Wild rice controls more root endophytic fungi than cultivated rice in the F1 offsprings after the crossbreeding

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Abstract

Background: Rice, which serves as a staple food for more than half of the world's population, has been planted all over the world. The hybridization of wild and cultivated rice has helped rice gain resistance to variable environmental conditions. Endophytic microbiomes have been known to be transferred along with the plants. However, the endophytic bacteria or fungi for the wild and cultivated rice, and their first crossbred generation have not been illustrated until now.

Results: In this study, we systematically compared the endophytic microbial community structures of Asian and African wild and cultivated rice species with their F1 offsprings. Results showed that both African and Asian wild rice controls more root endophytic fungi than cultivated rice in their first generation after crossbreeding. Furthermore, network analysis of the bacterial and fungal operational taxonomic units showed that Asian and African wild rice species can cluster and have more significant correlations than cultivated rice fungal species. The core bacterial species that connected wild rice with its F1 offsprings was Acidovorax, wherea the core bacterial species that linked cultivated rice to its F1 offsprings was Bradyrhizobium; and the core fungal species that can connect in wild rice and the F1 offsprings were Pleosporales, Myrothecium and Bullera, while the core fungal species that can connect in cultivated rice was those belonging to the Dendroclathra genus.

Conclusions: This study may provide the theoretical significance of the endophytic bacteria and fungi for wild and cultivated rice along with their F1 offsprings.

Background

Endophytic microbes inhabit plant tissues without causing any obvious damage to their host, and play crucial roles in plant growth, development, fitness and protection (Farrar et al. 2014; Truyens et al. 2015; Tian et al. 2020b). These endophytic microbes, including bacteria and fungi, spend a portion of their life cycle inside plants, normally residing on intercellular spaces and gaining carbohydrates, amino acids and inorganic nutrients from plants (Bacon and Hinton 2006). Despite their beneficial effects on plant growth and development, root-borne endophytic microbes are still largely unexplored in rice. Recent development in high-throughput technologies, such as next-generation sequencing (NGS), has enabled the investigations of endophytic microbiomes, facilitating sequencing of a larger number of microbes and encouraging in-depth analyses of microbial communities in taxonomic, phylogenetic, and evolutionary studies (Kaul et al. 2016; Carrión et al. 2019; Furtado et al. 2019).

Rice (Oryza sativa) is the main food staple for approximately half of the world's population; and thus, breeding for yield improvement to feed an ever-increasing world population is a critical goal for the rice research community (Zhang et al. 2016; Maurya et al. 2017; Tian et al. 2018; Tian et al. 2019). Wild rice (Oryza rufipogon), a relative of cultivated rice, possesses several unique attributes, including disease and lodging resistance as well as drought tolerance. Adverse conditions have seriously influenced rice growth and development, affecting rice yield worldwide (Prasad et al. 2017; Khumairoh et al. 2018; Mostofa et al.

2019). Wild rice, serving as the resource for the crossbreeding of rice, has improved traits of cultivated rice varieties due to the loses of valuable traits during the progress of domestication (Zhang et al. 2016; Tian et al. 2017; Shi et al. 2018). Several recent studies have shown that the endophytic microbial community associated with wild plant species may play important roles in their disease resistance (Pérez-Jaramillo et al. 2016; Tian et al. 2017; Tian et al. 2020b). Hybridization of cultivated plant species with wild species can transfer beneficial traits from both parents to the next generation, and may induce beneficial changes in the community composition of the endophytic microbiomes associated with the offspring to help them produce higher yields even under adverse environmental conditions (Goulet et al. 2017; Cao 2018).

However, it is not yet clear how the endophytic microbial community and diversity would change by the hybridization of wild and cultivated rice. Thus, a comparative analysis of the endophytic microbial community and diversity, which are associated with the cultivated and wild rice varieties, as well as their offsprings after crossbreeding, such as the F1 and F2 offsprings, may allow us to understand such acclimatization of the associated endophytic microbiomes, ultimately providing a meaningful solution for restructuring the endophytic microbial community to achieve a sustainable agricultural system at least for rice production.

Cultivated rice has two main species, namely Asian and African cultivated rice. The species of Asian cultivated rice were originated from nivara or common wild rice. African cultivated rice was originated from African wild rice. Presently, we have the plant resources of Asian cultivated rice, African cultivated rice, nivara wild rice, African wild rice, and their hybrid seeds for our study. Accordingly, the endophytic microbial diversity and community structures of Asian and African cultivated and wild rice along with their F1 offsprings were analyzed. We hypothesized that the microbial community structures of Asian/African wild rice are more similar with their F1 offsprings than those of Asian/African cultivated rice; and wild rice controls more root endophytic bacteria and fungi than cultivated rice in the F1 offsprings after the crossbreeding. Some shared species have changed among African and Asian wild and cultivated rice along with their F1 offsprings.

Materials And Methods

Plant materials

The plant materials of the Asian and African wild and cultivated rice along with their F1 generation used in this study are shown as Table 1. Seeds of wild rice (O. barthii, O. nivara and O. ruffipogon) and the F1 offsprings (African wild rice × African cultivated rice, nivara wild rice × Asian cultivated rice and common wild rice × Asian cultivated rice) were kindly provided by the International Rice Research Institute (IRRI) (Table 1). The seeds of Asian cultivated rice (O. sativa_subsp.indica and O. sativa_subsp.japonica) and O. glaberrima were collected from the Jiangxi Academy of Agricultural Sciences (Table 1). The germinated seeds (approximately three days after germination) were transplanted into pots containing 3 kg soil/pot with 4 seeds in each pot [18 cm high and 20 cm diameter (upper side)]. Subsequently, the pots were

placed in a growth chamber with a 16-h light/8-h dark photoperiod at 26/20°C with a relative humidity of 65%. The pots were watered once every 3 days with the same volume of Hoagland's nutrient solution to keep the relative soil water content at 13–15%. After 40 days from the beginning of the transplanting, the plants and roots were collected. The roots were cut and cleaned by tap water and sterilized by dipping into 1% sodium hypochlorite for 5 min. Then, the roots were cleaned by sterilized water.

Table 1
Summary of high-throughput sequencing data for bacteria and fungi.

