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Crop rotations alter bacterial and fungal diversity in paddy soils across East Asia

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ABSTRACT

Rice ecosystems are distributed broadly from temperate to tropical regions, but little is known about the underlying mechanisms shaping microbial communities and their network structure in paddy soils at the continental scale. Soil samples were collected from paddy fields across East Asia representing four types of crop rotations: single rice, rice–wheat rotation, double rice, and rice–legume–rice rotation. Here, we describe the roles that crop rotations, environmental heterogeneity and geographical distance play in determining the spatial distribution of microbial communities in paddy soils across East Asia. Our survey revealed remarkable differences in the diversity and composition of microbial operational taxonomic units (OTUs) among four crop rotations. The shared cosmopolitan OTUs Rhizobiales bacterium (genus Bradyrhizobium) and Hypocreales fungus played key-species roles in the ecological networks. A steeper slope of distance–decay for the fungal samples compared with the bacterial samples implies a faster turnover in fungal OTU composition across geographical zones. Bacterial communities were affected by soil environmental heterogeneity to an extent that overwhelmed the effect of geographical distance, whereas fungal communities were better predicted by geographical distance. The diversity and composition of bacterial and fungal communities corresponded strongly to soil pH but less strongly to total nitrogen. Remarkably, crop rotations played a key role in determining the changes in microbial diversity, community composition and networks. Taken together, these results provide a baseline ecological framework with which to pursue future research on soil microbial function in paddy soils.

1. Introduction

The traditional viewpoint of microbial biogeography 'everything is everywhere, but the environment selects' supports the unlimited dispersal capabilities of microorganisms and predicts that microbial community composition is driven solely by strong environmental conditions (Baas-Becking, 1934). However, it has previously been argued that dispersal limitation permits historical contingencies to substantially influence the structure of microbial communities (Rout and Callaway, 2012). Recent national and global surveys indicate that most bacteria, similarly to larger organisms, are restricted to broad habitat types, with little overlap among soil bacterial taxa (Griffiths et al., 2011; Nemergut et al., 2011). Conversely, some eukaryote and prokaryote taxa may in fact be cosmopolitan (Finlay, 2002; Barberán et al., 2014). The underlying processes shaping the ubiquitous or restrictive distribution patterns of microbial communities have not yet been clearly elucidated.

Both the deterministic niche and stochastic neutral processes control microbial community assembly (Caruso et al., 2011). The niche-driven community assembly is controlled by a hierarchical

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framework of dispersal limitation followed by species sorting through environmental and biotic filtering (Leibold and McPeek, 2006), while the stochastic community assembly by random processes of dispersal, birth sand deaths, and genetic drift (Bell, 2001). The relative importance of dispersal limitation versus environmental filtering for microbial community assembly has attracted substantial attention (Hanson et al., 2012). However, most studies examined the relative importance of abiotic and biotic interactions and attributed the remaining differences in composition to dispersal limitation with geographical distance (i.e. distance–decay) (Bell, 2010; Dumbrell et al., 2010). It has been established that historical contingencies such as dispersal limitation and changes in environmental states over time could potentially result in biogeographical endemism in microbial communities, and that the probability of such contingencies increases with spatial distance among communities (Martiny et al., 2006). If dispersal limitation is the prominent driver of biogeographical patterns, geographical distance would better predict genetic divergence between communities and habitats in close proximity that are more likely to share similar microbial taxa.

Active controversy continues regarding the relative contributions of environmental heterogeneity and geographical distance to microbial community dissimilarity. Many researchers have explored a significant relationship between microbial community composition and environmental properties (e.g. pH, salt and carbon) (Fierer and Jackson, 2006; Lozupone and Knight, 2007; Liu et al., 2015), supporting that microbial biogeographical patterns were primarily selected by contemporary environmental conditions (Hanson et al., 2012; Kivlin et al., 2014). However, the decrease in community similarity with increasing geographical distance is a prevalent biogeographical pattern observed in microbial communities (van der Gast et al., 2011). Whether geographical distance is a minor (Hazard et al., 2013), important (Griffiths et al., 2011), or dominant factor (Wu et al., 2013) compared with environmental factors remains unclear. The relative influence of environmental factors versus geographical distance on microbial β-diversity appears to depend largely on physiological traits, habitat type (Hanson et al., 2012), and spatial scale (Martiny et al., 2011).

Besides the diversity and composition of soil microbial communities, the interactions between microbial taxa which connected to microbial functional roles or environmental niches show patterns across spatial or temporal gradients (Barberán et al., 2012). An ecological network represents complex biological interactions in an ecosystem where species are linked by positive and negative interactions (Montoya et al., 2006). Although it is difficult to observe the biotic interactions in microbial communities, recent progress in association networks offers new insights into the structure of complex microbial communities and keystone populations (Barberán et al., 2012).

