

Comparison between wild and related cultivated rice species reveals strong impacts of crop domestication on methane metabolism of the rhizomicrobiome

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Research

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Abstract

Background

Rice root-inhabited microbial communities are seriously affected by domestication as evidenced by comparing the rhizomicrobiomes of wild and related cultivated rice species. While earlier studies focused on the structures of the rhizomicrobiomes, here, we compared the functioning of the microbial communities in the rhizosphere of wild versus that of related cultivated rice species, which were originated from Africa and Asia.

Results

The microbial functions involved in carbon metabolism and nucleotide metabolism were found to be enriched in wild rice species, whereas those involved in nitrogen metabolism, lipid metabolism, metabolism of cofactors and vitamins, and xenobiotic biodegradation were more enriched in cultivated rice species. Among the overall carbon metabolism, specifically, methane metabolism of the rhizomicrobiomes clearly differed between wild and cultivated rice. The key enzymes in methane production and utilization were overrepresented in wild rice species, suggesting that the rhizomicrobiome of wild rice maintained a better ecological balance for methane production and utilization than the related cultivated rice species.

Conclusions

For the first time, the impacts of rice domestication on the main metabolic pathways of the rhizomicrobiome were assessed, which revealed the strong impacts of rice domestication on methane metabolism that is one of the most critical functions of the microbial community of the rhizosphere of rice. The results provide important guidelines for future breeding and cultivation of rice in the framework of more sustainable rice production.

Background

Rice is one of the most important crops in the world, especially *Oryza sativa* that covers the food requirement of more than half of the world's population [1]. There are many wild and cultivated species in the *Oryza* genus, which are worldwide distributed. The genomes of *Oryza* species are categorized on the basis of the ability of their chromosomes to pair correctly during meiosis in the interspecies F1 hybrids [2]. All cultivated species and their wild progenitors are diploids and are among the species possessing the AA genome [3].

The development of cultivated rice from wild rice significantly altered desired traits, such as it increased yields and nutrient contents [4, 5]. African and Asian cultivated rice species were differently domesticated

[6–8]. 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. ruffipogon*) or nivara wild rice (*O. nivara*) [9–11]. *O. sativa* was continuously bred and adjusted, and ultimately evolved into two main varieties of indica and japonica [12].

The long-term processes of domestication and crop breeding have also led to great changes in the microbial communities surrounding the roots of many crop species, including common bean (*Phaseolus vulgaris*) [13], barley (*Hordeum vulgare*) [14], lettuce (*Lactuca sativa*) [15] and sunflower (*Helianthus annuus*) [16]. Generally, it can be presumed that wild plant species have characteristics that support the plants to survive under adverse natural conditions, including environmental stresses like drought, extreme temperatures, low-nutrient soils and the presence of soil-borne pathogens [17–19]. This higher acclimatization ability of wild species may be attributed to their associations with the microbial communities in the rhizosphere among many others [19–21].

Up to date, studies on the rhizosphere microbiomes comparing wild and domesticated crops are focused on the selection of different rhizosphere microbial communities in terms of community structure and only a small number of microbiological functions to reveal the reasons for plant and microbiome mutual selection [22–24]. With regard to the impact of rice domestication on the microbial diversity in the rhizosphere, Shenton et al. (2016) studied different genotypes of rice and found that the structure of the microbial communities in the rhizosphere of wild and cultivated rice significantly differed [25]. In particular, the taxa of the methanotrophic *Anaerolineae* were overrepresented in the rhizocommunities of wild rice as compared with those of cultivated rice. The paper suggested that the emissions of methane from the paddy fields could be altered by manipulating the plant genotypes through the introgression of wild rice genetic materials [25]. However, this implication was made based on the microbial community structure only and not on the assessments of the functionality of the rhizosphere communities [25], which therefore requires further studies. Yet, the implications of this study are highly relevant as the emission of methane from the huge areas of rice production all over the world are an important source of greenhouse gas emissions globally [26]. Thus, in this study we hypothesize that wild rice is overrepresented by rhizosphere communities that have potential functions related to methane metabolism. To test this hypothesis, we assessed the differential microbial functions in the rhizosphere of wild and domesticated rice species from Asia and Africa origins under field conditions using the metagenomics shotgun sequencing.

Results

Summary of metagenomic data set

Metagenomic analyses were used to assess the association of plant varieties and rhizomicrobiome functioning and structure of wild versus related cultivated rice. The raw sequencing data contained 40.7–153.0 million reads for each sample. Quality trimming of raw metagenomic data resulted in clean data sets with 39.2–147.7 million raw reads for each sample (Table S1). De novo co-assembly generated true

scaffolds for each sampling after discarding scaffolds shorter than 500 bp and contaminant scaffolds with a N50 value ranged from 583 to 690 bp (Table S1).

Functional profile of different wild and respective cultivated rice species

PCA analysis of microbial functional structures in the rhizosphere of wild and cultivated rice varieties is illustrated in Fig. 1. There is a clear separation in the functional profile of the rhizomicrobiome of wild rice species both from Africa and Asia from the cultivated rice varieties, although the separation in the overall functional profiles of wild rice from domesticated rice is explained by only 9.04% on PC1 (Fig. 1a).

However, when the functional profiles of the rhizomicrobiomes of the wild rice species were compared with the related cultivated species both from Africa and Asia the clustering of wild versus cultivated rice species was more pronounced as shown in Figs. 1b and 1c.

KOs enrichment in wild and cultivated rice

The KEGG enrichments of the different represented genes showed that there is a wide variety of pathways that may differ between wild and cultivated rice species both from Africa and Asia: the pathways methane metabolism, alanine, aspartate and glutamate metabolism, carbon fixation, lipopolysaccharide biosynthesis, nitrogen metabolism, pyruvate metabolism were enriched in African wild rice SW42 *O. barthii* (Af_W1-5) when compared to both African cultivated rice *O. glaberrima* (varieties No.2 and No.4; Af_C1-10) (Fig. 2a). Methane metabolism, pyruvate metabolism, glycolysis/gluconeogenesis, purine metabolism are enriched in both nivara wild rice (SW218 and SW223; As_W11-20) *O. nivara* when compared to two cultivated rice (106 indica and Meitezhen; As_C1-10) *O. sativa* subsp. *indica* (Fig. 2b). Methane metabolism, carbon fixation, phenylalanine, tyrosine and tryptophan biosynthesis, glycolysis/gluconeogenesis are enriched in both wild rice (common wild rice SW502 and SW499; As_W1-10) *O. ruffipogon* when compared to the two cultivated rice (Jiangxi japonica and Daohuaxiang japonica; As_C11-20) *O. sativa* subsp. *japonica* (Fig. 2c). Methane metabolism, pyruvate metabolism, glycolysis/gluconeogenesis, purine metabolism are enriched in both nivara wild rice (SW218 and SW223; As_W11-20) *O. nivara* when compared to two cultivated rice (Jiangxi japonica and Daohuaxiang japonica; As_C11-20) *O. sativa* subsp. *japonica* (Fig. 2d). Methane metabolism, ABC transporters, pyruvate metabolism are enriched in both wild rice (common wild rice SW502 and SW499; As_W1-10) *O. ruffipogon* when compared to two cultivated rice (106 indica and Meitezhen; As_C1-10) *O. sativa* subsp. *japonica* (Fig. 2e).