Species	Seed source	Sample name	Genome	Distribution
Oryza barthii (African wild rice)	IRRI	Af-W	AA	Western, eastern and southern Africa
Oryza glaberrima (African cultivated rice No. 2)	Jiangxi, China	AfC1	AA	West Africa
Oryza glaberrima (African cultivated rice No. 4)	Jiangxi, China	AfC2	AA	
African wild rice × African cultivated rice	IRRI	Af-H	AA	Western, eastern and southern Africa
Oryza nivara (nivara wild rice No.1)	IRRI	NW1	AA	Tropical and sub-tropical Asia
Oryza nivara (nivara wild rice No.2)	IRRI	NW2	AA	
nivara wild rice × Asian cultivated rice	IRRI	NW-H	AA	
Oryza ruffipogon (common wild rice No.1)	IRRI	CW1	AA	Tropical and sub-tropical Asia
Oryza ruffipogon (common wild rice No.2)	IRRI	CW2	AA	
common wild rice × Asian cultivated rice	IRRI	CW-H	AA	
Oryza sativa_subsp.indica (Asian cultivated rice, indica)	Jiangxi, China	InC	AA	China
Oryza sativa_subsp.japonica (Asian cultivated rice, japonica)	Jiangxi, China	JaC	AA	

Soil Dna Extraction And High-throughput Dna Sequencing

The sterilized roots in the above step were sunk into the liquid nitrogen and then ground into powder. Subsequently, the root DNA was extracted according to the protocol of the Fast DNA SPIN Kit (Catalog No. 6560 – 220, Germany) with 0.5 g soil for each sample. After dissolving in the DNA solution, the extracted DNA was quantified by NanoDrop 2000 (Thermo Scientific, Germany) before using for PCR. The

V3-V4 hypervariable regions of the 16S rRNA (341F 5'-ACTCCTACGGGAGGCAGCA-3' and 785R 5'-GGACTACHVGGGTWTCTAAT-3') were amplified for the analysis of the bacterial compositions while the ITS1 regions (ITSF 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITSR 5'-GCTGCGTTCTTCATCGATGC-3') were targeted for assessment of the fungal compositions. The 2 × 250 bp paired-end sequences of the PCR amplicons were sequenced using the HiSeq platform (Illumina, San Diego, CA, USA) at Beijing Biomarker Corporation.

QIIME software (http://qiime.org/) was used to qualify the sequences and to remove the barcodes and primers of the raw sequencing data. The clean data were subjected to an RDP classifier for taxonomic assignment with a minimum of 50 confidence estimates. Random resampling was performed using the smallest sequences of all the samples. Based on a 97% similarity level, the operational taxonomic units (OTUs) were classified using USEARCH (http://www.drive5.com/usearch/Usearch) after removing the singleton reads. A detrended correspondence analysis (DCA) was used to explore the changes in the overall microbial community composition. A partial Mantel test was performed to correlate the microbial communities with factors based on the Bray-Curtis distances. To clarify the relationships of the samples in the treatment clusters, a nonmetric multidimensional scaling analysis (NMDS) was used.

Statistical analysis

The principal component analysis (PCA), based on the OTU relative abundance, was performed by the PCA function in the FactoMineR package of R software version 3.2.1 (Feeley et al. 2011). The Euclidean distance was calculated and used for the PCA analysis. Differences in the diversity across samples were determined by SPSS 19.0 software using a one-way ANOVA followed by a least significant difference (LSD) test. The network analysis for the connection between bacterial and fungal communities was performed by dominated OTUs in each sample (Fagnan et al. 2012).

Results

Analysis of the Illumina sequencing data

Based on the Illumina sequencing data, with respect to bacteria, a total of 1,900,512 paired reads were received after unqualified sequences had been removed, with an average clean read number of 39,594 for each sequenced sample and a minimum read number of 30,750 (a total of 12 samples and each sample had 4 replicates) (Additional file 1: Table S1). The average length of the reads was 430 bp. The rarefaction curve displayed the correlation between the read number and observed species number, and reached the stable plateau phase, suggesting that the resulting number of current observed species number was reasonable for further statistical analysis (Additional file 2: Figure S1).

With regard to fungi, a total of 1,905,330 clean reads were recorded after the unqualified sequences had been filtered. The average read number for each sequenced sample was 47,860, and the lowest read number of 30,748 was received for only one sample. Furthermore, a total of 227 fungal OTUs were

produced based on 97% sequence similarity (Additional file 1: Table S1). The rarefaction curve describing the correlation of the fungal reads and observed species number showed that the resulting fungal observed species numbers were valid (Additional file 2: Figure S1).

Taxonomic Analysis

Viewing composition at higher levels (e.g. phylum) provides a better picture than at lower levels (e.g. species), when the number of species in a community is large and diversified. Additionally, such taxonomic abundance or composition can be viewed at the community level (all samples), sample group level (based on experimental factor) or at individual sample level. Taxa with very low read counts can also be collapsed into the 'Others' category using a count cutoff based on either the sum or median of their counts across all samples or all groups. Merging such minor taxa will help better visualize the significant taxonomic patterns in the data. Figure 1 shows the taxonomic composition using a stacked bar/area plot.

The bacterial and fungal communities were analyzed based on the phylum level. The main bacterial phyla in the samples were Proteobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, Acidobacteria, and Firmicutes, followed by Fibrobacteres and Verrucomicrobia (Fig. 1a), while the phyla of Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota were dominant for fungi (Fig. 1c). Furthermore, by comparing the relative abundances of the bacteria and fungi, we found that the relative abundance of Chloroflexi was significantly higher in both African and Asian cultivated rice than their respective wild rice (Fig. 1b), and the relative abundance of Basidiomycota was significantly higher in both African and Asian wild rice and F1 generation than in their respective cultivated rice (Fig. 1d). Although they had small relative abundances, the relative abundance of Glomeromycota was significantly higher in common wild rice than in the other samples (Fig. 1d).

Comparative alpha abundance and diversity analyses of the endophytic microbial communities of cultivated and wild rice plants, along with their F1 offsprings after crossbreeding

To analyze the differences in the bacterial and fungal community abundance and diversity of the wild and cultivated rice species along with their F1 generation, alpha analyses based on the Simpson, Chao1, ACE and Shannon indexes were performed. The results showed that the bacterial and fungal alpha abundances and diversities were not stabilized in the endophytes of both the cultivated and wild rice along with their F1 offsprings. As shown in Fig. 2, we observed significantly higher Chao1 and Shannon indexes in the bacterial community composition of both African and Asian cultivated rice than in that of their relative wild rice and respective F1 offsprings (Fig. 2b, d), whereas we did not find significant differences in the bacterial Simpson and ACE indexes of both African and Asian cultivated rice compared with their relative wild rice and respective F1 offsprings (Fig. 2a, c). The bacterial Chao1 and Shannon indexes in African cultivated rice (AfC1, AfC2) were significantly higher than in wild rice (Af-W) and their F1 offsprings (Af-H), whereas there was no significant difference between wild rice (Af-W) and the F1 offsprings (Af-H). Similarly, the bacterial Chao1, ACE and Shannon indexes in Asian cultivated rice (JaC,

