental conditions, clearly demonstrated that the domestication of rice originating from Asia and Africa impacted the functional potential of the rhizomicrobiomes (Fig. 1). Although the evolution of rice accessions in different geographical locations (Asia and Africa) led to the development of different rice accessions, a number of specific changes were observed in the functional potential of rhizosphere microbiota independently of the origin and type of rice accessions. Specific genes, such as those related to carbon metabolism and amino acid metabolism, were primarily enriched in wild rice, while others, such as those related to nitrogen metabolism, amino acid metabolism, lipid metabolism, metabolism of cofactors and vitamins, biodegradation of xenobiotics, and metabolism and biosynthesis of secondary metabolites, were enriched in cultivated rice, relative to wild rice (Fig. 2).

Notably, the methane metabolism pathway was significantly and consistently enriched in the rhizomicrobiomes of all wild accessions relative to the accessions and varieties of cultivated rice (Fig. 2). This was true for genes involved in methane production and those related to methane oxidation (Tables S2 and S3). Microbial methanogenesis accounts for approximately 74% of natural methane emission (Liu and Whitman, 2008), a process that plays a major role in global warming (Jiang et al., 2019). In fact, rice paddies have been shown to constitute a major source of anthropogenic methane emission (Cui et al., 2015; Chen et al., 2019). Methane that is primarily produced by rhizosphere methanogenic archaea and released during rice growth accounts for approximately 20% of global methane emission (Xiubin et al., 2014; Joseph et al., 2015).

Three types of methanogenic pathways have been identified, namely methanol to methane, $CO_2$ to methane, and acetate to methane (Evans et al., 2019). 18 common genes, which were more abundant in rhizomicrobiomes of wild rice versus those of cultivated rice, encode 16 KOs (Table S4), including 12 KOs that represent the