Rice can grow in diverse climates and soil types along the latitudinal gradient of East Asia. Rice ecosystems are considered a unique type of 'wetland' that is kept flooded during the period of rice cultivation. The flooded paddy rice fields produces anaerobic soil environment and represents a typical freshwater habitat for microorganisms, which influences microbial community composition (Prasanna et al., 2012; Schmidt and Eickhorst, 2013). However, the biogeographical patterns and network structure of microbial communities in paddy soils are poorly characterized across climate, soil and crop rotation gradients at the continental scale. In general, flooding selects for facultative and obligate anaerobic microorganisms (Sylvia et al., 2005). Therefore, we hypothesized that microbial communities exhibit ubiquitous taxa with special functions selected by flooded rice cultivation. The cosmopolitan species shared in different soils across different geoclimate regions could play important roles through functional compensation and complementarity (Resetarits and Chalcraft, 2007; Shade and Handelsman, 2012) and play unique functional roles in network structure of microbial communities (Wang et al., 2015a).

Soil bacterial and fungal communities drive the nutrient cycling and response to the long-term managements in paddy fields (Yuan et al., 2013; Zhao et al., 2014), while the archaea such as ammonia-oxidizing archaea functionally dominate nitrification only in the alkaline soils (pH > 8) (Wang et al., 2015b). It is crucial to understand the bacterial and fungal diversity and their functions in paddy soils under different managements. In this study, we examined the biogeographical patterns of microbial communities in paddy soils across East Asia. We selected multiple sites with paddy soils representing a range of soil types and crop rotations across latitudes with distinct climates with the following goals: (i) to identify the cosmopolitan OTUs in bacterial and fungal communities and their topological roles in ecological networks, (ii) to compare the distance–decay relationship of fungal community changing with distance to that of bacterial community, and (iii) to address the relative influences of soil properties, climatic factors, geographical distance, and crop rotations on bacterial and fungal communities in rice ecosystems. Here, we demonstrated that crop rotations play an important role in determining the diversity and composition of microbial community in paddy soils across a large spatial scale.

2. Materials and methods

2.1. Soil sample collection and soil chemical properties analysis

A total of 43 soil samples were collected from paddy fields along a north-south transect across East Asia (15.90°N to 47.47°N) (Koch et al., 1995), which represented four types of crop rotations (single rice, rice–wheat rotation, double rice, rice–legume–rice rotation), two soil groups and a wide range of climatic conditions (MAT ranged from 2 to 27.5 °C, MAP ranged from 500 to 2345 mm) (Fig. 1, Table 1, Table S1). In the temperate region with single rice and rice–wheat rotations, the rice planting continued for 30–50 years, and the soil was classified as Typical Hapli-Stagnic Anthrosols (CRGCST, 2001). The former land use was upland with crop rotation of wheat and/or maize. In the subtropical and tropical region, the rice planting continued for more than 50 years (even for several hundred years), and the soil was classified as Typical Fe-accumuli-Stagnic Anthrosols (CRGCST, 2001). The transect sampling of crop rotations along the latitude was combined with the representative sampling of soil types. This cluster sampling pattern along the environmental gradient could reveal the combined environmental and biotic filtering on soil microbial community at regional scale (Fierer et al., 2013; Jing et al., 2015). The soil sampling sites with a rice planting area more than 5 ha had consistent tillage and irrigation practices, and similar soil texture and terrain without crop patchiness. Soil samples were collected in October 2010 from paddy fields after the rice harvest for single rice and rice–wheat rotations, and after the late rice harvest for double rice and rice–legume–rice rotations. During the sampling period, the water layer was drained but soil water content was near field capacity. The global positioning system (GPS) coordinates recorded at each sampling site were imported into the NOAA website to calculate the pairwise geographical distance (http://www.nhc.noaa.gov/gccalc.shtml). At each sampling site, we established a transect in a 10 m × 100 m rectangular plot. Three sub-plots were randomly placed at least 40 m apart along the transect, and each sub-plot was in a circle with a 5 m diameter. Within each sub-plot, 20 soil cores (5-cm diameter) of the upper 15 cm soils were collected randomly and composited into a single bulk sample. All soil samples were chilled on ice immediately following collection in the field and transported in a