InC) were significantly higher than in nivara wild rice (NW1, NW2) and their F1 offsprings (NW-H), whereas there was no significant difference between nivara wild rice (NW1, NW2) and the F1 offsprings (NW-H). However, although the Shannon index in Asian cultivated rice (JaC, InC) was higher than common wild rice (CW1, CW2) and their F1 offsprings (CW-H), there were no significant differences in the bacterial Simpson, Chao1 and ACE indexes among Asian cultivated rice (JaC, InC), common wild rice (CW1, CW2) and their F1 offsprings (CW-H) (Fig. 2). For fungal alpha diversities, the results showed that there were no significant difference in the Simpson, Chao1, ACE and Shannon indexes among African cultivated rice (AfC1, AfC2), African wild rice (Af-W) and their F1 offsprings (Af-H) (Fig. 3a, b, c, d). In Asian rice group, the Simpson and Shannon indexes were significantly higher in nivara wild rice (NW1, NW2) and the F1 offsprings (NW-H) than in cultivated rice (JaC, InC) (Fig. 3a, d), while, the Chao1 and ACE indexes were higher in wild rice (CW1, CW2) than in cultivated rice (JaC, InC) and the F1 offsprings (CW-H) (Fig. 3b, c).

Beta Diversity Analysis

A PCA analysis based on the Euclidean distance of the plants based on endophytic microbiomes was performed together for cultivated and wild rice (Fig. 4). For bacteria, the examined Asian cultivated rice (JaC, InC) and African cultivated rice (AfC1, AfC2) species were clustered together while the indica and japonica species were grouped together (Fig. 4a). African wild rice (Af-W) can cluster together with the F1 offsprings derived from its crossbreeding with African cultivated rice; similar to nivara and common wild rice, the bacterial community compositions of nivara and common wild rice were more similar to their respective F1 offsprings, (Fig. 4a) than Asian cultivated rice. For fungi, the examined Asian (InC) and African (AfC1, AfC2) cultivated rice species were clustered together, and the two species of African cultivated rice were grouped together (Fig. 4b). NW2, NW-H, CW1, CW2 and CW-H can be grouped into cluster II, which showed similar community structures in African and Asian wild rice (NW2, CW1, CW2) and their F1 offsprings (NW-H, CW-H) obtained from the crossbreeding with Asian cultivated rice (Fig. 4b). Similar results also showed that the community structure of fungi is more similar in African wild rice (Af-W) with its F1 offsprings (Af-H) than African cultivated rice (AfC1, AfC2) (Fig. 4b). The Euclidean distances for PCA analysis were calculated to test the significance between the different comparisons of the wild and cultivated rice (Fig. 4c, d). Results showed that the differences in Euclidean distances for the bacterial community comparison between African wild rice and F1 offsprings were not significant. Likewise, the differences in Euclidean distances for the bacterial community comparison between nivara wild rice/common wild rice and the F1 offsprings were not significant either. The results showed that the Euclidean distance of the comparison between African wild rice and the F1 offsprings (Af-W vs Af-H) for the fungal community was lower than that between cultivated rice and the F1 offsprings (Af-H vs AfC1 and Af-H vs AfC2) (Fig. 4d). Similar results showed that the Euclidean distance of the fungal community comparison between cultivated rice and the F1 offsprings (JaC vs CW-H and InC vs CW-H) was higher than that between common wild rice and the F1 offsprings (CW1 vs CW-H and CW2 vs CW-H) (Fig. 4d), and the Euclidean distance of the fungal community comparison between cultivated rice and the F1

offsprings (JaC vs NW-H and InC vs NW-H) was higher than the comparisons between common wild rice and the F1 offsprings (NW1 vs NW-H and NW2 vs NW-H) (Fig. 4d).

Network Analysis Based On Bacterial And Fungal Otus

To determine the interaction mode of co-occurring community members and to infer the possible "cooperation" between different microbial groups, a Kamada-Kawai layout based on the relative OTU abundance of the samples for network analysis was performed (Fagnan et al. 2012). According to the relative abundance distribution of OTUs or each taxon in different samples, we can identify the microbial groups that are co-occurring with each other, and then construct the co-occurrence network of dominant microbial groups to explore their ecological significance based on the Kamada-Kawai layout based on the OTUs. The network analysis showed that Asian and African cultivated rice (Af-H, AfC2, JaC and InC) clustered and had more significant correlations than wild rice in both bacteria and fungi (Fig. 5a, b). However, African wild rice (Af-W) and common wild rice (NW2) had some common significantly correlated bacterial species (Fig. 5b), while African wild rice (Af-W) and common wild rice (NW1, NW2) had some common significantly correlated fungal species (Fig. 5b). Furthermore, there were more significantly correlated bacteria and fungi between both African and Asian wild rice and their F1 offsprings than between cultivated rice and their F1 offsprings (Fig. 5a, b). The core bacterial OTU that connected Asian/African wild rice and the F1 offsprings was OTU11459, which belongs to Acidovorax, while the core bacterial OTU that linked Asian/African cultivated rice to the F1 offsprings was OTU58789, which belongs to Bradyrhizobium (Fig. 5a). The core fungal OTUs that connected Asian/African wild rice with the F1 offsprings were OTU21966, OTU15713 and OTU6940, which belong to Pleosporales, Myrothecium and Bullera, respectively, while the core fungal OTU that linked Asian/African cultivated rice to the F1 offsprings was OTU19843, which belongs to Dendroclathra (Fig. 5b).

Discussion

The microbiome lives in an environment and can adapt to it (Elbeltagy et al. 2000; Wilkins et al. 2019; Zhang et al. 2019). Microbiomes have strong connections with their host plant, and they can affect the metabolism of their host plant and harm or benefit the host plant (Vandenkoornhuyse et al. 2015; Joshi et al. 2019; Yang et al. 2019). Furthermore, the culturing of plant varieties always causes some adverse stress effects due to the management of the planting (Hamid et al. 2017; Tian et al. 2020a). It is well known that hybridization can help promote the stress resistance in plants to their planting conditions (Nirmala et al. 2016; Yun et al. 2018). Studies have shown that the beneficial physiological traits of the hybridized plants are better than their original parents (Nirmala et al. 2016; Yun et al. 2018). However, no studies have demonstrated the relationship of the plants and their root endophytic microbiomes with hybridization.