Among all these differentially enriched pathways, methane metabolism and pyruvate metabolism were found to be enriched most consistently in the rhizomicrobiome of wild species with methane metabolism to be the most pronounced one (Fig. 2). The number of methane related genes was 30 in African wild rice *O. barthii* and African cultivated rice *O. glaberrima*, 54 in nivara wild rice *O. nivara* and cultivated *O. sativa* subsp. *indica*, 81 in *O. ruffipogon* and *O. Sativa* subsp. *japonica*, 91 in *O. nivara* wild rice and cultivated *O. Sativa* subsp. *japonica* and 64 in *O. ruffipogon* cultivated *O. sativa* subsp. *indica*.

Functional profile in methane metabolism of different wild species and cultivated rice varieties

As shown in Fig. 3a, the profiles of genes involved in methane metabolism in the rhizosphere of wild and cultivated rice species separate clearly (PC1 23.16%). Again, the separation between wild and cultivated species is even stronger when the related species are compared (African wild vs cultivated and Asian wild vs cultivated) (Figs. 3b and 3c).

A total of 81 KOs involved in methane metabolism (include methane synthesis and methane metabolism) are present in the metagenome dataset (Table S2). Among them, 16 KOs showed significant higher enrichments in wild versus cultivated rice species both from Africa and Asia; among these are K00024, K00170, K00201, K00399, K00402, K00443, K01007 and K03390 which are involved in methane synthesis, and K01622, K01834, K13831 and K16370 which are involved in methane metabolism.

The relative abundance of methanogens archaea and methane oxidation bacteria

Because the methane metabolism was overrepresented in the rhizomicrobiome of wild rice when compared to cultivated rice, we annotated the methane metabolism-related reads to taxonomy using metaphlan2 software. The annotation was mainly affiliated to Methylocystaceae (methane consumers) and Methanomicrobia (methane producers). The relative abundance of Methanomicrobia was significantly enriched in both African and Asian in the rhizomicrobiome of wild rice as compared to that of cultivated rice species (Figure S1). For the African wild species, the relative abundances in the rhizosphere communities of Methanomicrobia was higher than that in cultivated rice rhizospheres, 46% versus 21% (Figure S1A and B) while for the Asian rice species the relative abundances of Methanomicrobia were 34% versus 24% for the wild and the cultivated species respectively (Figure S1C and D). The relative abundances of Methylocystaceae were higher in Asian cultivated rice than in wild rice species whereas in the African rice species the relative abundances were higher in the rhizospheres of wild species than in the cultivated ones.

Co-occurrence structure of the other functional KOs in different pathways affected methane metabolism functional KOs of wild rice in compare with respective cultivated rice

Functional interactions play an important role in functional pathways assembly. Therefore, we explored whether there were differences in functional co-occurrence of KOs in different pathways with KOs of methane metabolism in the rhizomicrobiomes of wild rice versus cultivated rice (Fig. 4). The functional KOs correlations were characterized by SparCC analysis (SparCC's rho cut-off = 0.8, $P < 0.01$). The visualization of the interactions between methane metabolism functional KOs with the other functional

KOs in different pathways showed that the functional network was most complex in the rhizosphere of African wild rice, with the largest numbers of nodes and correlations. The network of the African cultivated rice rhizomicrobiomes were less complex, although still a relatively large number of interactions was observed. The co-occurrence networks in the Asian species were rather simple (Fig. 4). The network parameter characteristics of nodes, correlations, modularity and betweenness centrality distribution followed the order African wild rice (Af_W) > African cultivated rice (Af_C) > Asian wild rice (As_W) > Asian cultivated rice (As_C) (Table S3). The number of negative correlations in African wild rice and Asian wild rice were higher than that in African cultivated rice and Asian cultivated rice (Table S3).

Correlation test of related genes with methanomicrobia

Testing the correlation of the functional genes with the related archaea methanomicrobia Methanomicrobia and bacteria Methylocystaceae, showed that there are significant correlations between the relative abundance of methanomicrobia and overrepresentation of methyl-coenzyme M reductase gamma subunit (K00402), FO 2-phospho-L-lactate transferase (K11212), 5-amino-6-(D-ribitylamino)uracil –L-tyrosine 4-hydroxyphenyl transferase (K11781), and formylmethanofuran dehydrogenase subunit A (K00200). There are also significant linear relationships of K00402, K11212, K11781 and K00200, respectively with relative abundance of Methanomicrobia (Figure. 5). There is also a significant correlation between the TPM of K13812, which encodes for the bifunctional enzyme Fae/Hps, which is used for the assimilation of formaldehyde in ribulose monophosphate pathway and the relative abundance of Methylocystaceae (Figure. 5e).

Discussion

It is still a topic of debate whether or not domestication of crops led from wild ancestor species to cultivated species will have a specific impact on the microbial communities in the rhizosphere. Although the information revealed by different studies provides a variable picture, most studies do show that crop domestication will lead to directed selection of the rhizosphere microbiome, but such selection will vary depending on the environment [27–29]. One of the most interesting observations is that crop domestication seems to have less selective effect on bacteria than on fungi [30, 31] which is consistent with the results of our previous study on the comparison of the structure of the rhizomicrobiomes of wild and cultivated rice in natural environments [32].

Most comparable studies used next generation sequencing of amplicons of taxonomic phylogenetic markers of microbial diversity in order to assess the impact of crop domestication on the structure of the microbial communities in the rhizosphere. However, it is highly interesting and relevant for the future development of better crop varieties using the intrinsic characteristics of wild relatives to know how the functioning of the rhizomicrobiomes has been affected by domestication. Therefore, we applied DNA shotgun metagenome sequencing to assess the most pronounced potential rhizomicrobial functions affected by crop domestication. And, indeed, we showed that domestication of rice from different origins, Asia and Africa, has clearly impacted the potential functioning of the rhizomicrobiome. Although the

evolution of the rice species in different regions in Asia and Africa has led to clearly different rice plant species, we observed a number of specific changes in the microbial functions in the rhizosphere independent of the type of rice species. Certain genes such as those related to carbon metabolism, and amino acid metabolism were mainly enriched in wild rice, while others such as those related to nitrogen metabolism, amino acid metabolism, lipid metabolism, metabolism of cofactors and vitamins, xenobiotics biodegradation and metabolism and biosynthesis of other secondary metabolites were enriched in cultivated rice as compared to wild rice. With respect to the latter we can only speculate that this is a reflection of the specific conditions in which cultivated crops are growing including increased nutrient contents in soil as a result of fertilization and the presence of xenobiotics in the form of pesticides.