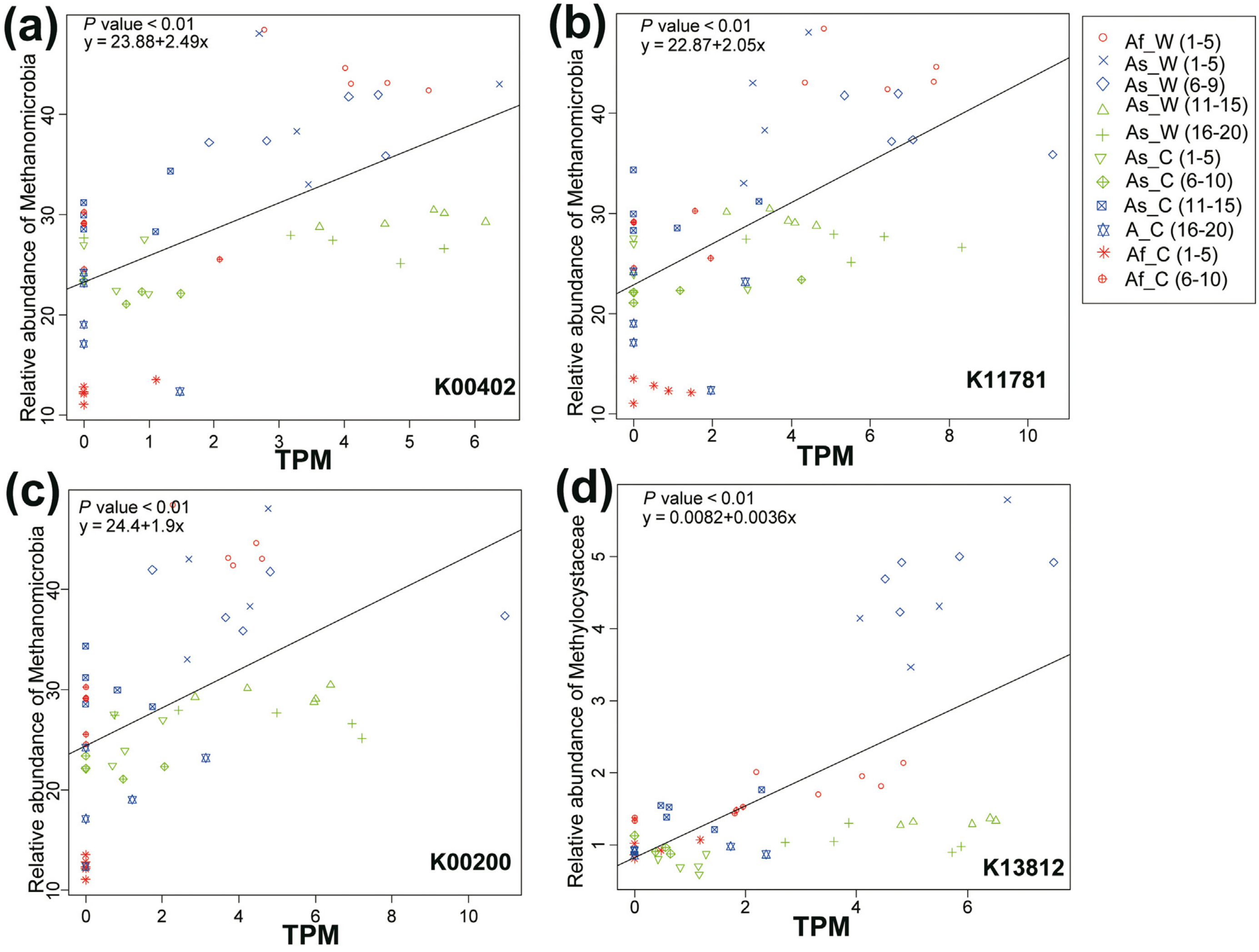


**Fig. 5.** (a–c) Correlations of the transcripts per million reads (TPM) of K00402 (a), K11781 (b), and K00200 (c) with the relative abundance of Methanomicrobia. (d) Correlation of the TPM of K13812 with the relative abundance of Methylocystaceae. The four groups were African wild rice *Oryza barthii* (Af_W1-5), African cultivated rice *O. glaberrima* (Af_C1-10), Asian wild rice (As_W1-9 and As_W11-20), and Asian cultivated rice varieties (As_C1-20). Af_W1-5, African wild rice; Af_C1-5, African cultivated rice LM8; Af_C6-10, African cultivated rice WH20; As_W1-5, Asian common wild rice SW499; As_W6-9, Asian common wild rice SW502; As_W11-15, nivara wild rice SW218; As_W16-20, nivara wild rice SW223; As_C1-5, 106 indica; As_C6-10, Meitezhen indica; As_C11-15, Jiangxi japonica; As_C16-20, Daohuaxiang japonica. Each rice accession has 5 replicates, except the common wild rice accession SW502 that has 4 replicates (As_W6-9).

three types of methanogenic pathways. More specifically, K00200, K00124, K00443, K06914, K18933 and K11781 of the $CO_2$-to-methane pathway were more enriched in the rhizomicrobiomes of wild rice than in those of cultivated rice (Fig. 6). This result suggests that the rhizomicrobiomes of wild rice has a greater potential to transform $CO_2$ into methane than those of cultivated rice. Similarly, K00197, K00194, K00170 and K00024 belonging to the acetate-to-methane pathway, while K00402 and K00400 participating in the methanol to methane pathway, were also more highly enriched in the rhizomicrobiomes of wild rice than in those of cultivated rice (Fig. 6).

Three types of methanotrophic pathways are recognized: the serine pathway, the ribulose phosphate pathway and the xylulose phosphate pathway (Kalyuzhnaya et al., 2015). Results of this study indicated that the serine pathway with associated genes K15633, K00058 and K00600, and the ribulose phosphate pathway with associated genes K13812 were enriched in the rhizomicrobiomes of wild rice than those of cultivated rice ($P < 0.05$) (Fig. 7, Table S4), which is consistent with the finding that methane oxidation is also more active in the rhizomicrobiomes of wild rice than those of cultivated rice accessions. Interactive networks showed that methane production-related KOs in the rhizomicrobiomes of wild rice were mainly associated with the carbon fixation pathway, pyruvate metabolism, glycolysis/gluconeogenesis and pyrimidine metabolism (Fig. 4), which can provide energy resources and intermediate molecules required for methane production (Lu et al., 2017; Staley et al., 2017; Miret-Casals et al., 2018). However, methane oxidation-related KOs in the rhizomicrobiomes of cultivated rice were mainly associated with phenylalanine, tyrosine and tryptophan biosyntheses, as well as retinol metabolism (Fig. 4). The aromatic amino acid phenylalanine, in addition to tyrosine and tryptophan, all are vital constituents of proteins and serve as precursors for thousands of indispensable metabolites (Maeda and Dudareva, 2012). Retinoids play an important role in regulating important biological processes, including morphogenesis, development, reproduction and apoptosis (Novák et al., 2008). Thus, their interaction with methane oxidation pathways may be indirect.

The greater abundance of genes involved in methane metabolism in the rhizomicrobiomes of wild rice accessions, relative to their abundance in the rhizomicrobiomes of cultivated relatives, suggests that both methane synthesis and oxidation are more pronounced in the rhizomicrobiomes of wild rice than cultivated rice (Figs. 6 and 7). In wild rice, the archaea present in the rhizomicrobiomes of wild rice can utilize $CO_2$, acetate and formate for methane synthesis more effectively than cultivated rice (Fig. 6) (Conrad et al., 2009; Maurer et al., 2018; Bhattacharyya et al., 2019). Such higher efficiency may allow wild rice accessions to reduce the level of acidification caused by anaerobic