Here, this work provided a preliminary proof of vertical transmission and heritability of specific endodermal microorganisms (fungi and bacteria) in plants. However, the propagation of microorganisms

through heritability is different from the clone network because this network is not composed of plant tissues; hence, the parental filter that occurs on microorganisms (i.e., wild type) is also different. Beta diversity has been popularly used for analyses of biological diversity among microbial community compositions along environmental gradients (Maaß et al. 2014; Rivest et al. 2019). Based on the bacterial and fungal community structures, the examined Asian and African cultivated rice species clustered together, and the indica and japonica species were grouped together (Fig. 2a, b); however, African wild rice clustered together with the F1 offsprings following its crossbreeding with African cultivated rice (Fig. 2a, b). Similar to nivara wild rice and common wild rice, the bacterial and fungal community compositions of nivara and common wild rice species were more similar to their respective F1 offsprings than Asian cultivated rice (Fig. 2a, b). Our demonstration of microbiol transmission supports the idea that microbial consortia and their host constitute a combined unit of selection.

Alpha-diversity measurement is particularly challenging for microbial communities (Haegeman et al. 2013; Flores-Rentería et al. 2016). Commonly, microbial diversity has been characterized as the diversity within a given community generally using the total number of OTUs (richness), their relative abundances (Shannon diversity), or indexes that combine these two dimensions (evenness). Studies have generally used microbial alpha diversity to explore the relationships between structure and functioning of microbial communities (Yuste et al. 2011; Flores-Rentería et al. 2016). The results showed that the bacterial and fungal alpha abundances and diversities were not stabilized in the endophytic microbiomes of both the cultivated and wild rice along with their F1 offsprings. Significantly higher Chao1 and Shannon indexes were found for the bacterial community composition of both African and Asian cultivated rice versus their relative wild rice and respective F1 offsprings (Fig. 2b, d), indicating that the bacterial community composition in cultivated rice had more bacterial species and evenness than wild rice. However, there were no stabilized significant differences in the Simpson, Chao1, ACE and Shannon indexes between African cultivated rice and African wild rice and their F1 offsprings (Fig. 2a, b, c, d). The fungal Simpson and Shannon indexes were significantly higher in nivara wild rice and the F1 offsprings than in cultivated rice and the F1 offsprings (Fig. 3a, d), indicating that nivara wild rice and its F1 offsprings retained more fungal diversity and evenness than Asian cultivated rice. Meanwhile, the Chao1 and ACE indexes in common wild rice were higher than in cultivated rice and the F1 offsprings (Fig. 3b, c). These results together indicated that there were more fungal species in common wild rice than in cultivated rice. In support of this finding, Tian et al. (2017) showed that the diversity and abundance indexes of common wild rice were higher than O. sativa for root-associated bacteria (Tian et al. 2017).

Results of PCA showed that African wild rice (Af-W) clustered together with the F1 offsprings after its crossbreeding with African cultivated rice. The bacterial community compositions of the respective F1 offsprings were more similar to that of nivara and common wild rice than to that of the cultivated rice indica and japonica species (Fig. 4a). For fungi, the examined Asian (InC) and African (AfC1, AfC2) cultivated rice species were clustered together, and the two species of African cultivated rice were grouped together (Fig. 4b). The fungal community structures of the nivara wild rice and common wild rice and their F1 offsprings were more similar than that observed between Asian cultivated rice species and their F1 offsprings (Fig. 4b). Likewise, results also showed that community structure of fungi between

African wild rice and its F1 offsprings was more similar than that noted between African cultivated rice and its F1 offsprings (Fig. 4b). Furthermore, Euclidean distance of African wild rice and the F1 offsprings (Af-W vs Af-H) for fungal community comparison was found to be lower than that between cultivated rice and the F1 offsprings (Fig. 4d), indicating that the F1 offsprings were more similar to African wild rice than to African cultivated rice in terms of fungal community composition. Similarly, the Euclidean distances of the fungal community comparison between cultivated rice and F1 offsprings were higher than that observed between common wild rice and F1 offsprings, demonstrating that the F1 offsprings showed higher relationship to common and nivara wild rice species than to cultivated rice indica and japonica species with respect to fungal community composition (Fig. 4d).

Network analysis for microbiomes has been used to explore the co-occurrence patterns among microbial taxa or functions (Ling et al. 2016). For plants, the transmission of a microbe along plant clonal networks extends the concept of physiological integration previously demonstrated for information and resources to microorganisms (Vannier et al. 2019). This integrated network structure and blueprint challenge the concept of a meta-holobiont organization in which plants can act as sinks or sources of microorganisms (Vannier et al. 2019). Such a structure can ensure communication between plants, especially between parent and offspring, to improve the adaptability or fitness of clones as a whole (Vannier et al. 2019). No study has performed yet a network analysis on wild and cultivated rice species along with their F1 offsprings. In this study, the network analysis showed that Asian and African cultivated rice clustered together and had more significant correlations than wild rice both in bacteria and fungi (Fig. 4a, b). However, African wild rice and common wild rice had some common significantly correlated bacterial species (Fig. 4b), while African wild rice and common wild rice had some common significantly correlated fungal species (Fig. 4b). Furthermore, there were more significantly correlated bacteria and fungi between both African and Asian wild rice and their F1 offsprings than between cultivated rice and their F1 offsprings (Fig. 4a, b). The bacterial population associated with the rhizosphere of wild rice species displayed differences with those associated with cultivated rice species, suggesting that the root traits selected in domestication could have significant influence on the rhizosphere microbial composition (Shenton et al. 2016). The network analysis showed significantly different correlated species among cultivated and wild rice along with their F1 offsprings (Fig. 5a, b). The core bacterial species that connected wild rice with its F1 offsprings was Acidovorax, wherea the core bacterial species that linked cultivated rice to its F1 offsprings was Bradyrhizobium (Fig. 5a). Acidovorax was detected as a pathogenic genus for plants in watermelon (Citrullus vulgaris), but these species may function in plant immune systems (Xu et al. 2008; Shavit et al. 2016). On the other hand, Bradyrhizobium can benefit plants in nitrogen utilization under more normal and adverse environmental stress conditions (Sulieman et al. 2015; Ambrosini et al. 2019; Kannan et al. 2019; Sulieman et al. 2019). Pleosporales, Myrothecium and Bullera were found to be the core fungal species that connected wild rice and the F1 offsprings, whereas Dendroclathra spp. were noted to be the core fungal species that linked cultivated rice to their F1 offsprings (Fig. 5b). Bullera spp. have been known to serve as biocontrol agents for benefiting plants in disease resistance and growth, explaining the greater relative abundance of Bullera in wild rice and the F1 offsprings (de Tenório et al. 2019).

