Supporting Information for

Investigation of rhizospheric microbial communities in wheat, barley and two rice varieties at seedling stage

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Supplementary materials and methods

OTU cluster and Species annotation

Once determined, 16S rRNA and ITS sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs). A representative sequence for each OTU was screened for further annotation. For each representative sequence, the RDP classifier (Version 11.4, https://github.com/rdpstaff/RDPTools) algorithm was used to annotate taxonomic information from the Greengenes Database http://greengenes.lbl.gov/cgi-bin/nph-index.cgi). To study the phylogenetic relationships between OTUs and the differences in the dominant species in different samples (groups), multiple sequence alignments were conducted using the MUSCLE software (Version 3.8.31, http://www.drive5.com/muscle/). OTU abundance information was normalized using a standard number of sequences, which was the number of sequences in the sample with the fewest sequences.

Diversity analysis

The alpha diversity describes the species diversity in a sample and is often represented by four metrics, including the observed species index, the Chao1 index, the Shannon index, and the Simpson index. These indicators were calculated for our samples by using QIIME (Version 1.9.1) and were displayed using R software (Version 3.2.2). Beta diversity analysis was used to evaluate differences in species complexity among samples. Beta diversity using both weighted UniFrac distances were also calculated by QIIME (Version 1.9.1).

PCA and PCoA plotting

Cluster analysis was preceded by principal component analysis (PCA), which was applied using the FactoMineR package and ggplot2 package in R software (Version 3.2.2) to reduce

the dimensions of the original variables. Principal Coordinate Analysis (PCoA) was performed to generate principal coordinates and visualize them from the complex, multidimensional data. The matrix of weighted or unweighted UniFrac distances between samples that was previously obtained was transformed to a new set of orthogonal axes whose first principal coordinate accounts for most of the variation in the samples; the second principal coordinate accounts for the second most variation, and so on. The results of the PCoA analysis were displayed by using the WGCNA package, stat packages and the ggplot2 package in R software (Version 3.2.2).

Metagenomic analysis

Data Filtration After DNA sequencing, quality filtering on the raw reads were performed under specific filtering conditions to obtain the high-quality clean data. (1) Trim low-quality bases (<=38) which go over the specific length threshold (40bp default). (2) Remove the reads with the base length of N over the specific length threshold (10bp default). (3) Remove the reads which with the overlap of adapter go over the specific length threshold (15bp default). (4) Remove the host contamination: use SoapAligner (http://soap.genomics.org.cn/soapaligner.html) to align the reads to the host genome.

Assembly Analysis After quality filtering, the valid sequences were assembled using SOAPdenovo (http://soap.genomics.org.cn/soapdenovo.html) with the parameters -K 49/55/59 (k-mer size) –R –M 3 –d 1 –u -F. The reads were compared with the contigs using SoapAligner (http://soap.genomics.org.cn/soapaligner.html) to detect unmap reads, and the unmap reads of every sample were cat together to be a mixture, the mixture is assembled using SOAPdenovo with the same parameters.

Gene Prediction Gene prediction from the assembled contigs was done with MetaGeneMark (http://topaz.gatech.edu/GeneMark/). Then align the reads to gene catalogue to get the reads number of gene of every sample. Filter the genes with the alignment reads less than 2. The abundance of genes for every sample was calculated by the follow formula:

$$G_k = \frac{r_k}{L_k} \cdot \frac{1}{\sum_{i=1}^{n} \frac{r_i}{L_i}}$$

(r represents the reads numbers aligmented, L represents the length of the gene).

The relative abundance of key genes of denitrifying bacteria measured by quantitative real-time polymerase chain reaction (qRT-PCR) The activity of denitrifying bacteria in the rhizosphere was inferred by measuring the relative abundance of denitrification-related genes (nirK/nirS/nosZ, genes that encode N_2O reductase).[1] qRT-PCR was performed in a previously reported manner.[2] Transcription of denitrification-related genes was normalized to that of 16S rRNA and was analyzed using the $2^{-\Delta\Delta Ct}$ method. qRT-PCR was performed with three independent replicates.

Supplementary results and discussion

Profiles of prokaryotic community differences in genus level (Fig. S4)

In our study, α-, β-, and γ-Proteobacteria were the largest contributors to the Proteobacteria population, whose abundance increased by more than 7.34% in the rhizospheres of BW group (wheat and barley). However, this population was only 3.84% greater in rice rhizospheres than in bulk soil. Within this phylum, the agriculturally important genus *Hydrogenophaga* and family Comamonadaceae were strongly enriched in rhizosphere environments. The relative

abundance of *Hydrogenophaga* in BW and rice rhizospheres was greater than that in bulk soil by 4.40% and 1.03%, respectively. The relative abundance of unidentified Comamonadaceae increased by 3.20% after barley cultivation and by 1.84%, 0.42% and 1.23 % in the wheat, *indica and japonica* rhizospheres, respectively. This genus and family are critical for nitrogen cycling and related to microbial denitrification.[3] Our qRT-PCR results showed that the abundance of the denitrification-related nirK/nirS/nosZ genes increased by 13.75-, 2.83- and 66.25-fold in the barley rhizosphere, respectively, compared with those in the bulk soil; the abundance of these genes increased slightly in the rhizosphere microorganisms of the other three tested plants (Fig. S7), indicating that plants can use their root exudates to exert a 'rhizosphere effect' on these functional communities that are involved in N-cycling. Denitrification is a respiratory microbial process that involves nitrous oxide (N_2O) reduction reactions, which is a potent greenhouse gas, contributing to global warming. Several previous studies demonstrated that root exudates can stimulate nitrate reduction or denitrification activity in soil by increasing the copy number of denitrification-related genes, including nirK/nirS/nosZ, and causing minor changes in denitrifier communities in maize.[4] Rhizobiales, another main Proteobacteria, with numerous symbiotic nitrogen-fixing bacteria that include the genera *Rhizobium*, *Rhodoplanes*, and *Devosia*, accounted for 9.78% of the prokaryotic abundance in bulk soil but only 3.44%, 1.61% and 1.89% in the rhizospheres of barley, *indica* and *japonica*, respectively. In contrast, its relative abundance increased slightly (0.34%) in the rhizosphere of wheat, indicating that the community of bacteria with nitrogen-fixing functions could also be regulated by plants in a strongly cultivar-dependent manner.[5] The decrease in the presence of Rhizobiales in the rhizosphere soil as well as the increase of

denitrifying bacteria could result in a loss of N or even greenhouse gas increases after plant cultivation.