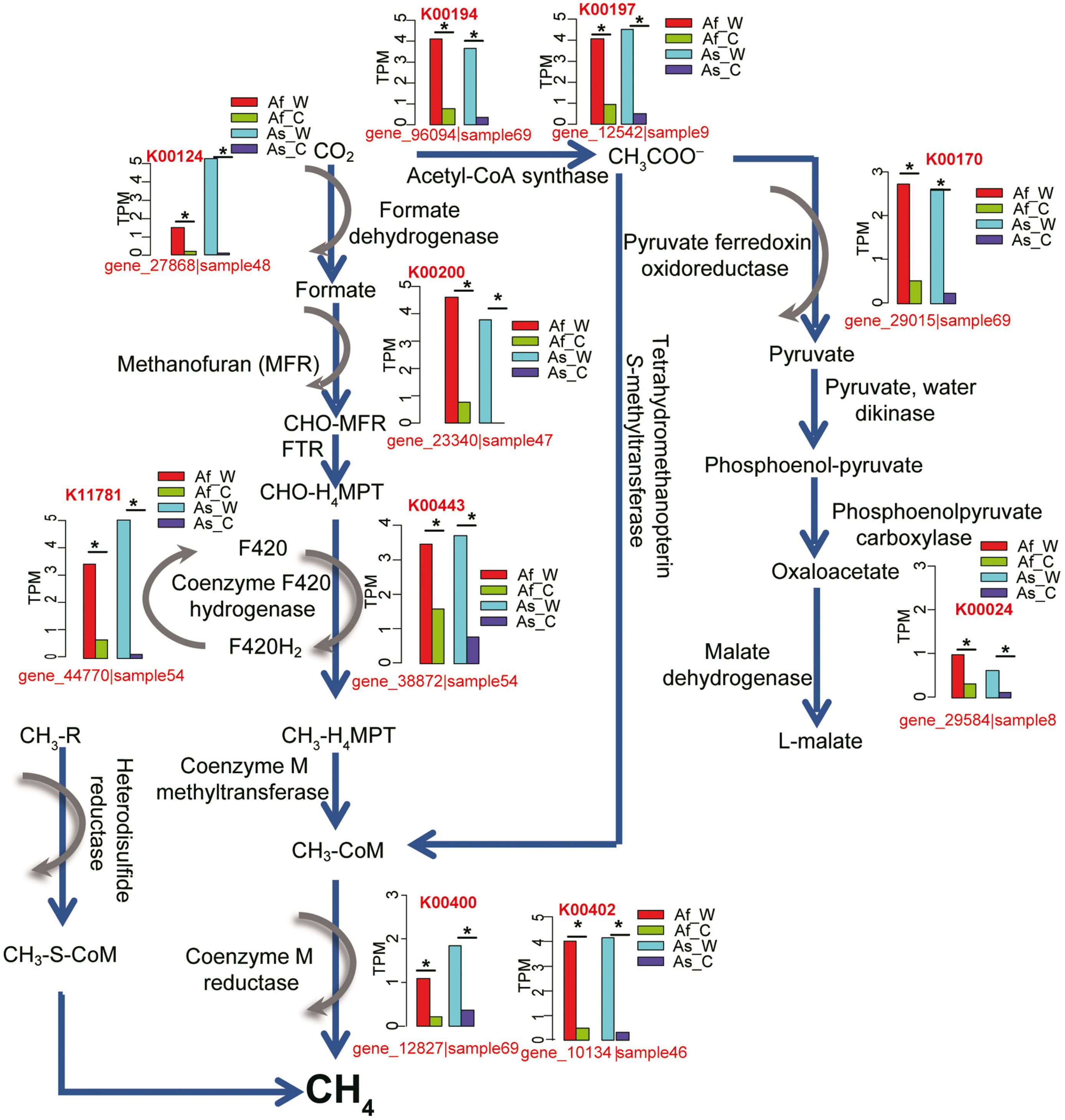


**Fig. 6.** Comparison of the relative abundances of genes encoding key enzymes involved in methane production in the rhizomicrobiomes of wild and cultivated rice. Asterisks indicate significant difference between the two groups (Student's *t*-test, $P < 0.05$). The four groups were African wild rice *Oryza barthii* (Af_W1-5), African cultivated rice *O. glaberrima* (Af_C1-10), Asian wild rice (As_W1-9 and As_W11-20), and Asian cultivated rice varieties (As_C1-20). Af_W1-5, African wild rice; Af_C1-5, African cultivated rice LM8; Af_C6-10, African cultivated rice WH20; As_W1-5, Asian common wild rice SW499; As_W6-9, Asian common wild rice SW502; As_W11-15, nivara wild rice SW218; As_W16-20, nivara wild rice SW223; As_C1-5, 106 indica; As_C6-10, Meitezhen indica; As_C11-15, Jiangxi japonica; As_C16-20, Daohuaxiang japonica. Each rice accession has 5 replicates, except the common wild rice accession SW502 that has 4 replicates (As_W6-9). CoM, coenzyme M; FTR, formylmethanofuran tetrahydromethanopterin N-formyltransferase; MPT, methanopterin; TPM, transcripts per million reads.

conditions in rhizosphere (Ma et al., 2020). The produced methane can then be utilized by bacteria capable of methane oxidation, helping reduce the level of methane emission released to the atmosphere (Fig. 7) (Bhattacharyya et al., 2019). Thus, the more prominent ability to produce and oxidize methane in the rhizomicrobiomes of wild rice can not only help plant growth, but also promote the growth of methane-oxidizing bacteria. It was also reported that the wild rice exuded more sugars, organic acids and amino acids than cultivated rice, and the root exudation is essential for methane production (Waschutza et al., 1992; Maurer et al., 2018). Additionally, the results of this study indicated that the rhizomicrobiomes of wild rice maintained a better ecological balance for methane production and utilization than those of their cultivated