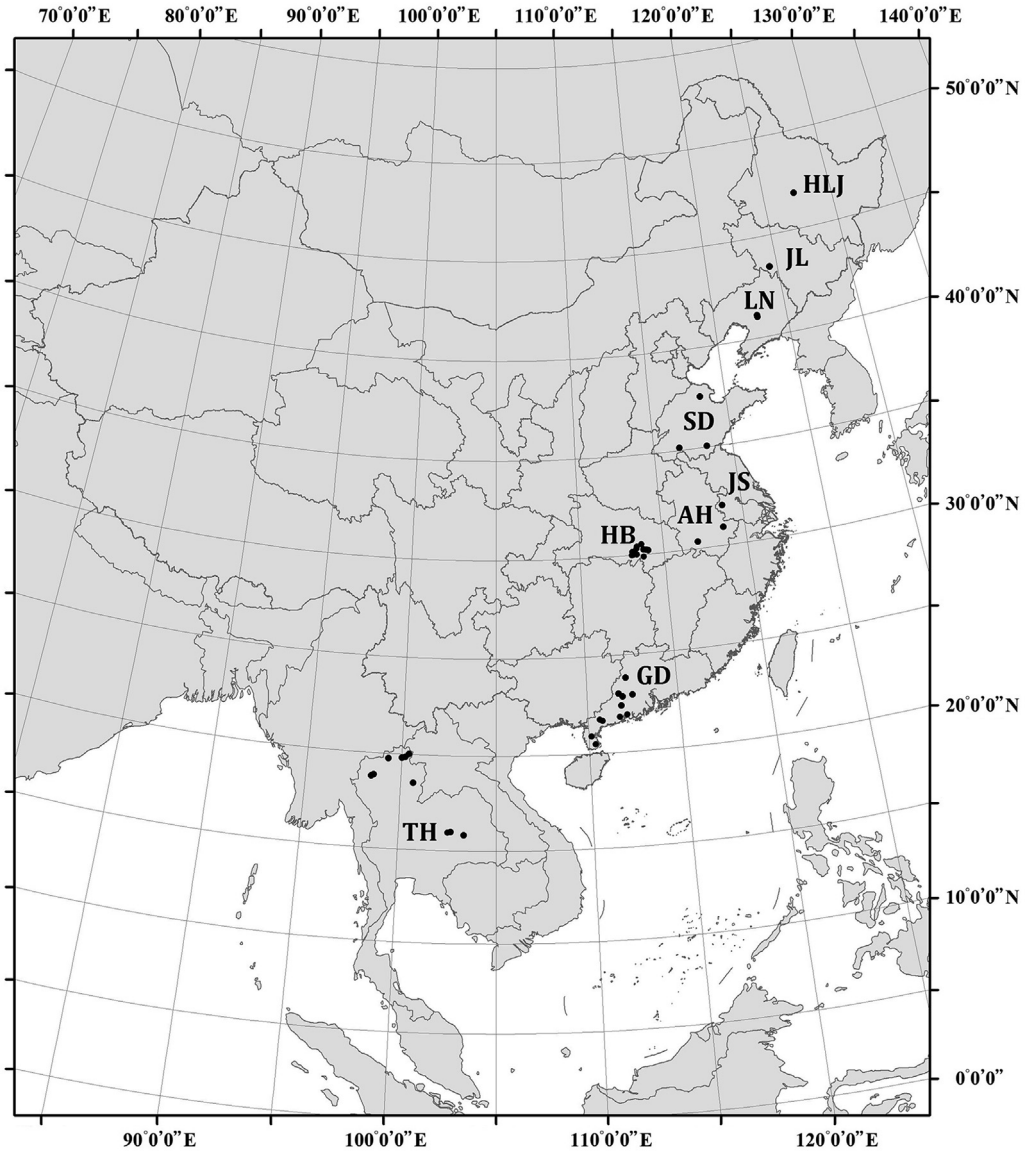

Fig. 1. Location of paddy soil sampling sites in East Asia representing four types of crop rotations: single rice (HLJ, JL and LN), rice–wheat (SD, JS and AH), double rice (HB and GD), and rice–legume–rice (TH). The provinces of China: HLJ, Heilongjiang; JL, Jilin; LN, Liaoning; SD, Shandong; JS, Jiangsu; AH, Anhui; HB, Hubei, GD, Guangdong. TH, Thailand.

cooler to the laboratory, where they were sieved to 4 mm to remove visible roots and residues, homogenized and subdivided into two subsamples. One subsample was air-dried to determine its edaphic properties, and the other was stored at −80 °C prior to DNA extraction.

Following the procedure described by Gregorich and Carter (2008), soil pH was measured using a glass electrode at a soil: water ratio of 1:2.5. Soil organic carbon content (SOC) was determined using wet digestion by the potassium dichromate method. The soil total N (TN) was measured by the micro-Kjeldahl method using digestion in H_2SO_4 followed by steam distillation. Soil total P (TP) and K (TK) were digested with HF–$HClO_4$, and plant available P (AP) and K (AK) were extracted with sodium bicarbonate and ammonium acetate, respectively, and then determined using the molybdenum-blue method with an atomic absorption spectrophotometer. Redox potential (Eh) was measured by using a combined platinum-calomel electrode connected to a pH/millivolt meter (S220-K, Mettler-Toledo, Switzerland) after 10 d waterlogging incubation (Jiang et al., 2012). Soil samples were treated with H_2O_2 and $(NaPO_3)_6$ to remove organic matter and disperse aggregates (Konert and Vandenberghe, 1997), and then the soil particle-size distribution was measured using a laser diffraction particle size analyzer (LS230, Beckman Coulter, Inc, USA).

2.2. Bar-coded pyrosequencing of bacterial and fungal communities

Total DNA was extracted from 0.5 g of soil after sampling using a Fast DNA Spin Kit for soil (MP Biomedicals, Santa Ana, CA) according to the manufacturer's instructions. The extracted soil DNA was dissolved in 100 μL of TE buffer and quantified using a spectrophotometer and stored at −20 °C until use. The 16S rRNA genes of bacteria were amplified from genomic DNA using primers 27F and 533R (Weisburg et al., 1991), and the 18S rRNA genes of fungi were amplified using primers 3NDfand V4_euk_R2 (Bråte et al., 2010). An 8-bp error-correcting tag was added to the forward primer. Samples were amplified following the thermal cycling protocol described previously (Fierer et al., 2008). Triplicate PCR reactions for each sample were pooled and purified using a

Table 1
Locations, crop rotations, soil type and characteristics of paddy soil samples.