The relative abundance of Bacteroidetes increased from 7.69% in the bulk soil to near 14.45% and 9.05% in the BW and rice rhizospheres, respectively. Similar to Proteobacteria, Bacteroidetes thrive under conditions of high nutrient availability and have important roles in biogeochemical cycling and the maintenance of plant health and productivity.[6] In the rhizosphere, the genus of Bacteroidetes whose abundance most greatly increased was *Flavihumibacter*, which increased by 3.19%, 2.61%, 1.50% and 1.12% in wheat, barley, *indica* and *japonica*, respectively. *Flavihumibacter* strains can degrade organic compounds from root exudates to form a variety of sole carbon sources that they can use.[7] The increased abundance of Proteobacteria and Bacteroidetes therefore represented an increase in the supply of labile C substrates in the soil.[6] The abundance of these two phyla in BW was higher than that in rice, implying that root excretions in BW contained a greater variety (or quantities) of C-containing compounds, which stimulates the accumulation of these kinds of bacteria.

Acidobacteria is one of the most abundant bacterial phyla in terrestrial ecosystems. The relative abundance of Acidobacteria was approximately 4.42% and 3.35% lower in BW and rice, respectively, than in bulk soil, and the plant genotype had only a minor effect on Acidobacteria abundance. Actinobacteria were also significantly depleted within the rhizospheres of 27 maize inbreds, compared with bulk soil.[8] This depletion suggests that this taxon is either actively excluded by the host immune system or negatively impacted by organic matter, which may be favorable for carbon polymer-degrading or fast-growing microorganisms that could presumably outcompete Acidobacteria.[9] The lower Acidobacteria

abundance was mainly attributed to Gaiellaceae, *Arthrobacter* and Nocardioidaceae species, which decreased by approximately 1% in all rhizosphere soils. Unfortunately, the specific roles of them in the soil ecosystem are relatively unknown.

The proportion of Planctomycetes (5.82-8.49%) in all sample rhizospheres was higher than that in bulk soil (4.15%). Within this phylum, the abundance of Gemmataceae, Pirellulaceae, *Planctomyces* and *Pirellula* increased by 0.20-1.15% in rhizospheres. Planctomycetes species are consistently present throughout plant development.[10] The increase in Planctomycetes was reported to be due to its ability to utilize a wide range of complex sugars and to reduce nitrates and nitrites.[11] Therefore, plant excretion was beneficial to this phylum and may provide them the advantage to survive in plant soil as compared to bulk soil.

The changes in these four core prokaryotes in the BW and rice rhizospheres are 26.51 and 13.58%, respectively, indicating that the four tested plants affected their rhizosphere communities to different degrees; the BW exerted stronger effects than the rice, resulting in dramatic changes in the rhizosphere communities. Several studies regarding bacterial rhizosphere communities demonstrated the key role of plant species in shaping bacterial communities in the rhizosphere. Mostly because plants provide exudates and other labile chemical resources, they exert considerable influence over the rhizosphere microbiome.[12] The composition of the core rhizosphere prokaryotes was significantly different from that of the core prokaryotes in bulk soil, suggesting that different plants select a subset of prokaryotes by applying different pressures. The bacterial community in rhizospheres constitutes part of a complex system that utilizes the N or C released by the plant. The availability of these

nutrients is the major driving force in the regulation of microbial diversity in the immediate vicinity of plant roots.[13]

Profiles of eukaryotic community differences in genus level (Fig. S6)

In Ascomycota, the dominate genera were *Humicola*, *Gloeotinia*, and *Haematonectria*. *Gloeotinia*, which is involved in seed disease, accounts for 17.68% of fungi in the bulk soil, but decreased by 11.51% and 2.78% in barley and wheat rhizospheres, respectively. In contrast, its relative abundance increased by 10.67% and 0.28% in *indica* and *japonica*, respectively. The other pathogen, *Haematonectria*, accounts for 4.02% of fungi in the bulk soil and decreased to 2.40% and 1.20% in barley and *indica* rhizospheres, respectively. Its relative abundance increased to 11.18% and 14.00% in wheat and *japonica*, respectively. *Humicola* abundance was lower by approximately 4.52% in 4 tested rhizospheres than in bulk soil. This genus was reported to be a parasite of cyst nematodes and an excellent producer of neutral thermostable cellulases and hemicellulases and thus might induce local and systemic resistance against pathogens.[14] These results suggest that plant species affected soil fungal community diversity and abundances, which may enhance resistance of the plants to fungal soil-borne disease.[15] As shown in Fig. 3b, the relative abundances of *Fusarium*, *Emericellopsis*, *Bionectria*, *Verticillium* and *Pyrenochaeta* genera in rhizospheres were 1-3% lower than in bulk soil, and some genera such as Chaetomium (0.14% in the bulk soil), an antibiotic producer that suppresses pathogens, were even completely undetectable in all rhizosphere samples.[16]

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Supplemental datasets list

Dataset 1 KOs information and pairwise comparison: KOs relative abundance of each sample (sheet 1) and pairwise comparison of KOs relative abundance of 2 groups (Indica & Japonica, Barley&Wheat) compared to the bulk soil (sheet 2, 3).

Dataset 2 Pairwise comparison of EC relative abundance of 2 groups (Indica & Japonica, Barley&Wheat) compared to the bulk soil.