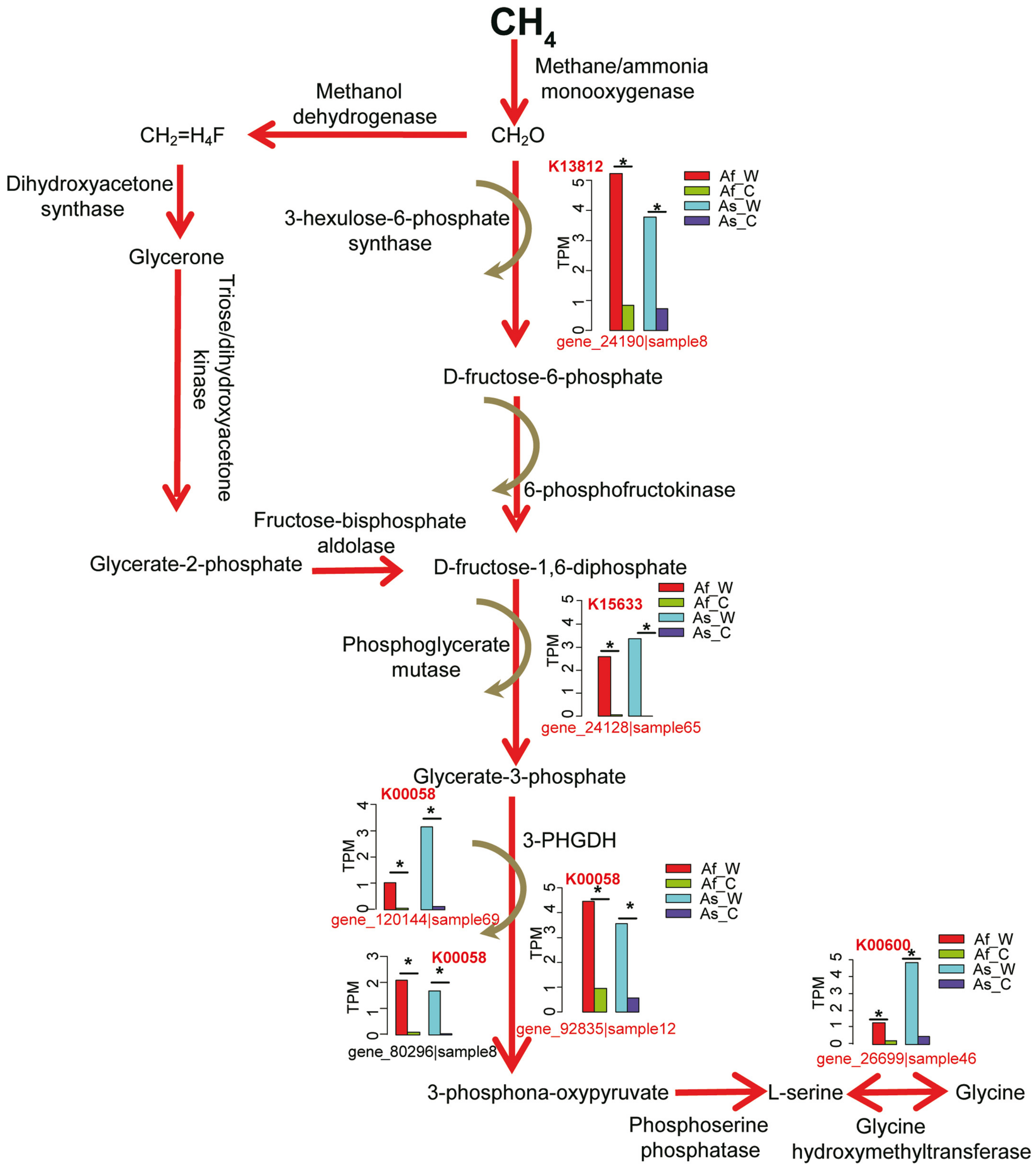


**Fig. 7.** Comparison of the relative abundances of genes encoding key enzymes involved in methane oxidation in the rhizomicrobiomes of wild and cultivated rice. Asterisks indicate significant difference between the two groups (Student's *t*-test, $P < 0.05$). The four groups were African wild rice *O. barthii* (Af_W1-5), African cultivated rice *O. glaberrima* (Af_C1-10), Asian wild rice (As_W1-9 and As_W11-20), and Asian cultivated rice varieties (As_C1-20). Af_W1-5, African wild rice; Af_C1-5, African cultivated rice LM8; Af_C6-10, African cultivated rice WH20; As_W1-5, Asian common wild rice SW499; As_W6-9, Asian common wild rice SW502; As_W11-15 nivara wild rice SW218; As_W16-20, nivara wild rice SW223; As_C1-5, 106 indica; As_C6-10, Meitezhen indica; As_C11-15, Jiangxi japonica; As_C16-20, Daohuaxiang japonica. Each rice accession has 5 replicates, except the common wild rice accession SW502 that has 4 replicates (As_W6-9). 3-PHGDH, D-3-phosphoglycerate dehydrogenase; TPM, transcripts per million reads.