Conclusions

In this study, we systematically compared the endophytic microbial community structures of Asian and African wild and cultivated rice along with their F1 offsprings. The results showed that both Asian and African wild rice controls more root endophytic fungi than the cultivated rice after their first crossbreeding. Furthermore, the network analysis showed that wild rice and the F1 offsprings were clustered together, and had significantly greater relative abundance of the core fungal species Pleosporales, Myrothecium and Bullera, while African and Asian cultivated rice can cluster together with the core fungal Dendroclathra species. This study may provide the theoretical significance of endophytic bacteria and fungi in wild and cultivated rice along with their F1 offsprings, which could be applied to isolating and screening useful bacterial and fungal resources for rice cultivation from its wide relatives.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All raw sequencing data or calculated data in this article can be offered if it is needed.

Competing Interests

The authors declare no conflict of interest.

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Authors' Contributions

CT designed and supported the experiment. LT performed the experiments; LT, XL, LJ and JC analyzed the data with the input of L-SPT; LT, XL, L-SPT and CT wrote the manuscript.

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Supplementary Files Legend

Additional files

Additional file 1: Table S1 Summary of high-throughput sequencing data for bacteria and fungi. Af-W, African wild rice; Af-H, F1 generation of the crossbreeding of African wild rice and African cultivated rice; AfC1, African cultivated rice No. 2; AfC2, African cultivated rice No. 4; NW1, nivara wild rice No. 1; NW2, nivara wild rice No. 2; NW-H, F1 generation of the crossbreeding of nivara wild rice and Asian cultivated rice; CW1, common wild rice No. 1; CW2, common wild rice No. 2; CW-H, F1 generation of the crossbreeding of common wild rice and Asian cultivated rice; JaC, Jiangxi japonica; InC, 106 indica. A, B, C and D represent the four replicates of each sample.

Additional file 2: Figure S1 Rarefication curves of bacterial (**a**) and fungal (**b**) composition communities. Af-W, African wild rice; Af-H, F1 generation of the crossbreeding of African wild rice and African cultivated rice; AfC1, African cultivated rice No. 2; AfC2, African cultivated rice No. 4; NW1, nivara wild rice No. 1; NW2, nivara wild rice No. 2; NW-H, F1 generation of the crossbreeding of nivara wild rice and Asian

cultivated rice; CW1, common wild rice No. 1; CW2, common wild rice No. 2; CW-H, F1 generation of the crossbreeding of common wild rice and Asian cultivated rice; JaC, Jiangxi japonica; InC, 106 indica.

Figures

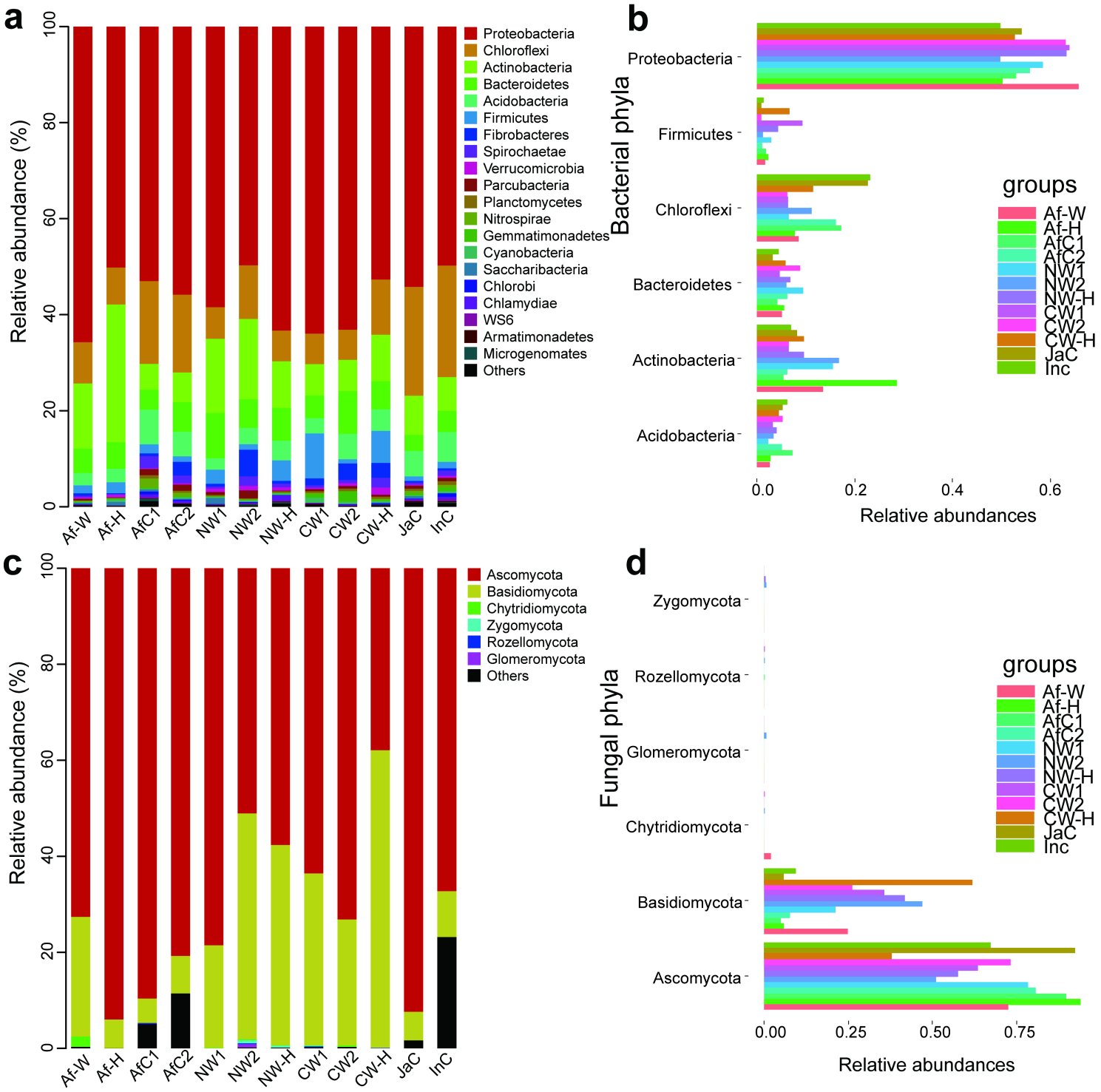

Figure 1

Taxonomies of endophytic bacteria (a, b) and fungi (c, d) at the phylum level. Af-W, African wild rice; Af-H, F1 generation of the crossbreeding of African wild rice and African cultivated rice; AfC1, African cultivated rice No. 2; AfC2, African cultivated rice No. 4; NW1, nivara wild rice No. 1; NW2, nivara wild rice No. 2; NW-H, F1 generation of the crossbreeding of nivara wild rice and Asian cultivated rice; CW1, common wild rice No. 1; CW2, common wild rice No. 2; CW-H, F1 generation of the crossbreeding of common wild rice and Asian cultivated rice; JaC, Jiangxi japonica; InC, 106 indica.