Supplemental Figures:

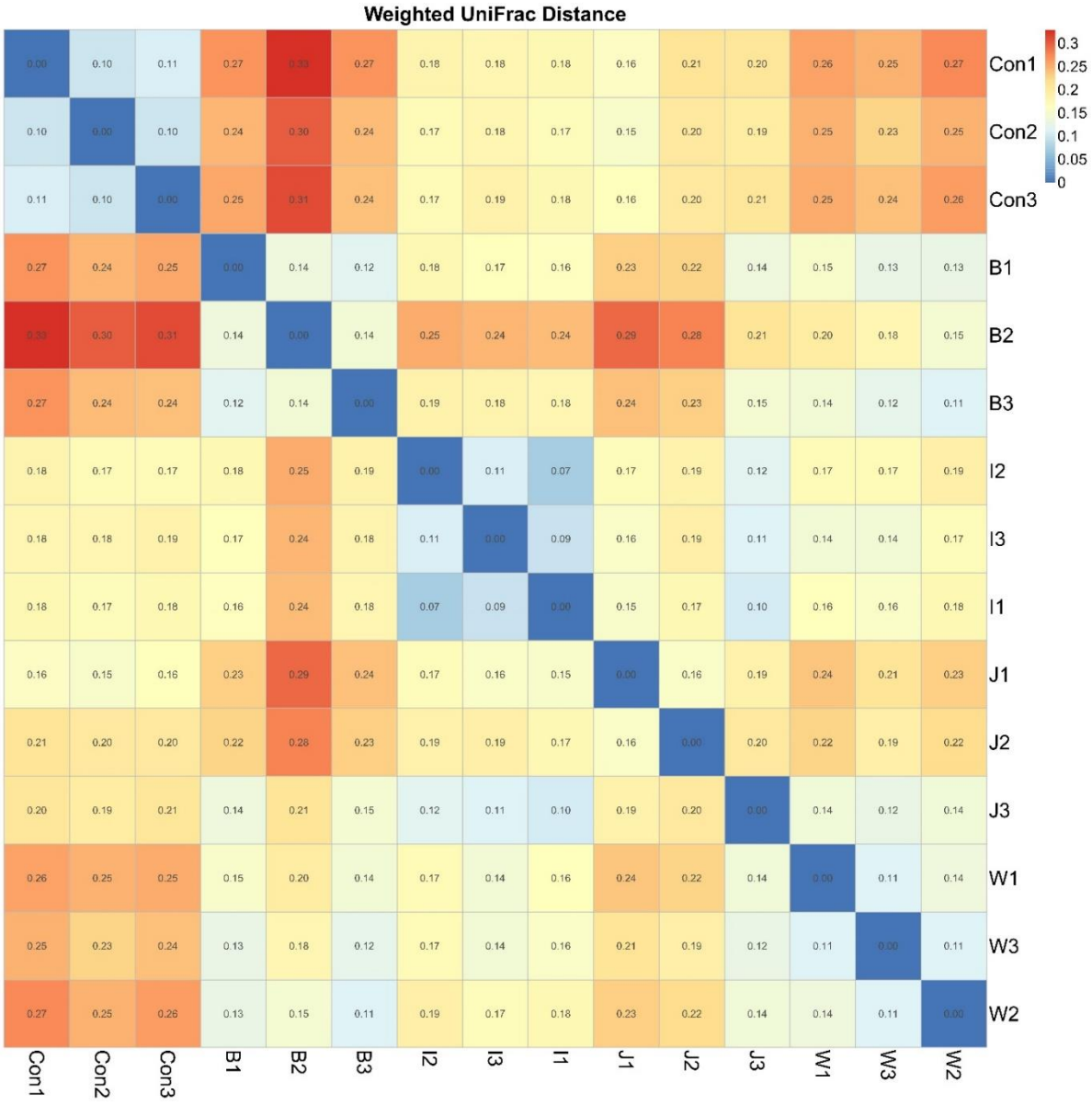

Fig. S1 weighted UniFrac distance (prokaryotic community) Con: bulk soil; B: barley; W: wheat; I: *indica* rice; J: *japonica* rice