relatives (Figs. 6 and 7). It may be therefore helpful to pay attention on exploring the genetic potential of wild rice, especially Asian wild rice, in breeding research to activate methane metabolism in rhizomicrobiomes of new varieties, thereby increasing their adaptability to soil and environmental conditions. Differences in the relative abundances of methanobacteria and methane-consuming methanotrophs between wild and cultivated rice accessions were not unambiguous (Fig. S2). The analysis of the archaeal and bacterial community composition in the rhizosphere of rice indicated that the relative abundance of Methanomicrobia was significantly higher in rhizomicrobial communities of both African and Asian wild rice accessions than in that of their related accessions of cultivated rice. The relative abundance of Methylocystaceae, which is the major family of methanotrophic bacteria, was significantly higher in the rhizomicrobiomes of both African and Asian wild rice than it was in related accessions of African and Asian cultivated rice (Fig. S2). Notably, differences in the abundances of KOs in the rhizomicrobiomes of wild versus cultivated rice were generally more pronounced in the African accessions than in the Asian accessions (Figs. 6 and 7). This may be due the larger sample representation of Asian rice accessions and the associated larger variability that was observed in the abundance data, although that was not entirely consistent as seen in Figs. 6 and 7. It is more likely, however, that the obtained data reflect the impact of differences in the genetic make-up of the examined rice accessions and the genetic differences on the structures of the rhizomicrobiomes. Our previous study showed that cultivated rice microbiome is more susceptible to shifts relative to wild rice microbiome, which may indicate that wild rice accessions maintain a more stable functional rhizomicrobiomes, especially in methane metabolism (Xu et al., 2019). The mechanisms associated with wild rice in regulating methane metabolism also include root oxidase activities, root exudates, aerenchyma gas spaces, plant growth parameters, biomass and grain yield and soil pH (Bhattacharyya et al., 2019), and the traits of few unproductive tillers, high root oxidative activity, small root system, and high harvest index in rice accessions are ideal for mitigating methane emission in rice fields (Wang and Adachi, 2000). Therefore, future studies on this topic should take these issues into account in the experimental design. In general, the findings of the present study indicate that the functional relationships between plants and their rhizomicrobiome may play an important role in co-selection (Mendes et al., 2014; Mendes and Raaijmakers, 2015). However, the limitation of the study based on the metagenomic data can only reflect the functional potential of the microbiota in the wild and cultivated rice at their flowering stage. Thus, further studies at different growth stages shall be considered in future.

## 5. Conclusions

This study, which investigated wild and cultivated rice under identical experimental conditions, demonstrated that the methane metabolism of the rhizomicrobiomes clearly differed between wild and cultivated rice. By comparing the African and Asian wild rice with their related cultivated species, the key enzymes for methane production and utilization were overrepresented in wild rice species, which indicated that the rhizomicrobiomes of wild rice maintained a better ecological balance for methane production and utilization than their related cultivated rice species. The results provide an important guideline for future breeding and cultivation of rice in the framework of more sustainable rice production.

## Availability of data and materials

All raw and processed data are available on NCBI and affiliated with bioproject number PRJNA632564.

## Funding

This work is financially supported by the National Natural Science Foundation of China (41920104008, 42007043), Science and Technology Project for Black Soil Granary (XDA28020400, XDA28080200), Special Foundation for Basic Research Program in Wild China of CAS (XDA23070501), Key Laboratory Foundation of Mollisols Agroecology (2020ZKHT-02). Publication number 7269 of the Netherlands Institute of Ecology (NIOO-KNAW).

## CRediT authorship contribution statement

CT, JZ, LJ designed the experiments and sample collection scheme. LT, JC, SS, YS, XL, HX, DC, YC, JW and LJ performed sample collection and processing. LT and JC performed data analyses and interpretation. LT and JC wrote the article with the input of JAV and EEK. All authors contributed to data interpretation and writing of the manuscript. All authors read and approved the final manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors would like to thank Rengang Zhang of Beijing Ori-Gene Science and Technology Co., Ltd. for providing advice on the data analysis.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150131.

## References

Bessho-Uehara, K., Furuta, T., Masuda, K., Yamada, S., Angeles-Shim, R.B., Ashikari, M., Takashi, T., 2017. Construction of rice chromosome segment substitution lines harboring *Oryza barthii* genome and evaluation of yield-related traits. Breed. Sci. 67, 408–415.

Bhattacharyya, P., Roy, K.S., Das, M., Ray, S., Balachandar, D., Karthikeyan, S., Nayak, A.K., Mohapatra, T., 2016. Elucidation of rice rhizosphere metagenome in relation to methane and nitrogen metabolism under elevated carbon dioxide and temperature using whole genome metagenomic approach. Sci. Total Environ. 542, 886–898.