Soil no[a]	Latitude	Longitude	Crop rotations	Soil type[b]	Soil characteristics[c]
HLJ-1	47.47°	127.99°	Single rice	Typical Hapli-Stagnic Anthrosols	Neutral pH; median Eh, SOC, TP, TK, AP, AK; low TN
JL-1	43.31°	124.36°	Single rice	Typical Hapli-Stagnic Anthrosols	Neutral pH; median Eh, SOC, TP, TK, AP, AK; low TN
LN-1	41.81°	123.43°	Single rice	Typical Hapli-Stagnic Anthrosols	Neutral pH; median Eh, SOC, TP, TK, AP, AK; low TN
LN-4	41.53°	123.35°	Single rice	Typical Hapli-Stagnic Anthrosols	Neutral pH; median SOC, TP, TK, AP, AK; low Eh, TN
LN-2	41.52°	123.32°	Single rice	Typical Hapli-Stagnic Anthrosols	Neutral pH; median SOC, TP, TK, AK; low Eh, TN, AP
LN-3	41.49°	123.29°	Single rice	Typical Hapli-Stagnic Anthrosols	Neutral pH; high AK; median Eh, SOC, TN, TP, TK, AP
SD-2	37.53°	118.30°	Rice–wheat	Typical Hapli-Stagnic Anthrosols	Neutral pH; median Eh, TP, TK, AP, AK; low SOC, TN
SD-3	35.11°	118.61°	Rice–wheat	Typical Hapli-Stagnic Anthrosols	Weak acid pH; median Eh, SOC, TP, TK, AP, AK; low TN
SD-4	35.02°	116.66°	Rice–wheat	Typical Hapli-Stagnic Anthrosols	Neutral pH; high SOC, TN, TP, AK; median Eh, TK, AP
JS-1	32.11°	118.88°	Rice–wheat	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; median Eh, SOC, TK, AK; low TN, TP, AP
AH-1	30.24°	117.00°	Rice–wheat	Typical Hapli-Stagnic Anthrosols	Neutral pH; median TP, AP, AK; low Eh, SOC, TN, TK
AH-2	30.82°	118.66°	Rice–wheat	Typical Hapli-Stagnic Anthrosols	Acid pH; median Eh; low SOC, TN, TP, TK, AP, AK
HB-10	30.73°	113.75°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; high Eh, AK; median SOC, TN, TP, TK; low AP
HB-9	30.47°	113.44°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; high TK, AK; median Eh, SOC, TN, TP; low AP
HB-8	30.36°	113.37°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; high SOC, TN; median Eh, TK, AK; TP; low AP
HB-7	30.31°	113.54°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Weak acid pH; high SOC, TN; median Eh, TK, AK; TP; low AP
HB-6	30.28°	113.73°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; high SOC, TN; median Eh, TK, AK; TP; low AP
HB-5	30.26°	114.00°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; high SOC, TN; median Eh, TK, AK; TP; low AP
HB-4	30.20°	113.26°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; high SOC, TN, AK; median TP, TK; low Eh, AP
HB-3	30.07°	113.44°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; high SOC, TN; median Eh, TP, TK; low AP, AK
HB-2	29.99°	113.23°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; median Eh, SOC, TN, TP, TK, AK; low AP
HB-1	29.88°	113.56°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Neutral pH; high SOC, TN; median Eh, SOC, TP, TK, AK; low AP
GD-32	24.01°	111.97°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high SOC, TN; median Eh, SOC, TP, TK, AP, AK
GD-35	23.17°	111.80°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high SOC, TP, AP; median Eh, TN, TK, AP, AK
GD-38	23.05°	112.69°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh, TK, AP, AK; high SOC, TN, TP
GD-29	22.90°	112.14°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh, SOC, TN, TP, TK; low AP, AK
GD-46	22.40°	111.90°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high TP; median Eh, SOC, TN, TK, AP, AK
GD-40	21.89°	111.89°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high Eh; median TK; low SOC, TN, TP, AP, AK
GD-42	21.83°	112.15°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh, SOC, TN, TK; low TP, AP, AK
GD-76	21.76°	110.69°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh, SOC, TN, TP, TK, AK; low AP
GD-75	21.74°	110.86°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high SOC, TP, AP; median Eh, TN, TK, AK
GD-63	20.89°	110.12°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh, SOC, TN, TP, TK, AP, AK
GD-57	20.47°	110.41°	Double rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high Eh, AP; median TP; low SOC, TN, TK, AK
TG-14	20.01°	100.43°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh; low SOC, TN, TP, TK, AP, AK
TG-16	19.90°	100.26°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high Eh; median TK; low SOC, TN, TP, AP, AK
TG-23	19.88°	100.06°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high Eh; median TK; low SOC, TN, TP, AP, AK
TG-27	19.87°	99.72°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high Eh, TK; low SOC, TN, TP, AP, AK
TG-35	18.80°	98.66°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh, TK, AK; low SOC, TN, TP, AP
TG-30	18.79°	98.12°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high Eh; median TK; low SOC, TN, TP, AP, AK
TG-47	18.68°	100.80°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high Eh; low SOC, TN, TP, TK, AP, AK
TG-41	15.90°	103.73°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; high Eh; median AK; low SOC, TN, TP, TK, AP
TG-4	15.98°	102.86°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh, AK; low SOC, TN, TP, TK, AP
TG-1	15.97°	102.62°	Rice–legume–rice	Typical Fe-accumuli-Stagnic Anthrosols	Acid pH; median Eh, TP, AK; low SOC, TN, TK, AP