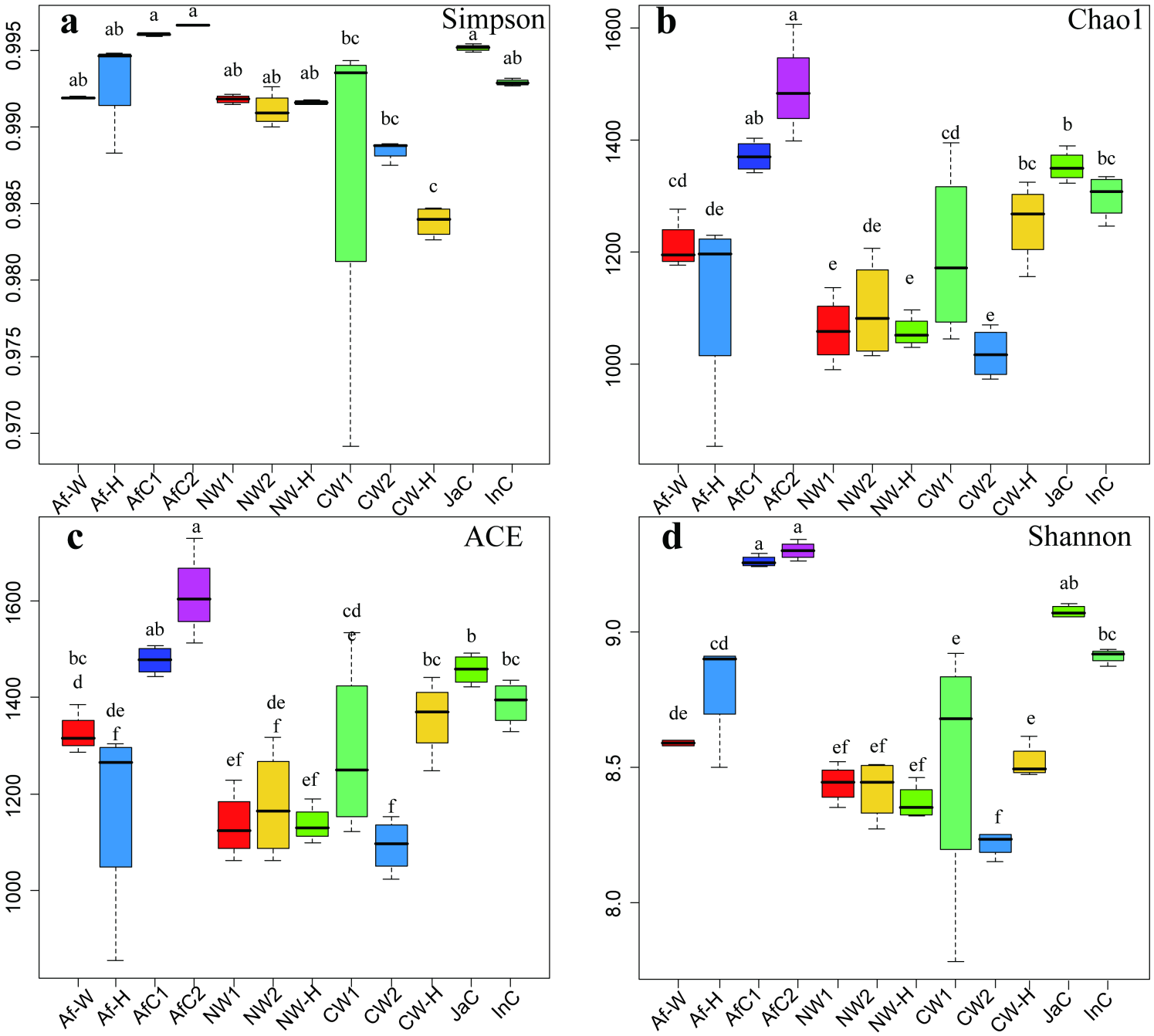

Figure 2

Alpha-diversity distribution using a box plot of the bacterial communities in the endophytic microbiomes of cultivated and wild rice plants along with their F1 offsprings for the Simpson (a), Chao1 (b), ACE (c) and Shannon indexes (d). Each boxplot represents the diversity distribution of a group present within four

replicates. Af-W, African wild rice; Af-H, F1 generation of the crossbreeding of African wild rice and African cultivated rice; AfC1, African cultivated rice No. 2; AfC2, African cultivated rice No. 4; NW1, nivara wild rice No. 1; NW2, nivara wild rice No. 2; NW-H, F1 generation of the crossbreeding of nivara wild rice and Asian cultivated rice; CW1, common wild rice No. 1; CW2, common wild rice No. 2; CW-H, F1 generation of the crossbreeding of common wild rice and Asian cultivated rice; JaC, Jiangxi japonica; InC, 106 indica.