It was remarkable that the pathway of methane metabolism was significantly and consistently enriched in the rhizomicrobiomes of all wild species as compared to those of cultivated rice. This held for both genes involved in methane production and genes related to methane oxidation. Microbial methanogenesis accounts for approximately 74% of natural methane emission. The process plays a major role in global warming. It has been shown that rice paddies constitute a major source of anthropogenic CH₄ emissions [33, 34]. Methane released during rice growth accounts for approximately 20% of global methane emissions which is mostly synthesized by rhizosphere methanogenic archaea [35, 36].

There were 81 KOs involved in methane metabolism (include methane synthesis and methane oxidation) and 16 KOs showed significant differences among African wild rice, African cultivated rice, Asian wild rice and Asian cultivated rice. Three types of methanogenic pathways are known: methanol to methane, CO₂ to methane, and acetate to methane [37]. We found that there were 9 significant differences KOs among African wild rice, African cultivated rice, Asian wild rice and Asian cultivated rice in the three types of methanogenic pathways. K00201, K00443 and K00399 of the CO₂ to methane pathway in wild rice were more enriched in the rhizomicrobiomes of wild rice than in those of in cultivated rice. Thus, to some extent, the ability of the rhizosphere communities of wild rice to transform carbon dioxide into methane is stronger than those of cultivated rice. The genes coded K00170, K00172, K01007, K01595 and K00024 belong to the acetate to methane pathway and were also more enriched in the rhizomicrobiomes of wild rice as compare to those of cultivated rice (Fig. 6).

Three types of methanotrophic pathways are known: the serine pathway, the ribulose-P pathway and the xylulose-P pathway [38]. We found that the genes coded K01834 and K00058 which belong to the serine pathway were significantly more enriched in the rhizosphere communities of wild rice as compare to those of cultivated rice (Figure. 7). Also K13831 and K16370, which belong to the ribulose-P pathway and K01622, which belongs to the xylulose-P pathway were significantly more enriched in the rhizomicrobiomes of wild versus those of cultivated rice ($P < 0.05$). We also explored the interactions of methane metabolism related functional KOs of wild rice versus cultivated rice with different functional KOs belonging to other pathways. Both in African and Asian wild rice the interactive networks were more complex than in the related cultivated rice. The methane metabolism related KOs of wild rice were mainly

affected by functional KOs belonging to carbon fixation pathway, pyruvate metabolism, glycolysis/gluconeogenesis and pyrimidine metabolism which can provide energy sources and intermediates for methane metabolism [39–41]. The methane metabolisms related KO of cultivated rice were mainly affected by functional KOs belonging to phenylalanine, tyrosine and tryptophan biosynthesis as well as retinol metabolism. The aromatic amino acid phenylalanine, as well as tyrosine and tryptophan are vital constituent of proteins in all living organisms and serve as precursor for thousands of indispensable metabolites [42] and retinoids play an important role in controlling such vital processes as morphogenesis, development, reproduction or apoptosis [43]. Thus, their interaction with methane oxidation pathways might be indirect.

The enriched abundance of genes involved in methane metabolism of the rhizomicrobiomes of wild rice species as compared to the abundances of these genes in the rhizomicrobiomes of cultivated relatives suggests that both methane synthesis and oxidation were more pronounced in the rhizomicrobiome of wild rice than that of cultivated rice. In wild rice, archaea utilize CO₂, acetate, and formate for methane synthesis, which can help wild rice to decrease the damage of acidification caused by anaerobic condition in rhizosphere. And the produced methane can be utilized by methane oxidizer bacteria, which can help to reduce methane emission to the atmosphere. Thus, the strengthening of the ecological balance of methane production and oxidation in wild rice not only helped plant growth, but also may have also promoted the growth of methane oxidizing bacteria. Most of our data supported this hypothesis, but there were contradictory data as well. In the first place the data on the relative abundance of methanobacteria and methane consuming methanotrophs were not unambiguously. By analyzing the archaeal and bacterial community compositions of the rice rhizosphere, the relative abundance of Methanomicrobia was significantly higher in the microbial communities of the rhizospheres of both the African and Asian wild rice than in that of their related cultivated rice species. Also, the relative abundance of Methylocystaceae, which are the major methanotrophic bacteria, in the rhizomicrobiome of African wild rice was significantly higher in the that of the related cultivated rice species. However, the relative abundance of Methylocystaceae was lower in the rhizosphere communities of Asian wild rice than in that of the Asian cultivated rice. Moreover, the differences in the abundances KO's in the rhizomicrobiomes of wild versus cultivated rice were, generally speaking, more pronounced for the African species than for the Asian ones. This could be due to the larger sample size in case of the Asian rice species and associated larger variability in the data on the abundances, although that was not consistent as seen in Figs. 6 and 7, but it is more likely that that is a reflection of differences in the genetic make-up of the rice species and thus, their association with the rhizosphere microbial communities. It is therefore, recommended that in future studies these aspects are dealt with in details. It may also suggest that the functional matching between plant and rhizomicrobiomes may be greater than the taxonomic selection effect [44, 45].

Conclusions

In this study, it has 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 cultivated species respectively, the key enzymes were overrepresented for methane production and utilization in wild rice species, which indicated that the rhizomicrobiome of wild rice maintained a better ecological balance for methane production and utilization than the 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.

Materials And Methods

Site description, plant materials and experimental design

The study area was located in the rice experimental region of 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 1,347.5 mm, of which 85% were during the months from May to October. The soil chemical properties of the experimental field were: 14.67 g·kg⁻¹ soil organic carbon (SOC), 1.45 g·kg⁻¹ total nitrogen (TN), 0.51 g·kg⁻¹ total phosphorus (TP), 28.46 mg·kg⁻¹ available phosphorus (AP) and 157.64 mg·kg⁻¹ available potassium (AK). Five experimental field plots were planted for each rice species, each of them 4 square meters. The experiment was arranged in a randomized block design with a distance of 30 cm between the plots.

Three wild rice species (three wild species, namely African wild rice SW42, Asian common wild rice SW499 and SW502, and Asian nivara wild rice SW218 and SW223) and two cultivated rice species (*Oryza glaberrima* and *Oryza sativa*) with a total of 11 varieties were used in this study (Table 1). For the comparison of African wild and cultivated rice, one African wild rice SW42 (*Oryza barthii*) and two African cultivated rice (*Oryza glaberrima* No.2 and No.4) were used (Table 1). The seeds of wild and cultivated African rice were kindly provided by the International Rice Research Institute (IRRI). For the comparison of Asian wild and cultivated rice, two common wild rice (*Oryza ruffipogon*) SW499 and SW502, two nivara wild rice (*Oryza nivara*) SW218 and SW223 and four Asian cultivated rice (*Oryza sativa*) 106 (indica), Meitezhen (indica), Jiangxi (japonica) and Daohuanxiang (japonica) were used. The seeds of Asian cultivated rice were collected from Jiangxi Academy of Agricultural Sciences (Table 1).