Bhattacharyya, P., Dash, P.K., Swain, C.K., Padhy, S.R., Roy, K.S., Neogi, S., Berliner, J., Adak, T., Pokhare, S.S., Baig, M.J., Mohapatra, T., 2019. Mechanism of plant mediated methane emission in tropical lowland rice. Sci. Total Environ. 651, 84–92.

Bin Rahman, A.N.M.R., Zhang, J., 2016. Flood and drought tolerance in rice: opposite but may coexist. Food Energy Secur. 5, 76–88.

Brisson, V.L., Schmidt, J.E., Northen, T.R., Vogel, J.P., Gaudin, A.C.M., 2019. Impacts of maize domestication and breeding on rhizosphere microbial community recruitment from a nutrient depleted agricultural soil. Sci. Rep. 9, 15611.

Bulgarelli, D., Garrido-Oter, R., Munch, P.C., Weiman, A., Droge, J., Pan, Y., McHardy, A.C., Schulze-Lefert, P., 2015. Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe 17, 392–403.

Cardinale, M., Grube, M., Erlacher, A., Quehenberger, J., Berg, G., 2015. Bacterial networks and co-occurrence relationships in the lettuce root microbiota. Environ. Microbiol. 17, 239–252.

Carlson, K.M., Gerber, J.S., Mueller, N.D., Herrero, M., MacDonald, G.K., Brauman, K.A., Havlik, P., O'Connell, C.S., Johnson, J.A., Saatchi, S., West, P.C., 2017. Greenhouse gas emissions intensity of global croplands. Nat. Clim. Chang. 7, 63–68.

Carvalhais, L.C., Rincon-Florez, V.A., Brewer, P.B., Beveridge, C.A., Dennis, P.G., Schenk, P.M., 2019. The ability of plants to produce strigolactones affects rhizosphere community composition of fungi but not bacteria. Rhizosphere 9, 18–26.

Cen, W., Liu, J., Lu, S., Jia, P., Yu, K., Han, Y., Li, R., Luo, J., 2018. Comparative proteomic analysis of QTL CTS-12 derived from wild rice (*Oryza rufipogon* Griff.), in the regulation of cold acclimation and de-acclimation of rice (*Oryza sativa* L.) in response to severe chilling stress. BMC Plant Biol. 18 (163).

Chang, T.-T., 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. Euphytica 25, 425–441.

Chen, Y., Li, S., Zhang, Y., Li, T., Ge, H., Xia, S., Gu, J., Zhang, H., Lü, B., Wu, X., Wang, Z., Yang, J., Zhang, J., Liu, L., 2019. Rice root morphological and physiological traits interaction with rhizosphere soil and its effect on methane emissions in paddy fields. Soil Biol. Biochem. 129, 191–200.

Cheng, L., Kim, K.-W., Park, Y.-J., 2019. Evidence for selection events during domestication by extensive mitochondrial genome analysis between japonica and indica in cultivated rice. Sci. Rep. 9, 10846.

Conrad, R., Klose, M., Claus, P., Dan, J., 2009. Activity and composition of the methanogenic archaeal community in soil vegetated with wild versus cultivated rice. Soil Biol. Biochem. 41, 1390–1395.

Cui, W., Qi, F., Zhang, Y., Cao, H., Zhang, J., Wang, R., Shen, W., 2015. Methane-rich water induces cucumber adventitious rooting through heme oxygenase1/carbon monoxide and Ca(2 ) pathways. Plant Cell Rep. 34, 435–445.

Edwards, J.A., Santos-Medellin, C.M., Liechty, Z.S., Nguyen, B., Lurie, E., Eason, S., Phillips, G., Sundaresan, V., 2018. Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice. PLoS Biol. 16, e2003862.

Evans, P.N., Boyd, J.A., Leu, A.O., Woodcroft, B.J., Parks, D.H., Hugenholtz, P., Tyson, G.W., 2019. An evolving view of methane metabolism in the Archaea. Nat. Rev. Microbiol. 17, 219.

Grochowski, L.L., Xu, H., White, R.H., 2005. Ribose-5-phosphate biosynthesis in methanocaldococcus jannaschii occurs in the absence of a pentose-phosphate pathway. J. Bacteriol. 187, 7382–7389.

Jiang, Y., Qian, H., Wang, L., Feng, J., Huang, S., Hungate, B.A., van Kessel, C., Horwath, W.R., Zhang, X., Qin, X., Li, Y., Feng, X., Zhang, J., Deng, A., Zheng, C., Song, Z., Hu, S., van Groenigen, K.J., Zhang, W., 2019. Limited potential of harvest index improvement to reduce methane emissions from rice paddies. Glob. Chang. Biol. 25, 686–698.

Joseph, E., Cameron, J., Christian, S.M., Eugene, L., Natraj Kumar, P., Srijak, B., Eisen, J.A., Venkatesan, S., 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl. Acad. Sci. U. S. A. 112, E911.

Kalyuzhnaya, M.G., Puri, A.W., Lidstrom, M.E., 2015. Metabolic engineering in methanotrophic bacteria. Metab. Eng. 29, 142–152.

Khush, G.S., 2005. What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol. Biol. 59, 1–6.