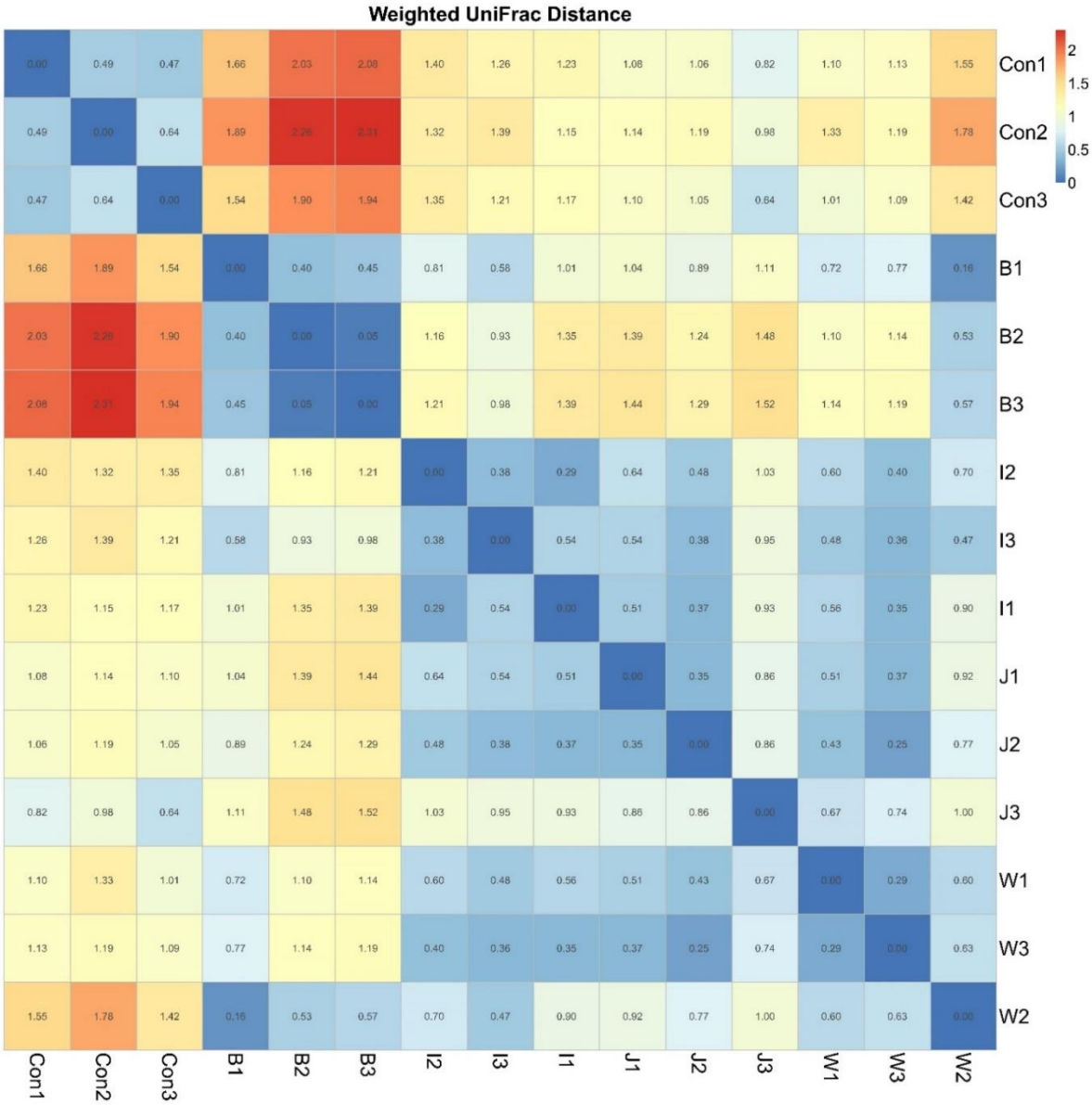

Fig. S2 weighted UniFrac distance (eukaryotic community) Con: bulk soil; B: barley; W: wheat; I: *indica* rice; J: *japonica* rice

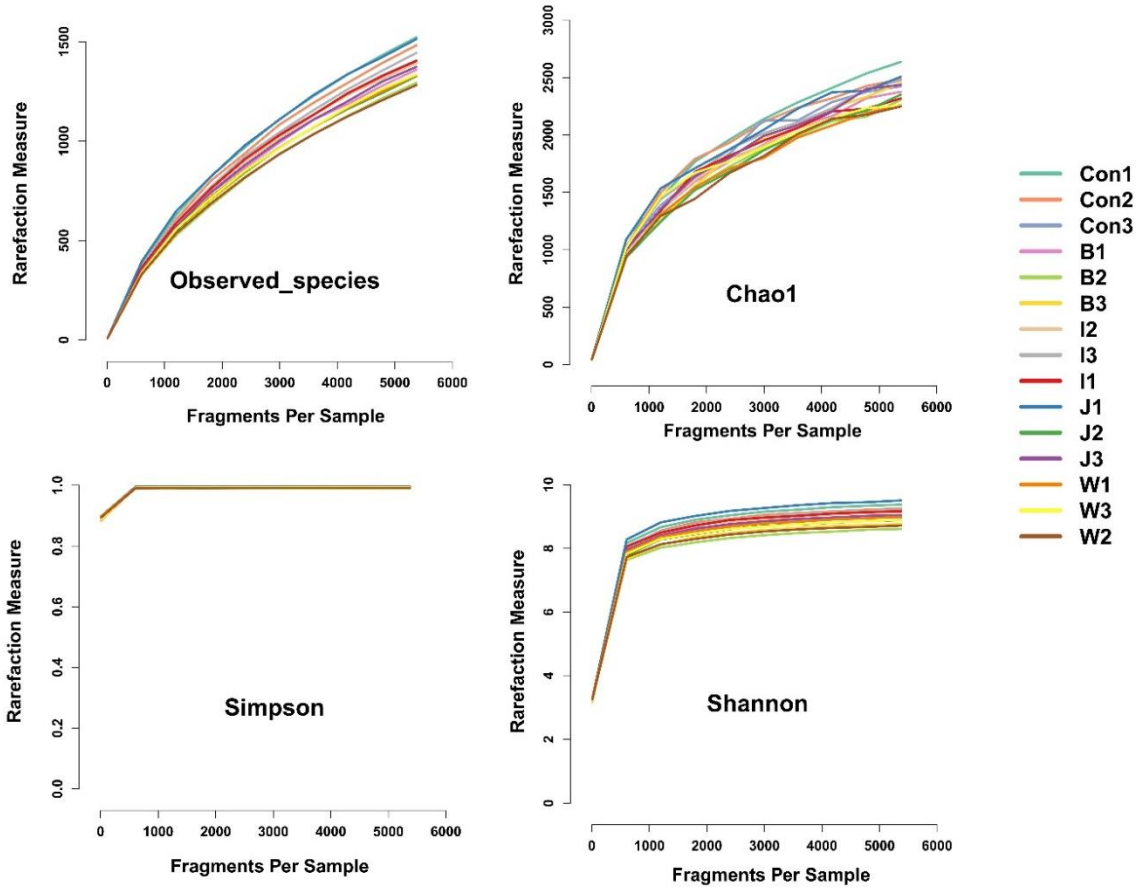

Fig. S3 alpha diversity (prokaryotic community) Con: bulk soil; B: barley; W: wheat; I: *indica* rice; J: *japonica* rice