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

<i>Origin</i>	<i>Cultivar name</i>	<i>Species</i>	<i>Interinational code</i>	<i>Sample name</i>	<i>Distribution</i>
Africa	African wild rice SW42	<i>Oryza barthii</i>	106238	Af-W(1 ~ 5)	Western, eastern, southern Africa
	African cultivated rice No. 2	<i>Oryza glaberrima</i>	-	Af-C(1 ~ 5)	Western Africa
	African cultivated rice No. 4	<i>Oryza glaberrima</i>	-	Af-C(6 ~ 10)	Western Africa
Asia	Common wild rice SW502	<i>Oryza ruffipogon</i>	106452	As-W(1 ~ 4)	Tropical and sub-tropical Asia
	Common wild rice SW499	<i>Oryza ruffipogon</i>	106286	As-W(6 ~ 10)	
	Nivara wild rice SW218	<i>Oryza nivara</i>	86655	As-W(11 ~ 15)	Tropical and sub-tropical Asia
	Nivara wild rice SW223	<i>Oryza nivara</i>	88949	As-W(16 ~ 20)	
	106 indica	<i>Oryza sativa subsp.indica</i>	-	As-C(1 ~ 5)	China
	Meitezhen indica	<i>Oryza sativa subsp.indica</i>	-	As-C(6 ~ 10)	China
	Jiangxi japonica	<i>Oryza sativa subsp.japonica</i>	-	As-C(11 ~ 15)	China
	Daohuaxiang japonica	<i>Oryza sativa subsp.japonica</i>	-	As-C(16 ~ 20)	China

Note: all cultivars possess the AA genome type (Vaughan et al. 2008).

DNA extraction, sequencing and data analysis

For each sample, 4 plants were harvested during flowering stage, and the rhizosphere soils were then carefully collected by brushing [46]. Then 0.5 g soil from each sample was used for microbial DNA extraction following the protocol of the Fast DNA SPIN Kit (Catalog No. 6560 – 220, Germany). The concentration and quality of the extracted DNA samples were determined by NanoDrop 2000 Spectrophotometer (Thermo Scientific, Germany). Total DNA was fragmented by ultrasonic with average 300 bp length, after which the DNA fragments were added with adapter for the DNA library construction. The total DNA library was submitted for shotgun sequencing using Illumina HiSeq X TEN (San Diego, CA, USA) in paired-end method. The raw metagenomic sequencing data were quality-controlled by fastp (<https://github.com/OpenGene/fastp>). Clean data were assembled by megahit [47, 48] to obtain the

contigs. The contigs ≥ 500 bp were used for gene prediction by MetaGeneMark [49]. Cd-hit was used to remove the redundancy of the predicted genes in coverage 90% and 90% similarity [50]. Bowtie 2 was used for the sequencing blast to the cleandata, and Samtool was used for transferring sam files into bam files [51, 52]. Subsequently, the fragments per kilobase per million reads mapped (FPKM) were calculated by eXpress software (<https://github.com/adarob/eXpress>) to quantify the abundance of genes. The differentially metagenome profiles were calculated by R 3.3.1 (<https://www.r-project.org/>). The sequences of the microbial genes were blasted against KAAS (KEGG Automatic Annotation Server) (<https://www.genome.jp/tools/kaas/>) to obtain the KEGG orthology (KO) number [53]. The package GOstats in R 3.3.1 bioconductor was used for KEGG enrichment in order to allow for the comparisons between the cultivated and wild rice samples.

Statistical analysis

Principal component analysis (PCA) based on the KEGG data with KO numbers and methane related KO numbers was conducted using the PCA function in R 3.3.1 with the FactoMineR package. Bubble diagram was made by R 3.3.1 using the enriched KEGG pathways of wild versus cultivated rice. One-way ANOVA with Turkey tests were used to test the difference of functional KOs among different varieties of wild and cultivated rice ($P < 0.05$). The rhizosphere functional KOs correlations were characterized by SparCC analysis (SparCC's rho cut-off = 0.8, $P < 0.01$). The network analysis of the connection between bacterial and fungal communities was performed by Gephi 0.9.2 based on the correlation coefficient [54].

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

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

Competing Interests

The authors declare no conflict of interest.

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Authors' contributions

CT, JZ, LJ designed the experiments and sample collection scheme. LT, JC, SS, XL 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 JV and EK. All authors contributed to data interpretation and writing of the manuscript. The authors read and approved the final manuscript.