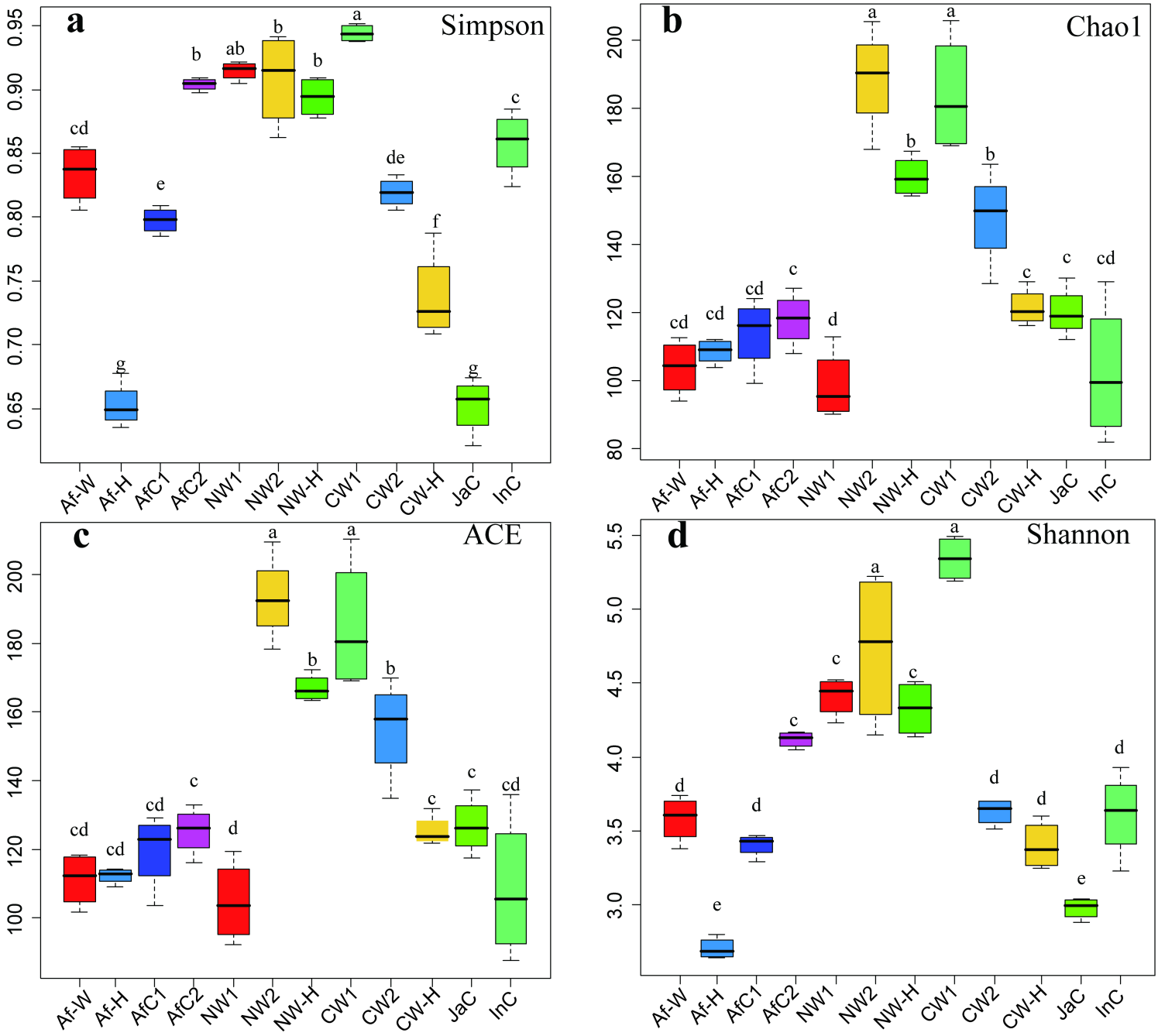

Figure 3

Alpha-diversity distribution using a box plot of the fungal communities in the endophytic microbiomes of cultivated and wild rice plants with their F1 offsprings for the Simpson (a), Chao1 (b), ACE (c) and Shannon indexes (d). Each boxplot represents the diversity distribution of a group present within four replicates. Af-W, African wild rice; Af-H, F1 generation of the crossbreeding of African wild rice and African

cultivated rice; AfC1, African cultivated rice No. 2; AfC2, African cultivated rice No. 4; NW1, nivara wild rice No. 1; NW2, nivara wild rice No. 2; NW-H, F1 generation of the crossbreeding of nivara wild rice and Asian cultivated rice; CW1, common wild rice No. 1; CW2, common wild rice No. 2; CW-H, F1 generation of the crossbreeding of common wild rice and Asian cultivated rice; JaC, Jiangxi japonica; InC, 106 indica.