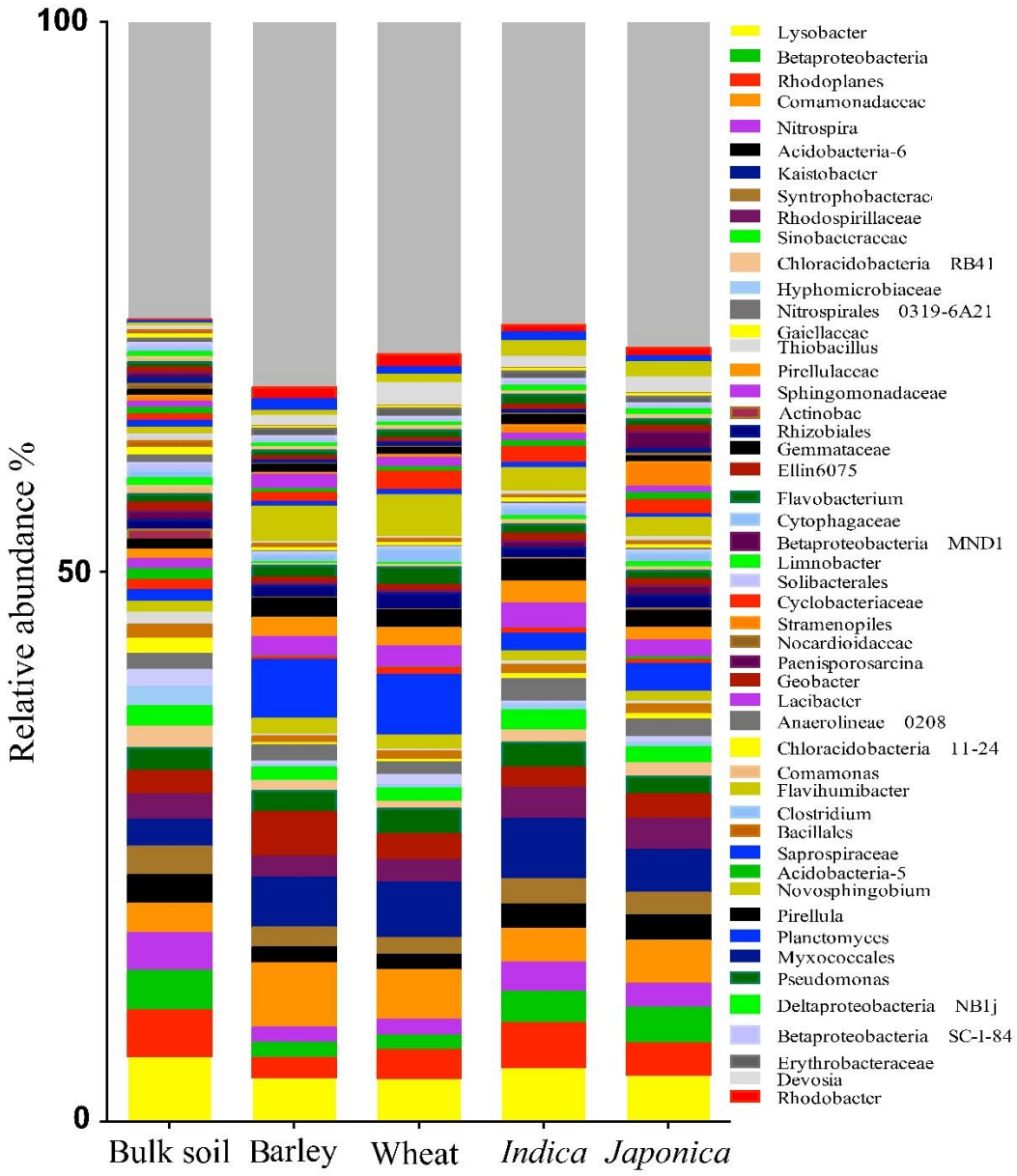

Fig. S4 Prokaryotic community profiles (relative abundance) in bulk soil, wheat, barley, indica and japonica rice rhizospheres at the genus level; here the microbial names of order, class and family levels represent unidentified microbes in this taxon. Values are means of biological replicates, n = 3 for all groups.

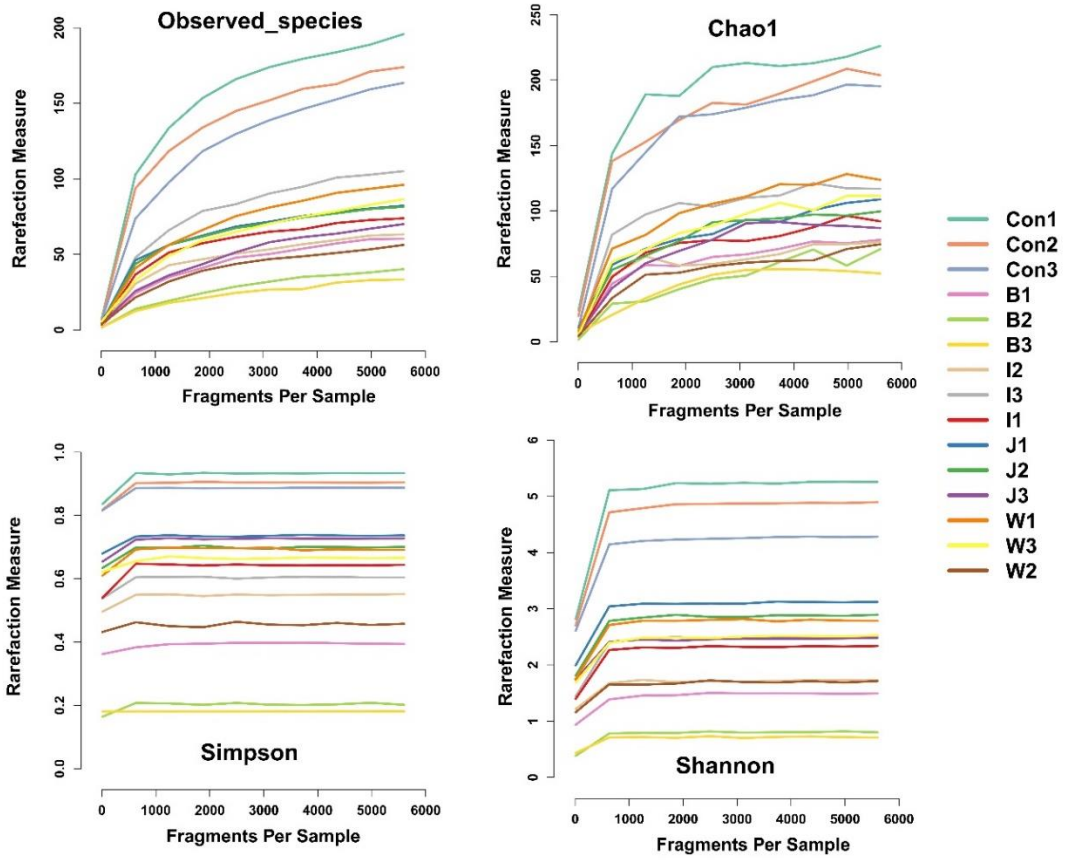

Fig. S5 alpha diversity (eukaryotic community) Con: bulk soil; B: barley; W: wheat; I: *indica* rice; J: *japonica* rice