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Figures

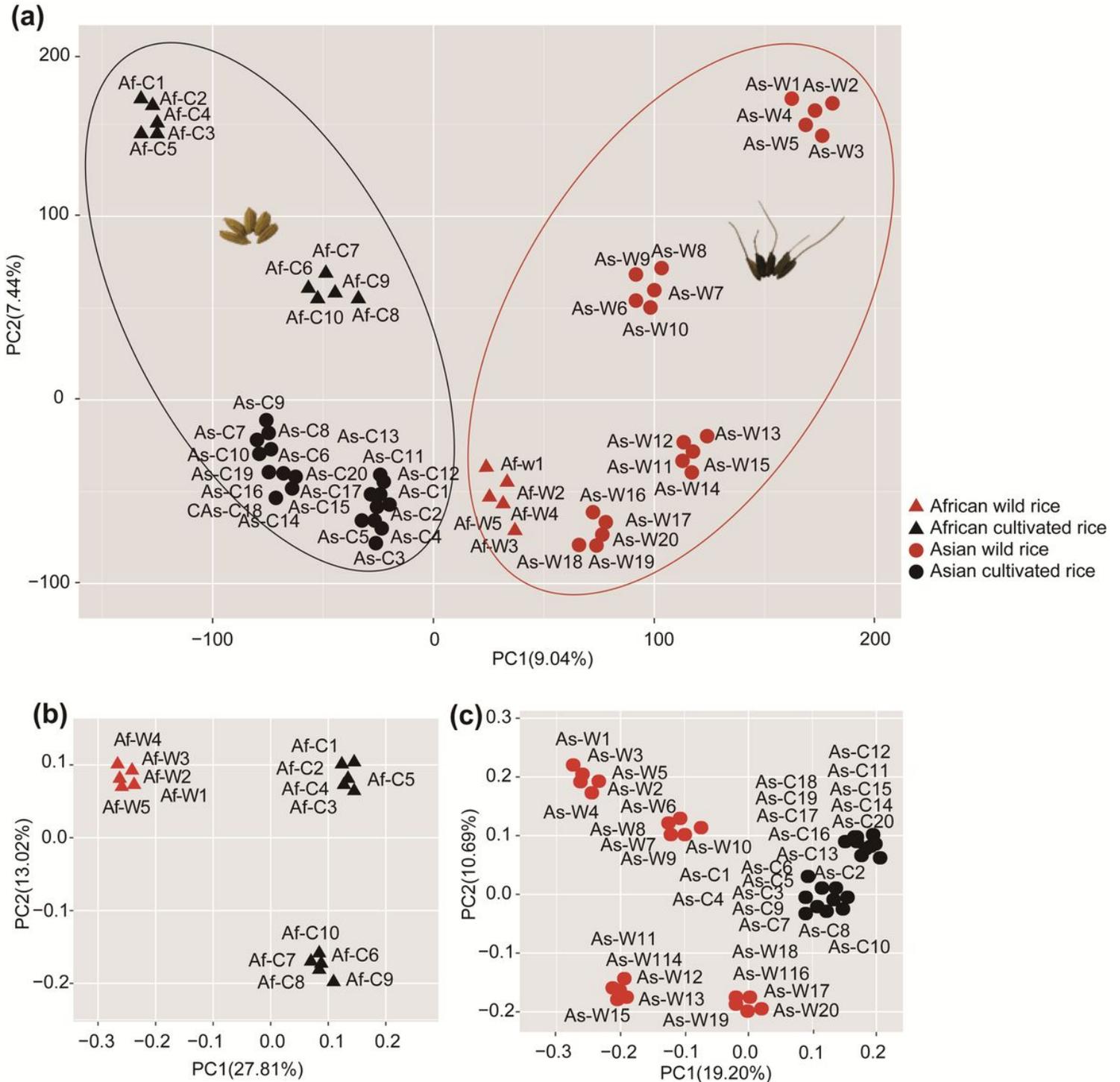


Figure 1

PCA analysis of the samples based on KEGG functional profiles of rhizosphere microbiomes. (a) Functional profiles of rhizomicrobiome for all samples, (b) Functional profiles of rhizomicrobiome of wild and cultivated African rice, and (c) Functional profiles of rhizomicrobiome of wild and cultivated Asian rice.

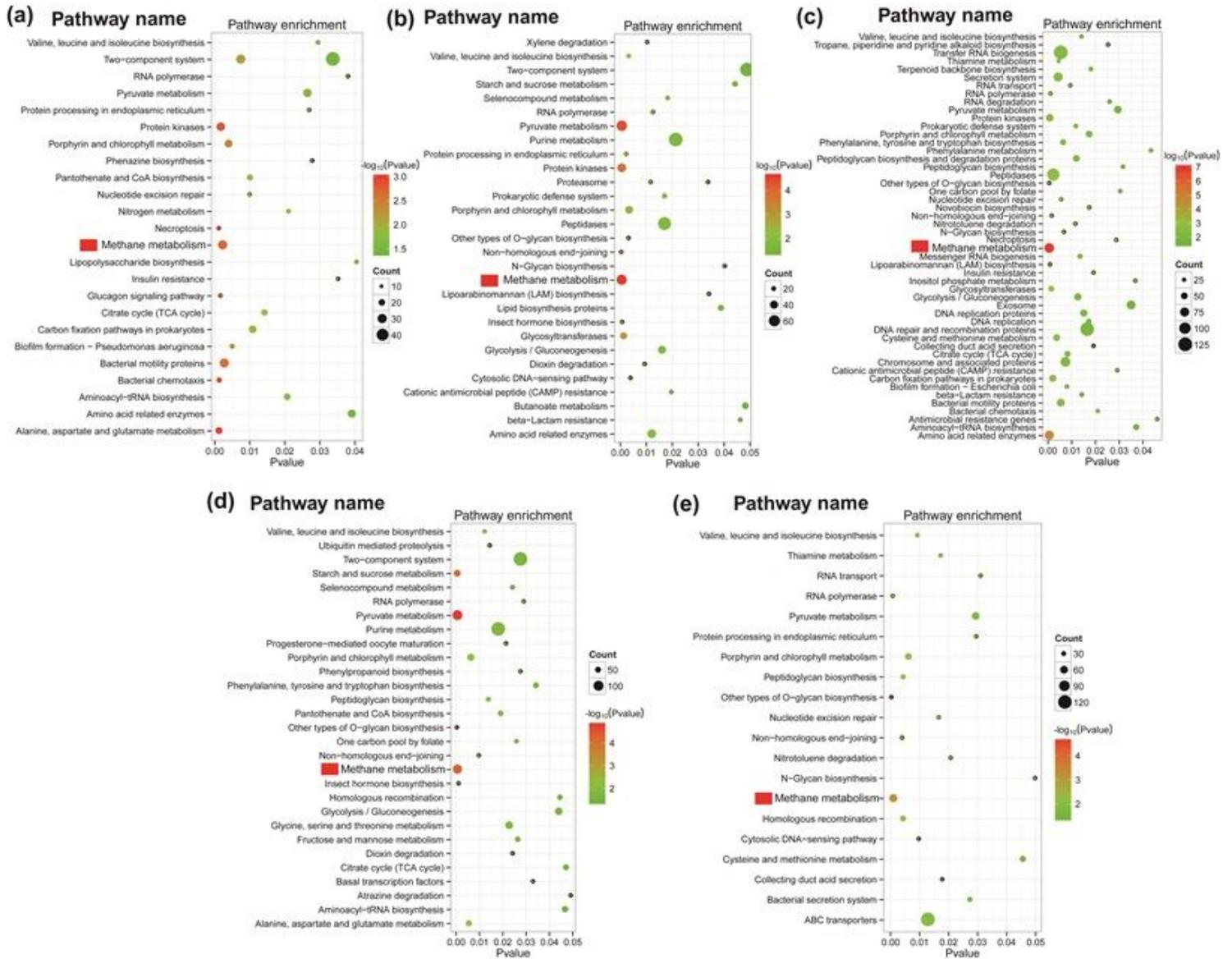


Figure 2

Microbial KOs enrichment in rhizospheres of wild rice versus cultivated rice. Comparisons of : (a) African wild rice SW42 *O. barthii* VERSUS African cultivated rice *O. glaberrima* (varieties No.2 and No.4); (b) nivara wild rice species, SW218 and SW223, *O. nivara*, versus two cultivated rice species 106 indica and Meitezhen, *O. sativa* subsp. indica; (c) common wild rice, SW502 and SW499, *O. ruffipogon*, versus two cultivated rice, Jiangxi japonica and Daohuaxiang japonica, *O. sativa* subsp. japonica. (d) nivara wild rice, SW218 and SW223, *O. nivara*, versus two cultivated rice Jiangxi japonica and Daohuaxiang japonica, *O. sativa* subsp. Japonica. (e) common wild rice , SW502 and SW499, *O. ruffipogon*, versus two cultivated rice species ,106 indica and Meitezhen, *O. sativa* subsp. Japonica.