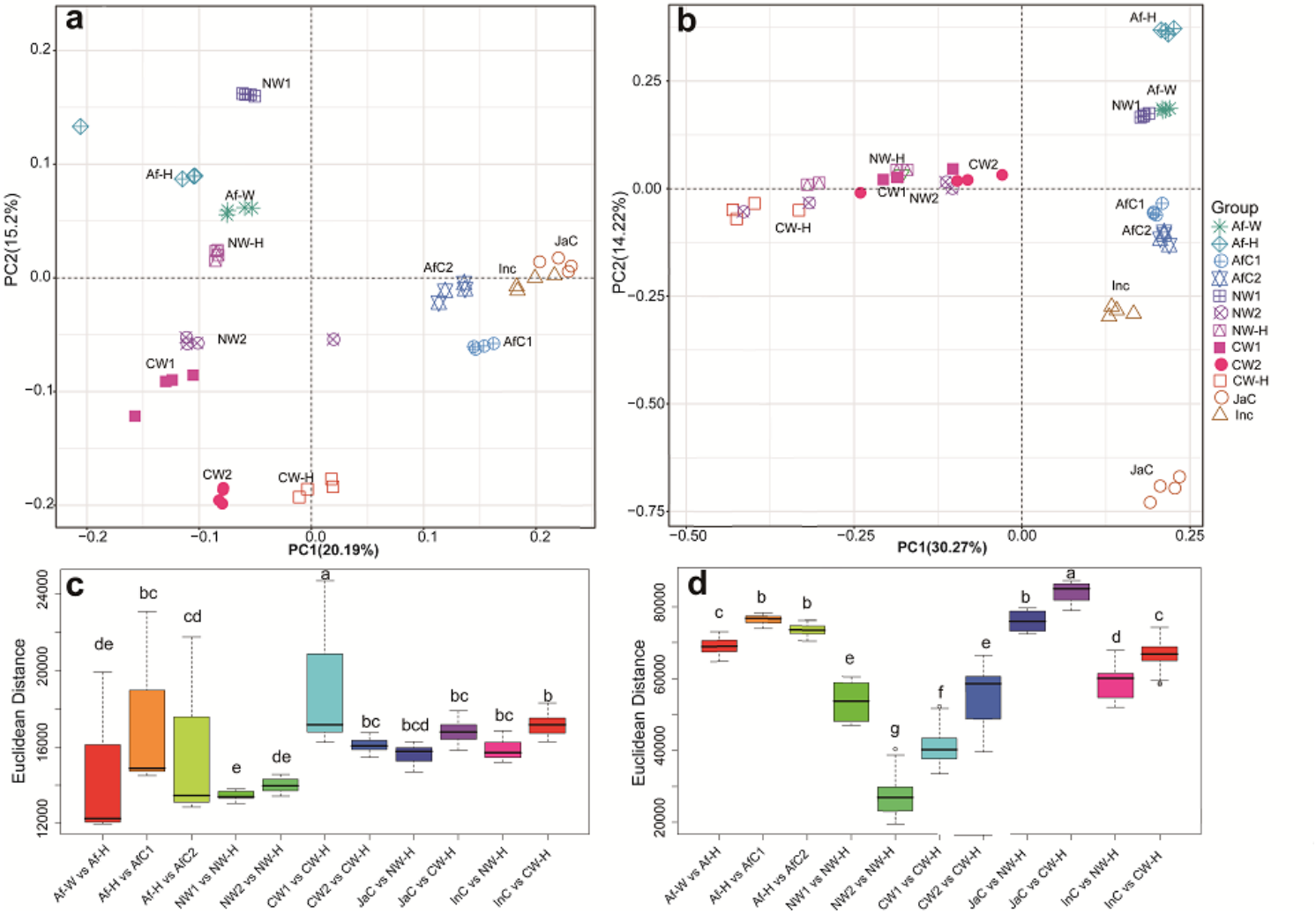

Figure 4

Principal component analysis of the bacterial (a) and fungal (b) communities; and Euclidean distance of the bacterial (c) and fungal (d) based on the comparison of the F1 offsprings with their wild and cultivated parental rice. Each boxplot represents the diversity distribution of a group present within four replicates. Af-W, African wild rice; Af-H, F1 generation of the crossbreeding of African wild rice and African cultivated rice; AfC1, African cultivated rice No. 2; AfC2, African cultivated rice No. 4; NW1, nivara wild rice No. 1; NW2, nivara wild rice No. 2; NW-H, F1 generation of the crossbreeding of nivara wild rice and Asian cultivated rice; CW1, common wild rice No. 1; CW2, common wild rice No. 2; CW-H, F1 generation of the crossbreeding of common wild rice and Asian cultivated rice; JaC, Jiangxi japonica; InC, 106 indica.

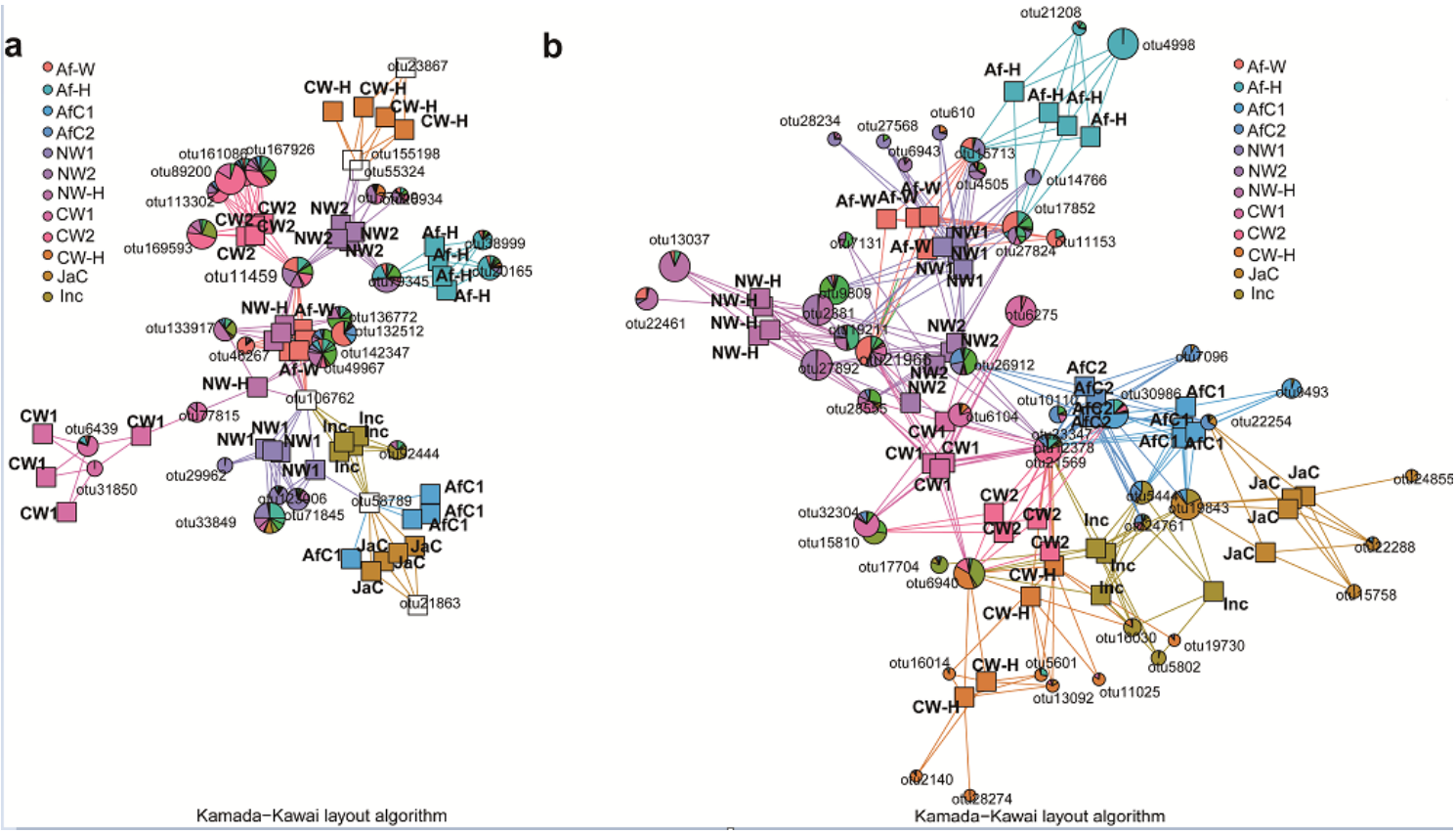

Figure 5

Co-occurrence network of bacterial (a) and fungal (b) communities in the endophytic microbiomes of cultivated and wild rice plants with their F1 offsprings. The dot size corresponds with the operational taxonomic unit (OTU) abundance (log2-transformed). Co-occurrence may be exaggerated among the large number of rare OTUs due to the dominance of zeros for rare OTUs in most samples. Each boxplot represents the diversity distribution of a group present within four replicates. Af-W, African wild rice; Af-H, F1 generation of the crossbreeding of African wild rice and African cultivated rice; AfC1, African cultivated rice No. 2; AfC2, African cultivated rice No. 4; NW1, nivara wild rice No. 1; NW2, nivara wild rice No. 2; NW-H, F1 generation of the crossbreeding of nivara wild rice and Asian cultivated rice; CW1, common wild rice No. 1; CW2, common wild rice No. 2; CW-H, F1 generation of the crossbreeding of common wild rice and Asian cultivated rice; JaC, Jiangxi japonica; InC, 106 indica.

Supplementary Files

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