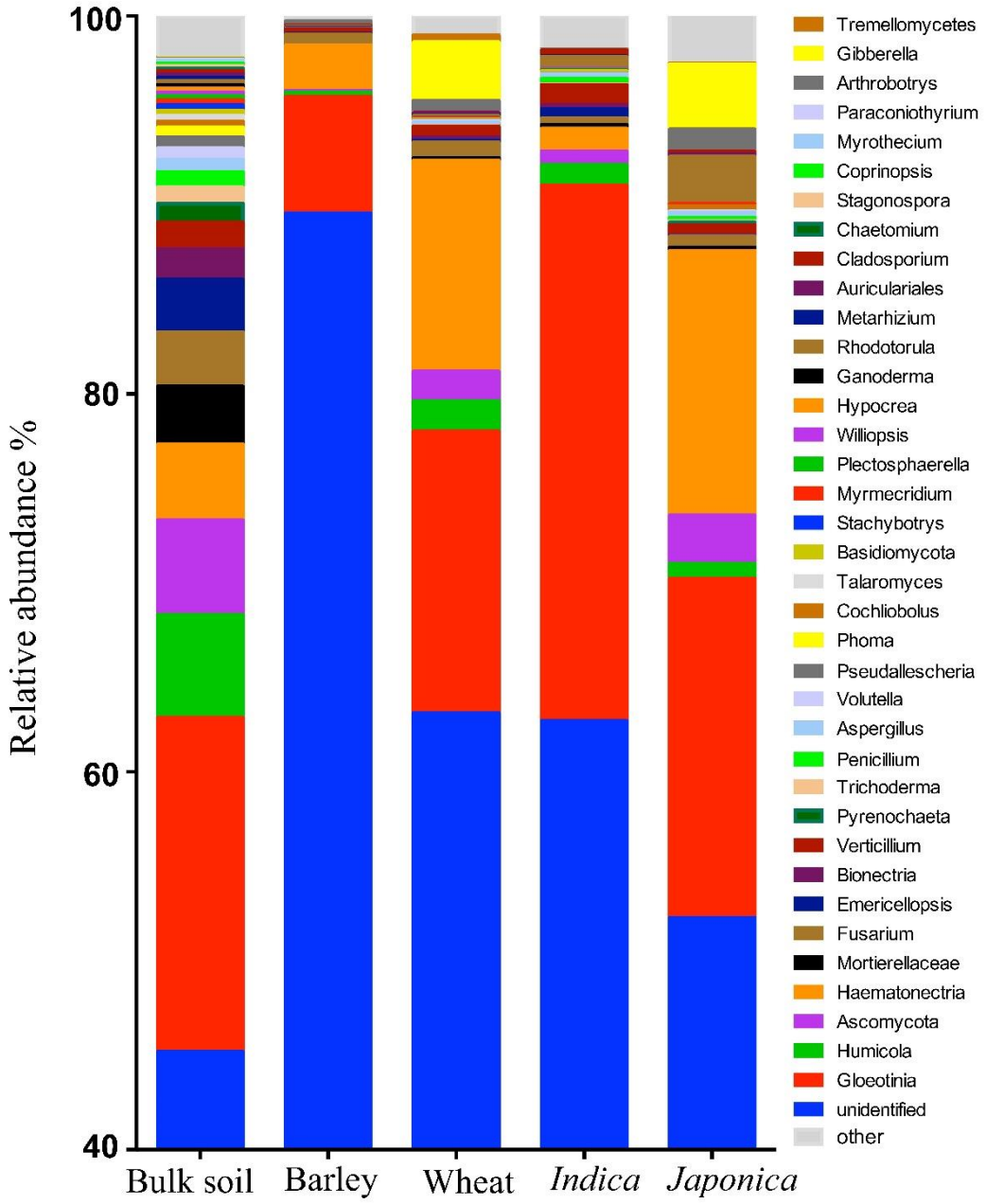

Fig. S6 Eukaryotic community profiles (relative abundance) in bulk soil, wheat, barley, *indica* and *japonica* rice rhizospheres at the genus level; here the microbial names of order, class and family levels represent unidentified microbes in this taxon. Values are means of biological replicates, n = 3 for all groups.