Kurata, N., 2008. Chromosome and genome evolution in rice. In: Hirano, H.-Y., Sano, Y., Hirai, A., Sasaki, T. (Eds.), Rice Biology in the Genomics. Era. Springer Berlin Heidelberg, Berlin, Heidelberg.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357.

Leff, J.W., Lynch, R.C., Kane, N.C., Fierer, N., 2016. Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, Helianthus annuus. New Phytol. 214.

Leff, J.W., Lynch, R.C., Kane, N.C., Fierer, N., 2017. Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, Helianthus annuus. New Phytol. 214, 412–423.

Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22, 1658–1659.

Li, C., Zhou, A., Sang, T., 2006. Genetic analysis of rice domestication syndrome with the wild annual species, *Oryza nivara*. New Phytol. 170, 185–194.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079.

Li, D., Liu, C.-M., Luo, R., Sadakane, K., Lam, T.-W., 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31, 1674–1676.

Liu, Y., Whitman, W.B., 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann. N. Y. Acad. Sci. 1125, 171–189.

Lu, X.-M., Chen, C., Zheng, T.-L., 2017. Metagenomic insights into effects of chemical pollutants on microbial community composition and function in estuarine sediments receiving polluted river water. Microb. Ecol. 73, 791–800.

Ma, J., Wei, H., Su, Y., Gu, W., Wang, B., Xie, B., 2020. Powdered activated carbon facilitates methane productivity of anaerobic co-digestion via acidification alleviating: microbial and metabolic insights. Bioresour. Technol. 313, 123706.

Maeda, H., Dudareva, N., 2012. The shikimate pathway and aromatic amino acid biosynthesis in plants. Annu. Rev. Plant Biol. 63, 73–105.

Maurer, D., Kiese, R., Kreuzwieser, J., Rennenberg, H., 2018. Processes that determine the interplay of root exudation, methane emission and yield in rice agriculture. Plant Biol. 20, 951–955.

Mendes, R., Raaijmakers, J.M., 2015. Cross-kingdom similarities in microbiome functions. ISME J. 9.

Mendes, L.W., Kuramae, E.E., Navarrete, A.A., van Veen, J.A., Tsai, S.M., 2014. Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J. 8, 1577–1587.

Miret-Casals, L., Sebastián, D., Brea, J., Rico-Leo, E.M., Palacín, M., Fernández-Salguero, P.M., Loza, M.I., Albericio, F., Zorzano, A., 2018. Identification of new activators of mitochondrial fusion reveals a link between mitochondrial morphology and pyrimidine metabolism. Cell Chem. Biol. 25 (268–278), e264.

Moner, A.M., Furtado, A., Henry, R.J., 2018. Chloroplast phylogeography of AA genome rice species. Mol. Phylogenet. Evol. 127, 475–487.

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A.C., Kanehisa, M., 2007. KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res. 35, W182–W185.

Nallanchakravarthula, S., Mahmood, S., Alström, S., Finlay, R.D., 2014. Influence of soil type, cultivar and *Verticillium dahliae* on the structure of the root and rhizosphere soil fungal microbiome of strawberry. Plos One 9, e111455.

Nayar, N.M., 2012. Evolution of the African rice: a historical and biological perspective. Crop Sci. 52, 505–516.

Novák, J., Beníšek, M., Hilscherová, K., 2008. Disruption of retinoid transport, metabolism and signaling by environmental pollutants. Environ. Int. 34, 898–913.

Pérez-Jaramillo, J.E., Mendes, R., Raaijmakers, J.M., 2016. Impact of Plant Domestication on Rhizosphere Microbiome Assembly and Functions. 90. Springer Open Choice.

Perez-Jaramillo, J.E., de Hollander, M., Ramirez, C.A., Mendes, R., Raaijmakers, J.M., Carrion, V.J., 2019. Deciphering rhizosphere microbiome assembly of wild and modern common bean (*Phaseolus vulgaris*) in native and agricultural soils from Colombia. Microbiome 7, 114.

Schlemper, T.R., van Veen, J.A., Kuramae, E.E., 2017a. Co-variation of bacterial and fungal communities in different sorghum cultivars and growth stages is soil dependent. Microb. Ecol. 76, 1–10.

Schlemper, T.R., MFA, Leite, Lucheta, A.R., Shimels, M., Bouwmeester, H.J., van Veen, J.A., Kuramae, E.E., 2017b. Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. FEMS Microbiol. Ecol. 93.

Shenton, M., Iwamoto, C., Kurata, N., Ikeo, K., 2016. Effect of wild and cultivated rice genotypes on rhizosphere bacterial community composition. Rice 9, 42.

Shi, S., Tian, L., Nasir, F., Li, X., Li, W., Tran, L.-S.P., Tian, C., 2018. Impact of domestication on the evolution of rhizomicrobiome of rice in response to the presence of *Magnaporthe oryzae*. Plant Physiol. Biochem. 132, 156–165.

Staley, C., Ferrieri, A.P., Tfaily, M.M., Cui, Y., 2017. Diurnal cycling of rhizosphere bacterial communities is associated with shifts in carbon metabolism. Microbiome 5, 65.