[a] HLJ, Heilongjiang; JL, Jilin; LN, Liaoning; SD, Shandong; JS, Jiangsu; AH, Anhui; HB, Hubei, GD, Guangdong; TH, Thailand.
[b] Soil type was classified according to CRGCST (2001).
[c] Soil characteristics was detailed in the HYPERLINKTable S1. The soil characteristics were separated into different groups based on the following standards: (1) pH: acidic (<6.0), weak acidic (6.0–6.5), neutral (6.5–7.5); (2) soil redox potential (Eh): high (−38 to −113 mV), median (−113 to −188 mV), low (−212 to −188 mV); (3) soil organic carbon (SOC): high (36.1–49.0 g kg^{-1}), median (23.3–36.1 g kg^{-1}), low (10.4–23.3 g kg^{-1}); (4) total nitrogen (TN): high (2.2–3.0 g kg^{-1}), median (1.5–2.2 g kg^{-1}), low (0.7–1.5 g kg^{-1}); (5) total phosphorus (TP): high (0.94–1.33 g kg^{-1}), median (0.54–0.94 g kg^{-1}), low (0.15–0.54 g kg^{-1}); (6) total potassium (TK): high (24.0–34.0 g kg^{-1}), median (13.9–24.0 g kg^{-1}), low (3.9–13.9 g kg^{-1}); (7) available phosphorus (AP): high (47.1–69.9 mg kg^{-1}), median (24.2–47.1 mg kg^{-1}), low (1.3–24.2 mg kg^{-1}); (8) available potassium (AK): high (125.2–172.5 mg kg^{-1}), median (78.1–125.2 mg kg^{-1}), low (30.8–78.1 mg kg^{-1}).

QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA, USA). A single composite sample for pyrosequencing was prepared by combining approximately equimolar amounts of the PCR products from each sample. Amplicon pyrosequencing of the PCR products was conducted on a 454 Life Sciences GS FLX (Roche Diagnostics, Indianapolis, IN, USA). After sequencing was completed, all sequence reads were quality checked using the Mothur software (Schloss et al., 2009). Raw sequence reads were filtered before subsequent analyses to minimize the effects of random sequencing errors. Briefly, we eliminated low-quality sequences using the following criteria: (i) did not perfectly match the proximal PCR primer, (ii) were less than 200 bp, (iii) contained one or more ambiguous base(s), or (iv) were flagged as chimeric artifacts. The remainder of the sequences was trimmed and classified using a Bayesian approach with the Silva database (Pruesse et al., 2007) as a template and a cutoff of 80%. To correct for survey effort, we used randomly selected subsets of 4500 and 2000 sequences from bacterial and fungal samples, respectively, to compare relative differences between samples. The number of sequences per sample enabled us to hold the effects of the survey effort at the same level when comparing the diversity indices and Bray–Curtis dissimilarities. The bacterial and fungal operational taxonomic units (OTUs) were defined by using Mothur. Sequence similarity thresholds of 97%, 95%, 92% and 89% were applied for assignment at species, genus, family and order level, respectively (Nemergut et al., 2011; Thomson et al., 2015). The 454 pyrosequencing results have been deposited in the DNA Data Bank of Japan (DDJB) under accession numbers DRA001139 and DRA001141 for 16S rRNA and 18S rRNA genes, respectively.

2.3. Statistical analysis

We used randomly selected subsets of same sequences from bacterial and fungal samples to calculate the Shannon diversity

indices (α-diversity) and Bray–Curtis dissimilarities between samples (β-diversity). To evaluate the differences among crop rotations with unequal number of samples, one-way analyses of variance (ANOVA) followed by Bonferroni's test (Winer et al., 1991) was performed by using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The phylogenetic molecular ecological networks (pMENs) between bacteria and fungi were constructed using the random matrix theory (RMT)-based network approach (Luo et al., 2007). The pMEN construction and analyses were performed using a pipeline written in Java and Perl scripts (Zhou et al., 2011). We standardized the distribution matrix of bacteria and fungi into relative abundances for subsequent Pearson correlation analysis. The networks between bacteria and fungi were constructed across all sampling sites and within crop rotations. The OTUs detected in more than half of the sampling sites were kept for the network construction. The missing values were filled through the nearest neighbors method (Troyanskaya et al., 2001), which calculated the mean of the remained values for the missing positions. Various indexes, including the average geodesic distance (*GD*), average clustering coefficient (*avgCC*) and modularity of the pMEN were used as values to test the differences in crop rotations. Average connectivity (*avgK*) was calculated to measure the complexity of a network structure. The module is a group of OTUs which are well connected among themselves but are less linked with OTUs belonging to other modules (Olesen et al., 2007). The role of each OTU was determined based on its position compared with other OTUs in its own module and how well it connected to nodes in other modules. Therefore, the role of OTU *i* in the network was characterized by its within-module connectivity (Z_i) and among-module connectivity (P_i) (Guimera and Amaral, 2005). According to the simplified criteria, all species were sorted into four subcategories: peripherals, connectors, module hubs, and network hubs (Olesen et al., 2007).