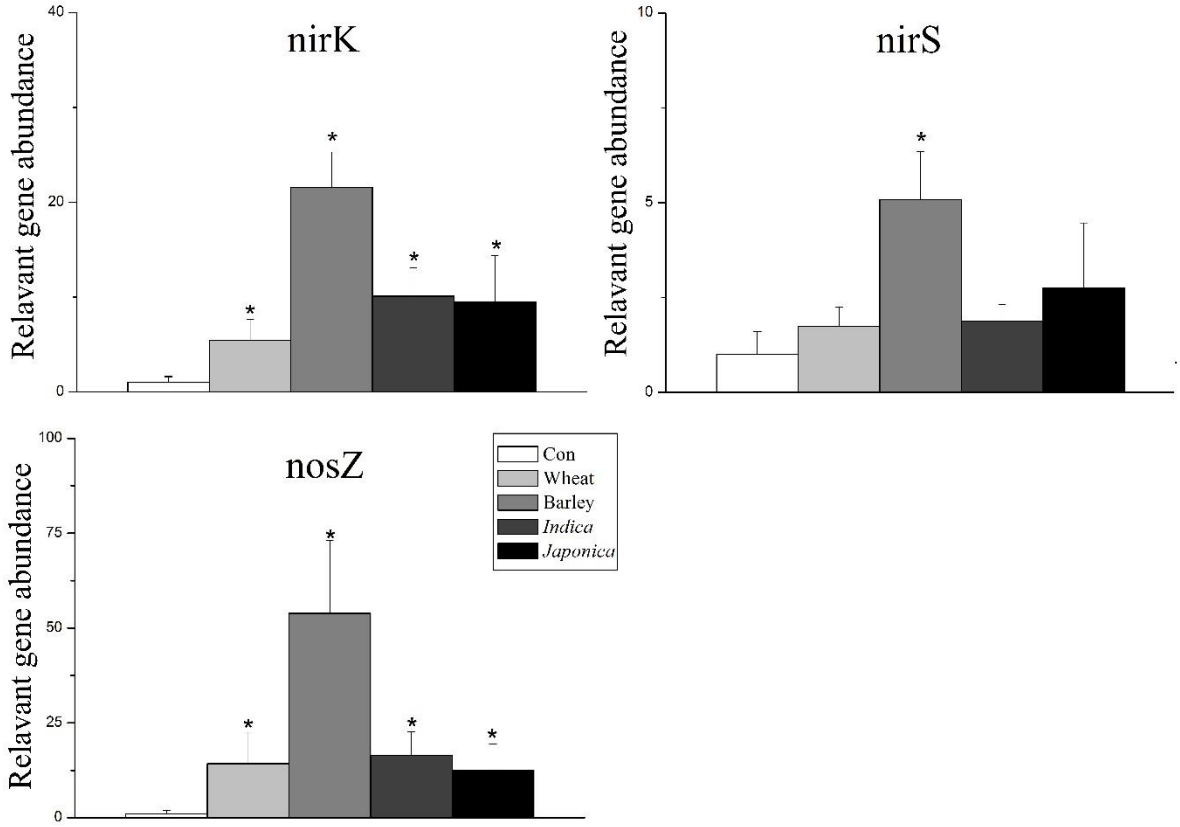

Fig. S7 The abundance of functional genes (nirK, nirS, nosZ) involved in denitrification normalized to the abundance of 16s rRNA gene. Statistical significance among treatments was tested by analysis of variance (ANOVA) followed by the Dunnett's post hoc test using StatView 5.0 program. Asterisks represent statistically significant differences at $p < 0.05$ between 4 crops and Con group (bulk soil).