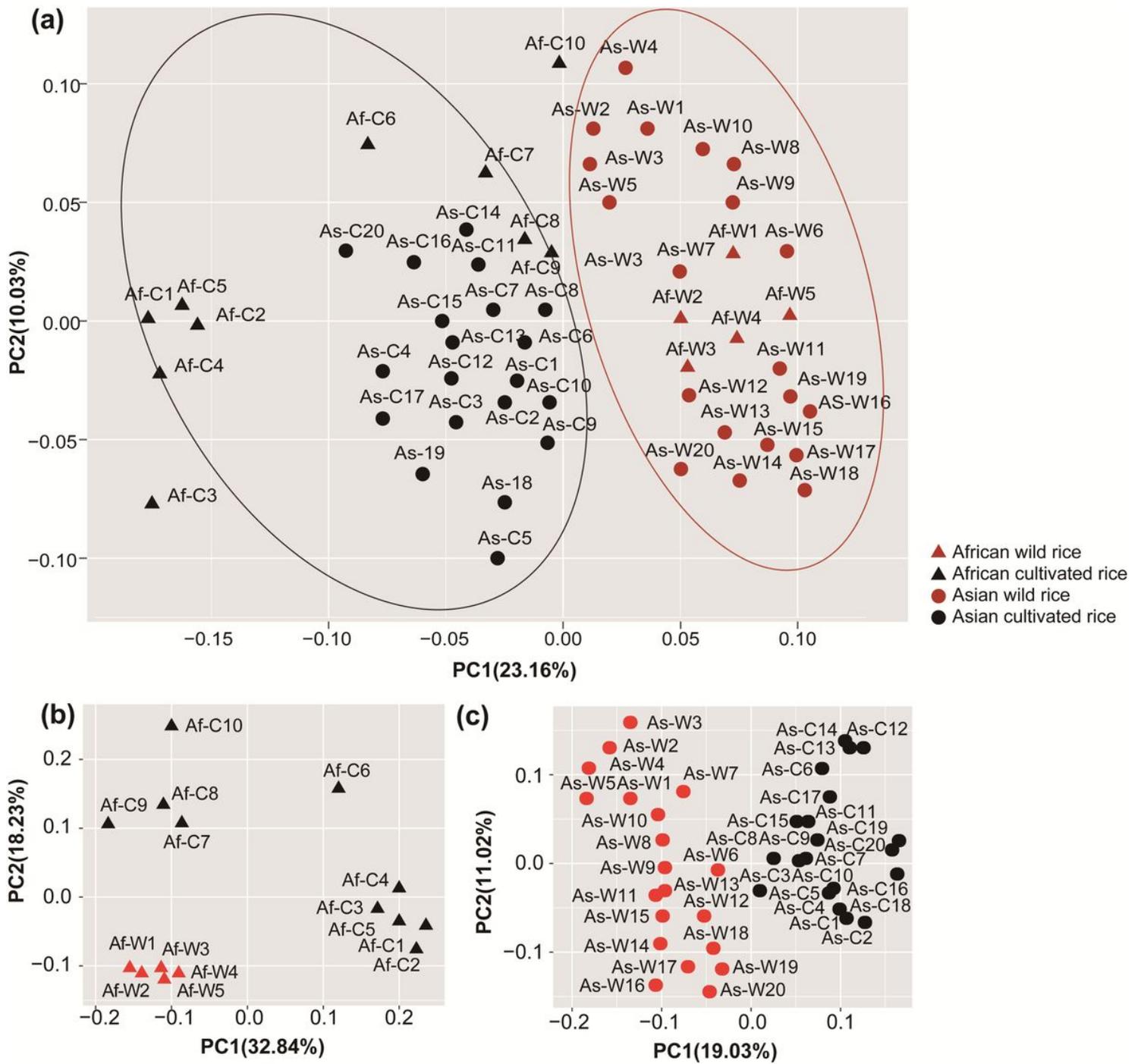


Figure 3

PCA analysis of KOs of methane metabolism (including methane synthesis and methane oxidation) of rhizosphere microbiomes. (a) methane metabolism KOs distribution for all samples, (b) and (c) methane metabolism KOs of wild and cultivated African rice.