Su, J., Hu, C., Yan, X., Jin, Y., Chen, Z., Guan, Q., Wang, Y., Zhong, D., Jansson, C., Wang, F., Schnürer, A., Sun, C., 2015. Expression of barley SUSIBA2 transcription factor yields high-starch low-methane rice. Nature 523, 602–606.

Su, J., Shan, Z., Chen, Z., 2018. Effects of heterogeous expression of Hvsusiba2 rice on methane mitigation and related micro-organism abundance in paddy fields. Chin. J. Eco-Agric. 26, 1333–1342.

Tian, L., Zhou, X., Ma, L., Xu, S., Nasir, F., Tian, C., 2017. Root-associated bacterial diversities of *Oryza rufipogon* and *Oryza sativa* and their influencing environmental factors. Arch. Microbiol. 199, 1–9.

Tian, L., Shi, S., Nasir, F., Chang, C., Li, W., Tran, L.-S.P., Tian, C., 2018a. Comparative analysis of the root transcriptomes of cultivated and wild rice varieties in response to *Magnaporthe oryzae* infection revealed both common and species-specific pathogen responses. Rice 11, 56.

Tian, L., Shi, S., Ma, L., Nasir, F., Li, X., Tran, L.-S.P., Tian, C., 2018b. Co-evolutionary associations between root-associated microbiomes and root transcriptomes in wild and cultivated rice varieties. Plant Physiol. Biochem. 128, 134–141.

Tian, L., Shi, S., Ma, L., Tran, L.-S.P., Tian, C., 2020a. Community structures of the rhizomicrobiomes of cultivated and wild soybeans in their continuous cropping. Microbiol. Res. 232, 126390.

Tian, L., Lin, X., Tian, J., Ji, L., Chen, Y., Tran, L.-S.P., Tian, C., 2020b. Research advances of beneficial microbiota associated with crop plants. Int. J. Mol. Sci. 21, 1792.

Truong, D.T., Franzosa, E.A., Tickle, T.L., Scholz, M., Weingart, G., Pasolli, E., Tett, A., Huttenhower, C., Segata, N., 2015. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat. Methods 12, 902–903.

Vaughan, D., Ge, S., Kaga, A., Tomooka, N., 2008. Phylogeny and biogeography of the genus Oryza. In: Hirano, H.Y., Sano, Y., Hirai, A., Sasaki, T. (Eds.), Rice Biology in the Genomics Era. Biotechnology in Agriculture and Forestry. vol. 62. Springer, Berlin, Heidelberg.

Wang, B., Adachi, K., 2000. Differences among rice cultivars in root exudation, methane oxidation, and populations of methanogenic and methanotrophic bacteria in relation to methane emission. In: Wassmann, R., Lantin, R.S., Neue, H.-U. (Eds.), Methane Emissions From Major Rice Ecosystems in Asia. Springer, Netherlands, Dordrecht, pp. 349–356.

Waschutza, S., Hofmann, N., Niemann, E.-G., Fendrik, I., 1992. Investigations on root exudates of Korean rice. Symbiosis 13, 181–189.

Waters, D.L.E., Nock, C.J., Ishikawa, R., Rice, N., Henry, R.J., 2012. Chloroplast genome sequence confirms distinctness of Australian and Asian wild rice. Ecol. Evol. 2, 211–217.

Xiubin, K., Yahai, L., Ralf, C., 2014. Different behaviour of methanogenic archaea and Thaumarchaeota in rice field microcosms. FEMS Microbiol. Ecol. 87, 18–29.

Xu, S., Tian, L., Chang, C., Li, X., Tian, C., 2019. Cultivated rice rhizomicrobiome is more sensitive to environmental shifts than that of wild rice in natural environments. Appl. Soil Ecol. 140, 68–77.

Yamanaka, S., Nakamura, I., Nakai, H., Sato, Y.-I., 2003. Dual origin of the cultivated rice based on molecular markers of newly collected annual and perennial strains of wild rice species, *Oryza nivara* and *O. rufipogon*. Genet. Resour. Crop. Evol. 50, 529–538.

Yan, Y., Kuramae, E.E., de Hollander, M., Klinkhamer, P.G.L., van Veen, J.A., 2016. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. ISME J. 11, 56.

Yang, X., Wang, B., Chen, L., Li, P., Cao, C., 2019. The different influences of drought stress at the flowering stage on rice physiological traits, grain yield, and quality. Sci. Rep. 9, 3742.

Zhao, Q., Feng, Q., Lu, H., Li, Y., Wang, A., Tian, Q., Zhan, Q., Lu, Y., Zhang, L., Huang, T., Wang, Y., Fan, D., Zhao, Y., Wang, Z., Zhou, C., Chen, J., Zhu, C., Li, W., Weng, Q., Xu, Q., Wang, Z.-X., Wei, X., Han, B., Huang, X., 2018. Pan-genome analysis highlights the extent of genomic variation in cultivated and wild rice. Nat. Genet. 50, 278–284.

Zhu, W., Lomsadze, A., Borodovsky, M., 2010. Ab initio gene identification in metagenomic sequences. Nucleic Acids Res. 38, e132.