The Bray–Curtis distances were visualized using principal coordinates analysis (PCoA) to determine the influence of crop rotations on the beta diversity of bacterial and fungal community (Anderson and Willis, 2003). Variation partitioning analysis (VPA) was performed to quantify the relative influence of crop rotations, environmental variables, climatic variables, and spatial variables on the composition of bacterial and fungal communities based on partial canonical correspondence analysis (pCCA) (Oksanen et al., 2013). To minimize the autocorrelation between spatial distance and environmental variables, spatial variables were derived from the analysis of principal coordinates of neighbor matrices (PCNM; Borcard and Legendre, 2002). The nonmetric multidimensional scaling (NMDS) ordination was used to characterize the overall variations in bacterial and fungal community composition with soil variables. The relationships between the diversity of microbial community and geographical distance were measured by linear mixed models used to take spatial autocorrelation into account (Pinheiro et al., 2012). All of these procedures were implemented using the R Version 2.15.1 (http://www.r-project.org) software with the packages vegan and nlme. Partial Mantel tests enabled us to determine the correlations between the distances separating microbial communities and soil variables, climatic variables or spatial patterns in PASSAGE (Rosenberg and Anderson, 2011).

3. Results

3.1. Soil geochemical characteristics

The major geographical and physicochemical characteristics of paddy soils were listed in Table 1 and Table S1. Soil characteristics varied considerably with sampling sites, following a gradually changing trend. Soil pH increased from the south to the north of China, ranging from 4.72 to 7.40. Soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), and available phosphorus (AP) were lower in rice–legume–rice rotation than those in other crop rotations, while redox potential (Eh) followed an opposition trend.

3.2. Taxon distribution and core OTU occurrences across four crop rotations

The pyrosequencing methods used in our study allowed the presence of soil microorganisms to be compared among geographically distant zones across East Asia (Fig. 1, Table 1). Bacterial communities were dominated by the Chloroflexi (15.8%), Actinobacteria (15.1%), Betaproteobacteria (12.1%), Acidobacteria (9.6%), Deltaproteobacteria (8.2%), Firmicutes (7.9%), and Alphaproteobacteria (7.7%), and fungal communities were dominated by the Ascomycota (77.9%) (Fig. S1). When using 97% similarity as the definition of an OTU, there was an unexpected result that only 2 (2592 sequences) of 37,337 bacterial OTUs, identical to the order Rhizobiales in Alphaproteobacteria, were common to all sampling sites. The majority (62.2%) of bacterial OTUs, representing 30,703 sequences, were present at only a single location. Similar to the bacterial OTUs, only 1 (4163 sequences) of the 2916 fungal OTUs, affiliated with the order Hypocreales in Ascomycota, was common to all sites, whereas more than half (54.8%) of the fungal OTUs, representing 4637 sequences, were restricted to one location. Furthermore, there were more shared bacterial and fungal OTUs identified at lower levels of phylogenetic resolution. At genus, family and order levels, 3, 19 and 38 shared bacterial OTUs were identified, while 3, 4 and 6 shared fungal OUT were identified, respectively (Table S3).

3.3. Visualization of topological roles of individual nodes

The topological properties commonly used in network analysis were calculated to describe the complex patterns of interrelationships between bacteria and fungi at the applied analysis threshold (Table 2). The values of average clustering coefficient (*avgCC*), average geodesic distance (*GD*) and modularity of empirical networks were significantly higher than the values for their corresponding random graph with identical sizes, suggesting the observed networks possessed typical small-world and modular characteristics (Table 2). Furthermore, we determined the topological role of each OTU in microbial networks consisting of all soil samples via RMT-based network analysis (Fig. 2). Three of the ten bacterial module hubs were affiliated with Alphaproteobacteria and Actinobacteria, respectively, while the others belonged to different taxa, including Chloroflexi, Firmicutes, Acidobacteria, and Nitrospirae. Three fungal module hubs were exclusively affiliated with the specific Ascomycota phylum. Particularly, one of the shared bacteria (order Rhizobiales, genus Bradyrhizobium) was categorized as a connecter, whereas the shared fungus (order Hypocreales) was categorized as a module hub (Figs. 2 and 3).

3.4. Relationship of microbial communities to soil chemical properties, climatic factors and geographical distance

Soil microbial diversity was estimated by the Shannon index to calculate α diversity. Soil bacterial and fungal α diversity were significantly related to Eh ($r^2 = 0.28$, $P = 0.015$ and $r^2 = 0.15$, $P = 0.028$), rather than soil texture (sand content, $P > 0.05$; clay content, $P > 0.05$; slit content, $P > 0.05$). Soil pH was the best predictor of bacterial ($r^2 = 0.42$, $P < 0.001$) and fungal ($r^2 = 0.27$, $P = 0.016$) α diversity, with the lowest levels of α diversity observed in acid paddy soils (Fig. 4). Furthermore, the bacterial community composition responded significantly to soil pH ($r^2 = 0.29$, $P < 0.001$)

Table 2
Topological properties of the empirical molecular ecological networks (MENs) between bacterial and fungal communities and their associated random MENs under different cropping rotations.