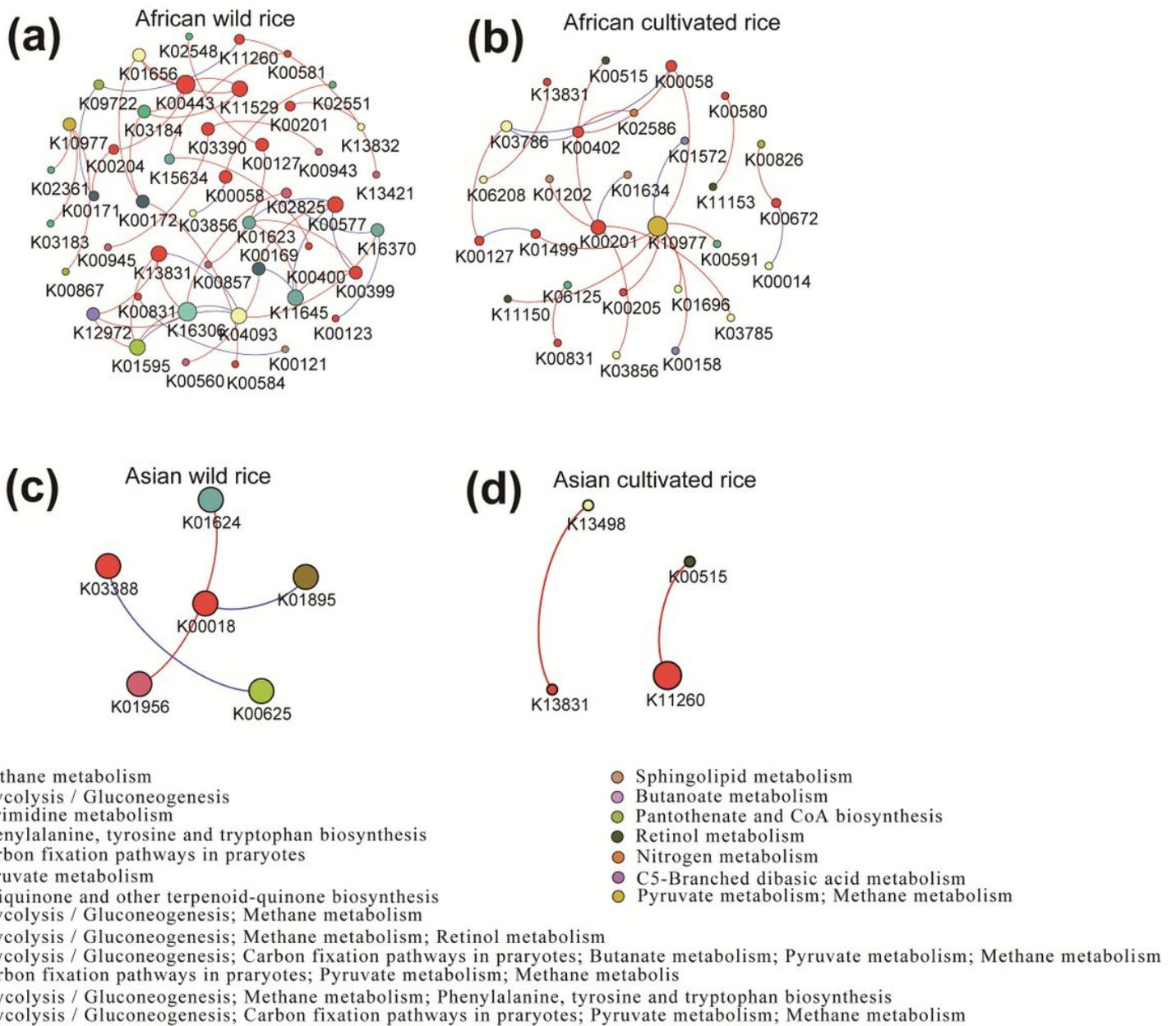


Figure 4

Network based on SparCC correlation coefficients (SParCC's rho cut-off = 0.8, $P < 0.01$), showing how functional KOs of other pathways affected the KOs of methane metabolism of the rhizomicrobiomes of wild rice versus cultivated rice. 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.

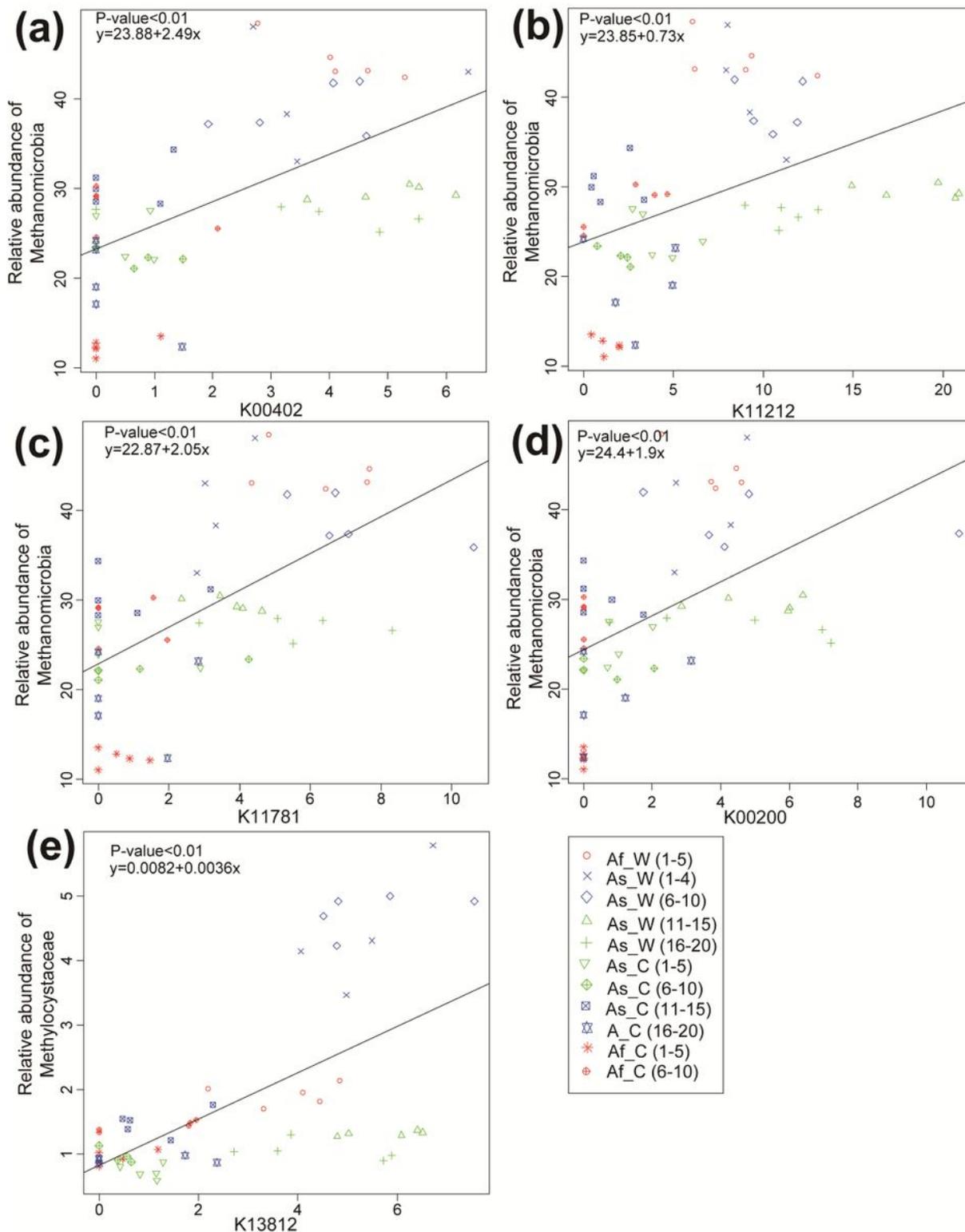


Figure 5

Correlation of the TPM of K00402 (a), K11212 (b), K11781 (c), and K00200 (d) respectively with relative abundance of Methanomicrobia, and correlation of the TPM of K13812 with the relative abundance of Methylocystaceae (e). TPM: transcripts per million reads. Af_W1-5, African wild rice; As_W11-15 nivara wild rice No. 1; As_W16-20, nivara wild rice No. 2; As_W1-4, common wild rice No. 1; As_W6-10, common

wild rice No. 2; As_C1-5, 106 indica; As_C11-15, Jiangxi japonica; Af_C1-5, African cultivated rice No. 2; As_C6-10, Meitezhen indica; Af_C6-10, African cultivated rice No. 2; As_C16-20, Daohuaxiang japonica.