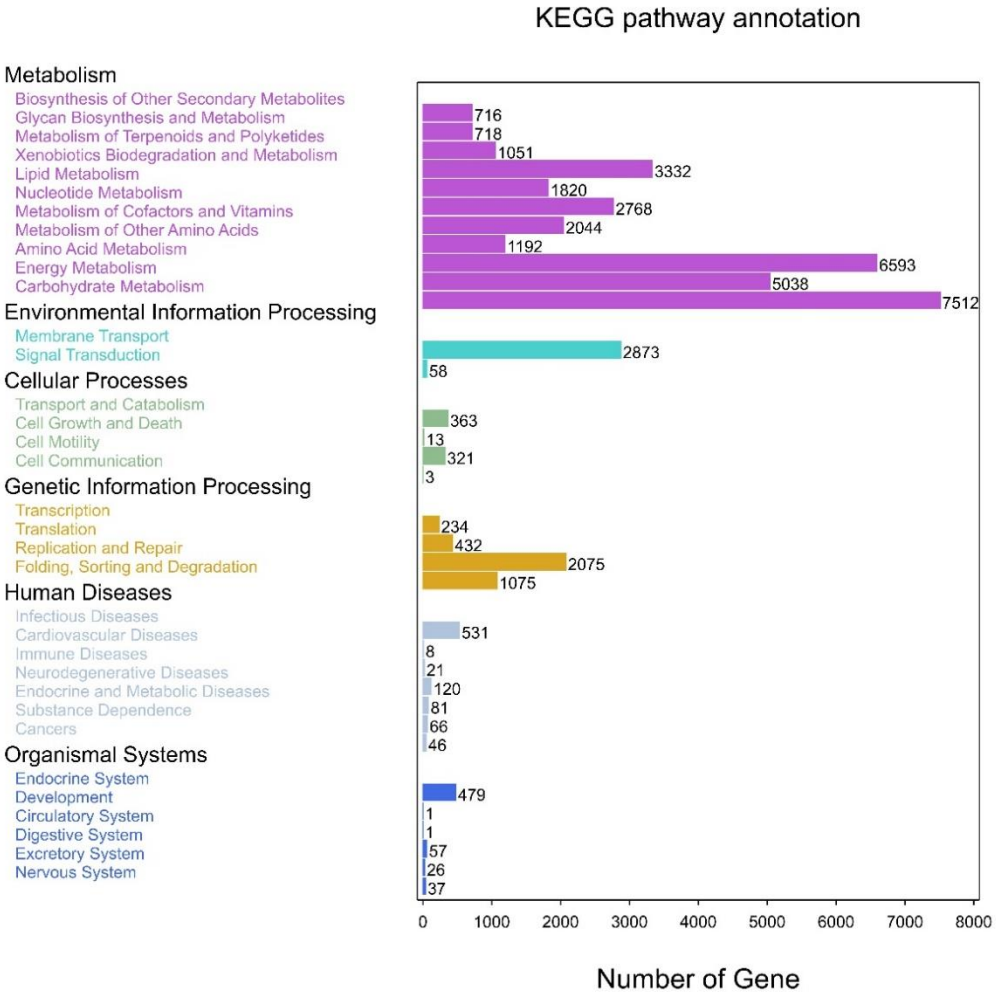

Fig. S8 KEGG pathway annotation

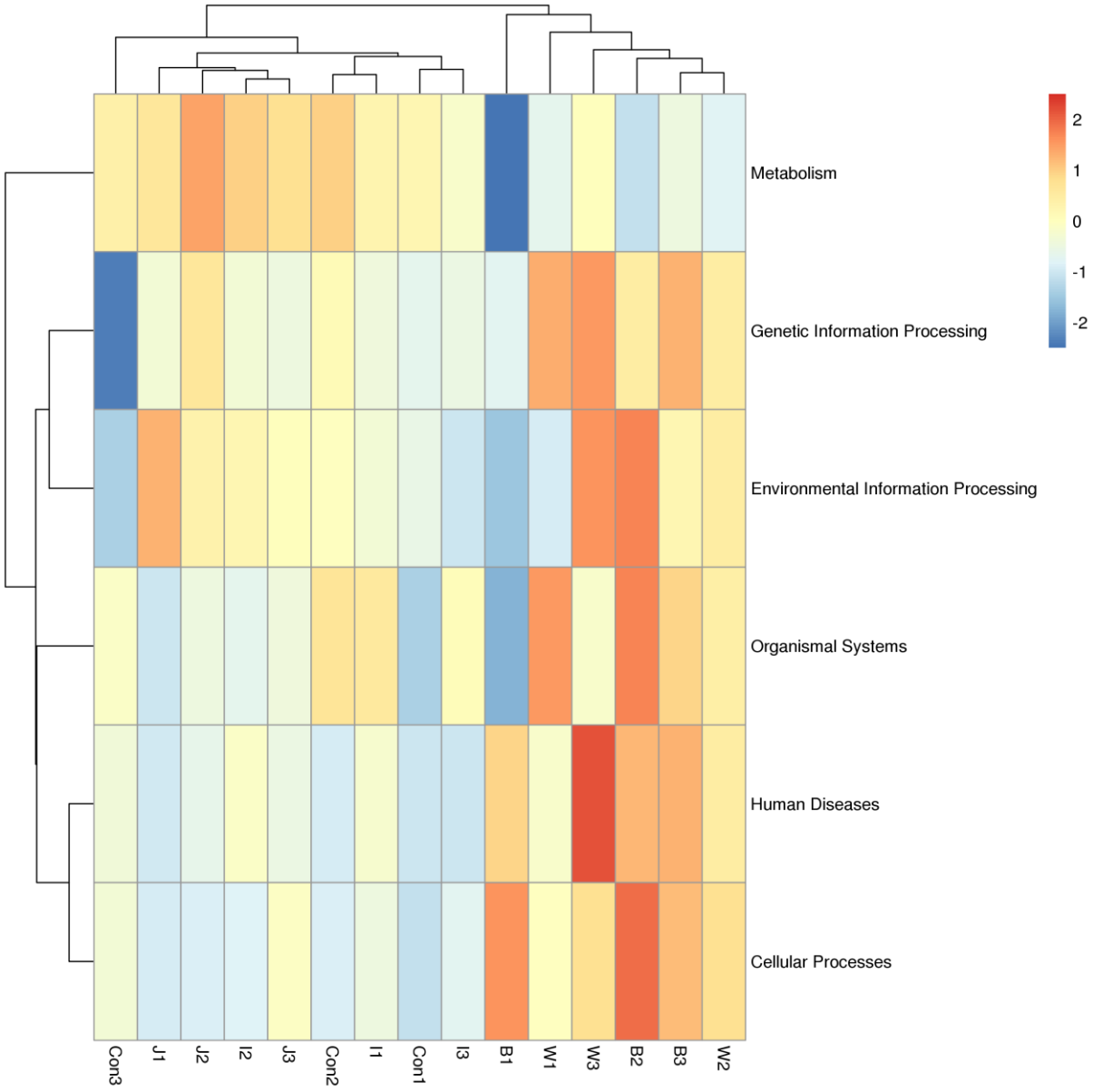

Figure S9 Relative abundance of functional categories heatmap in the KEGG databases (level 1).

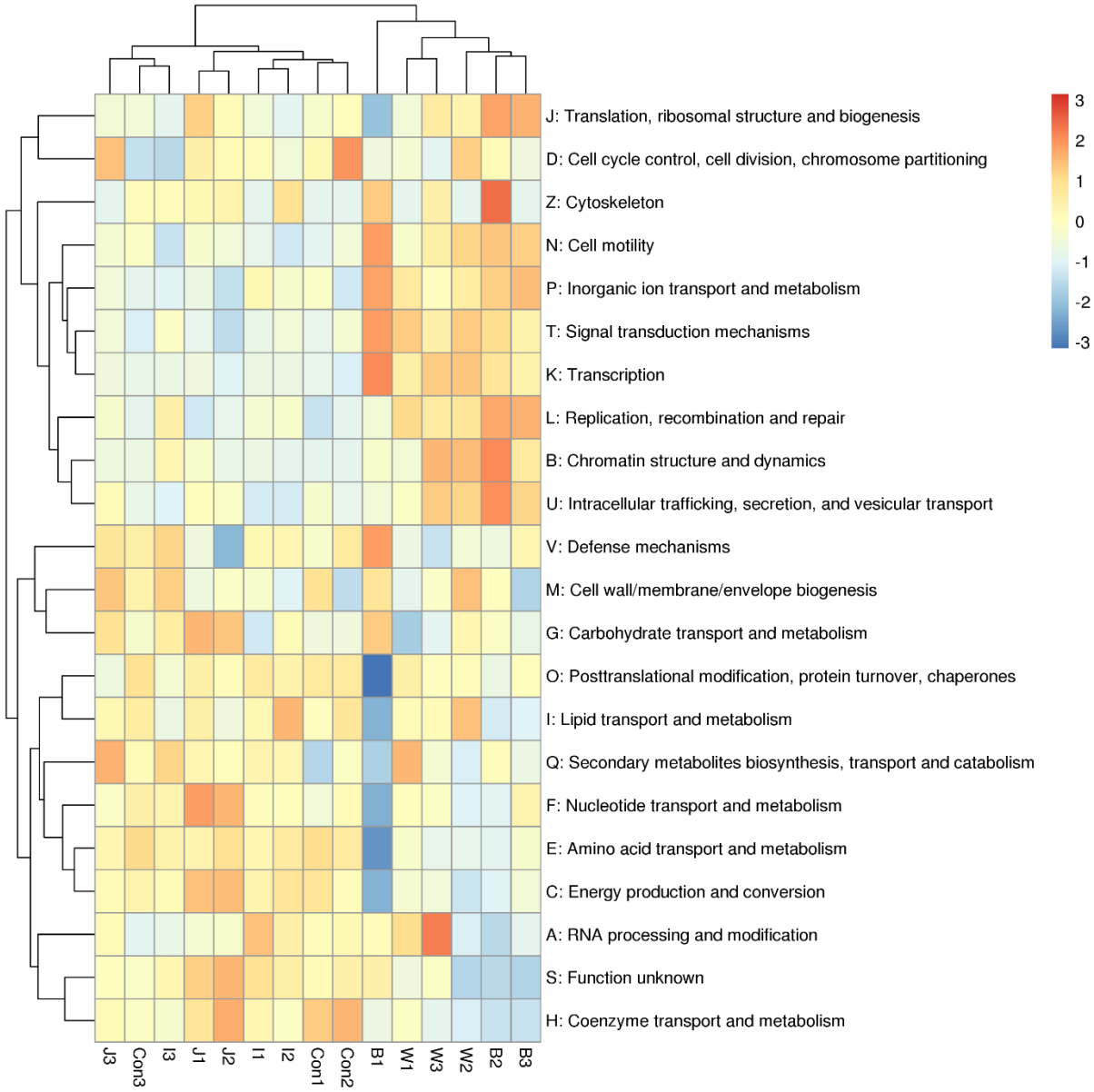

Figure S10 Relative abundance of functional categories heatmap in the eggNOG databases (level 1).