Crop rotations	Empirical networks[a]							Random networks		
	s_t	Network size	Link	*avgK*	GD	*avgCC*	Modularity	GD ± SD	*avgCC* ± SD	Modularity ± SD
Whole	0.74	457	1183	5.18	8.53	0.41	0.82	3.48 ± 0.03	0.068 ± 0.003	0.58 ± 0.01
Single rice	0.89	468	1219	5.21	5.34	0.43	0.78	2.48 ± 0.07	0.047 ± 0.005	0.43 ± 0.01
Rice–wheat	0.89	535	1585	5.92	5.21	0.47	0.82	2.97 ± 0.03	0.054 ± 0.004	0.57 ± 0.01
Double rice	0.90	561	2057	7.33	5.33	0.45	0.77	2.81 ± 0.04	0.046 ± 0.004	0.59 ± 0.01
Rice–legume–rice	0.89	622	2379	7.65	7.37	0.41	0.81	3.24 ± 0.06	0.060 ± 0.005	0.64 ± 0.01

[a] s_t, similarity threshold; network size, the number of OTUs (e.g. nodes) in a network; *avgK*, average connectivity; GD, average geodesic distance, *avgCC*, average clustering coefficient.

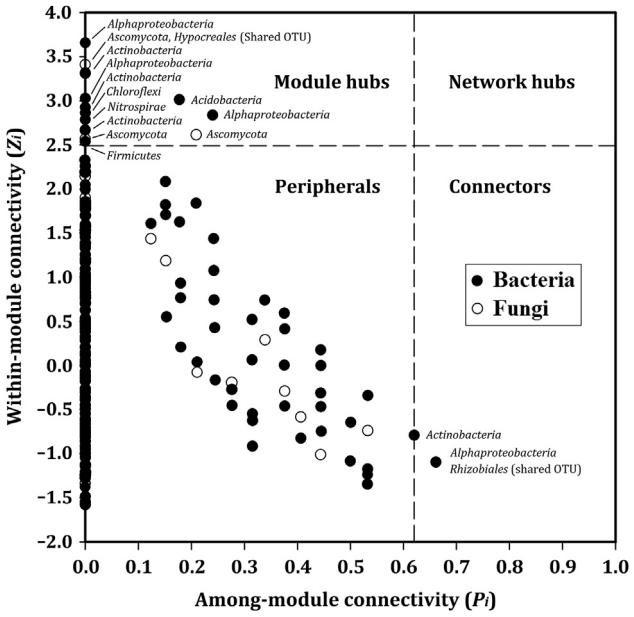

Fig. 2. Z_i–P_i plot showing the distribution of OTUs based on their topological roles in networks between bacteria and fungi. Each symbol represented an OTU in the bacterial (filled circle) or fungal (empty circle) network. The threshold values of Z_i and P_i for categorizing OTUs were 2.5 and 0.62, respectively.

and TN ($r^2 = 0.02$, $P < 0.001$) across all sampling sites when controlling for all other geochemical variables using a partial Mantel test. Analogously, the fungal community composition exhibited a stronger correlation with pH ($r^2 = 0.05$, $P < 0.001$) than with TN ($r^2 = 0.03$, $P < 0.001$), although the relationship was far weaker than that for bacterial communities. Among all of the soil and site characteristics examined, the primary axes (NMDS1) indicated a stronger correlation with soil pH than with TN (Fig. S2).

A plot of community similarity using the Bray–Curtis index versus log-transformed distance for each pair of samples revealed that bacterial communities displayed a significant, negative (slope = −0.055, $P < 0.001$) distance–decay curve across the scales from 1 km up to approximately 4500 km (Fig. 5). The distance–decay slope of fungal communities was significantly (slope = −0.081, $P < 0.001$) steeper than the bacterial slope. When the four crop rotations were analyzed separately, the relationship between the slopes of bacterial and fungal communities indicated relatively similar trends (Fig. S3). Over all of the spatial zones, both soil chemical properties and geographical distance appeared to influence bacterial and fungal β diversity. The partial Mantel test determined that the similarity of bacterial and fungal community composition between samples was highly correlated with soil variable distance and geographical distance but not with climatic variable distance (Table S2). The variation partitioning analysis (VPA) indicated that soil variables explained 27.3% and 21.4% of the variations in bacterial and fungal community compositions, respectively, and that geographical distance explained 9.8% and 22.7%, respectively (Table 3).

3.5. Changes in bacterial and fungal communities with crop rotations

Crop rotations significantly influenced not only bacterial and fungal diversity (Fig. S4, $P < 0.05$) but also the abundances of specific bacterial (Betaproteobacteria and Gammaproteobacteria) and fungal (Hypocreales) taxa (Fig. 6, $P < 0.05$). The soil bacterial diversity under single rice was significantly higher than that under double rice and rice–legume–rice rotations, while the soil fungal diversity under single and double rice were intermediate between rice–wheat and rice–legume–rice rotations (Fig. S5, $P < 0.05$). Additionally, crop rotations strongly affected microbial community compositions and ecological networks across East Asia (Fig. S6, Table 2). The value of average connectivity (*avgK*) showed a decrease order of rice–legume–rice > double rice > rice–wheat > single rice. The VPA indicated that crop rotations explained the largest proportion of the variance in bacterial (34.1%) and fungal (28.3%) community structures (Table 3).