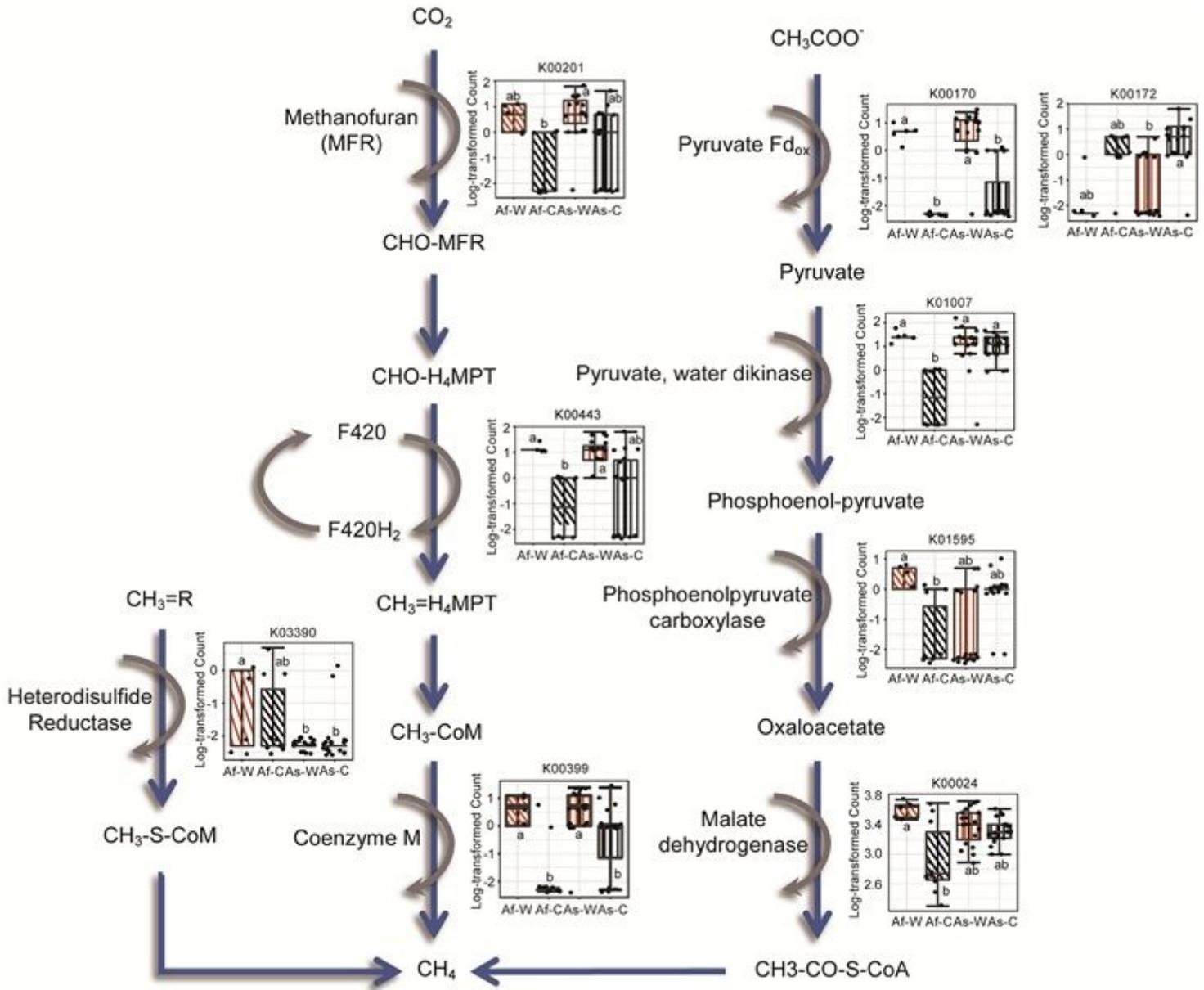


Figure 6

The comparison of the relative abundances of key enzymes involved in methane production in the rhizospheres of wild and cultivated rice.

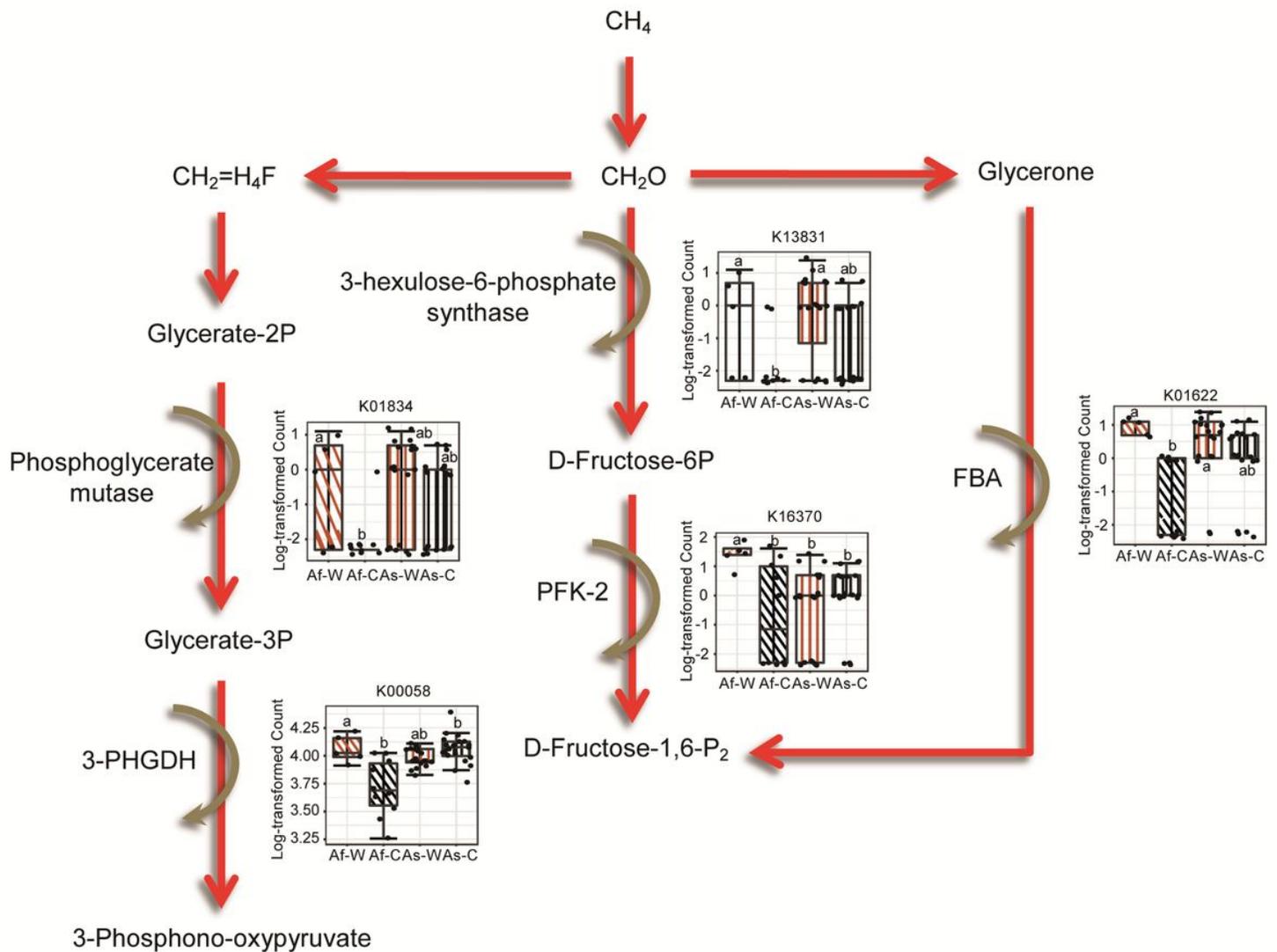


Figure 7

The comparison of the relative abundances of key enzymes involved in methane oxidation in the rhizomicrobiomes of wild and cultivated rice.

Supplementary Files

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