4. Discussion

The pyrosequencing methods allowed the presence of soil microorganisms to be compared among geographically distant zones. For the bacterial community, Chloroflexi, Actinobacteria, Acidobacteria, and Betaproteobacteria were the predominant groups, and these phyla are commonly found in paddy soils, as reported in previous studies (Hussain et al., 2011). For the fungal community, Ascomycota were found to be predominant across all samples in our study, suggesting the ubiquity of this fungal group and an important role in rice ecosystems (Carlile et al., 2001). The existence and extent of cosmopolitanism among bacteria and fungi is frequently discussed and has important implications for our understanding of microbial evolution and diversity. In the current study, ubiquitous taxa were extremely rare in bacterial and fungal communities. Bacteria (Bell et al., 2005) and fungi (Taylor et al., 2006) previously believed to be cosmopolitan have commonly been redefined as assemblages of species with limited biogeographical distributions, and only a few free-living fungi are presumed to be truly cosmopolitan (Pringle et al., 2005).

The shared cosmopolitan OTUs Rhizobiales bacteria and Hypocreales (Ascomycota: Sordariomycetes) fungi have been observed to play important roles in denitrification in paddy soil and other soil ecosystems (Risgaard-Petersen et al., 2006; Yoshida et al., 2009). Floodwaters buffer soil temperature and allow ample growth of N_2-fixing microorganisms (Roger, 1996). The bacteria of the genus

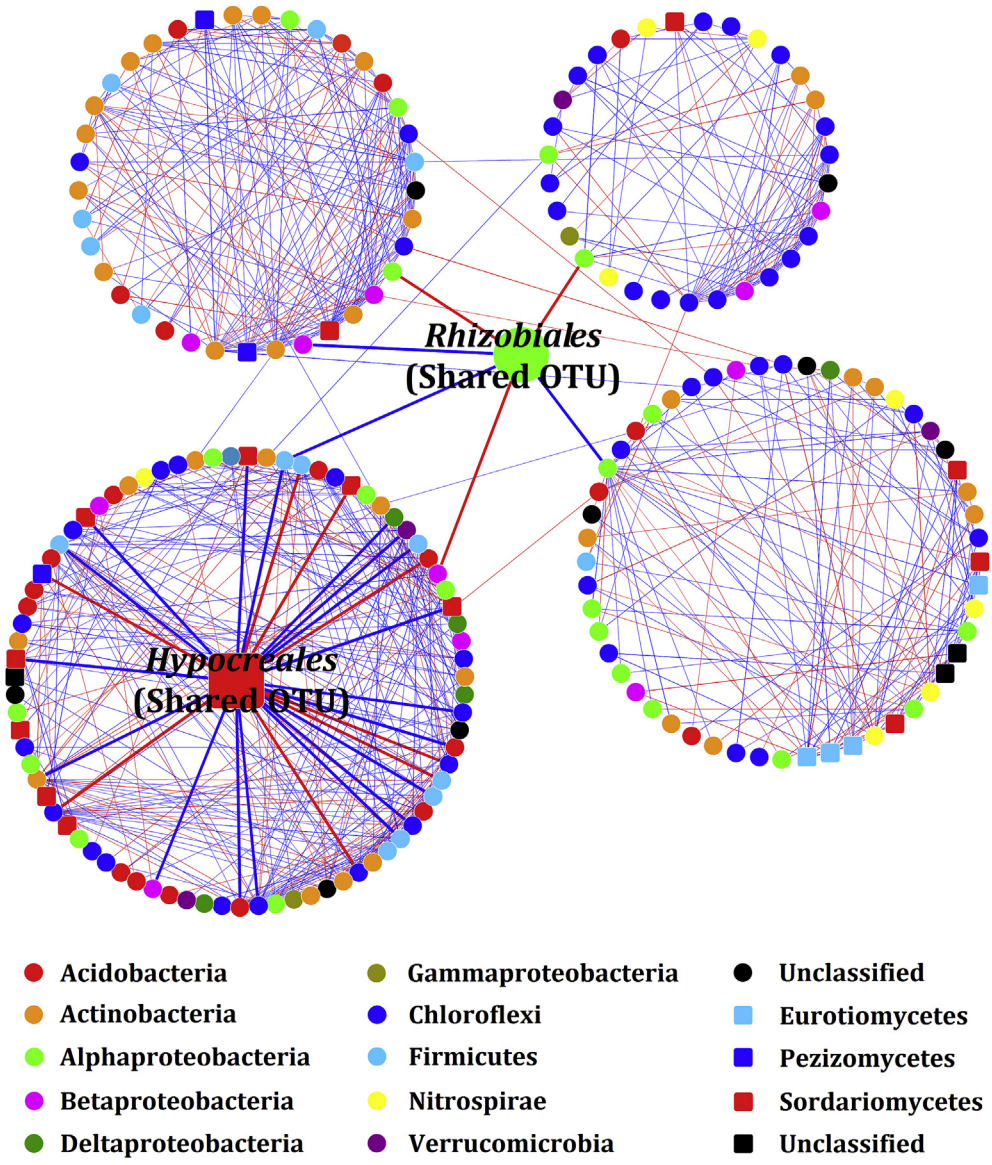

Fig. 3. The shared bacterial and fungal OTUs categorized as the connector and module hub in ecological network between bacteria (circle) and fungi (square), respectively. A blue edge indicated a positive interaction between two individual nodes, while a red edge indicated a negative interaction. The edges between the shared OTUs and other nodes were in bold.

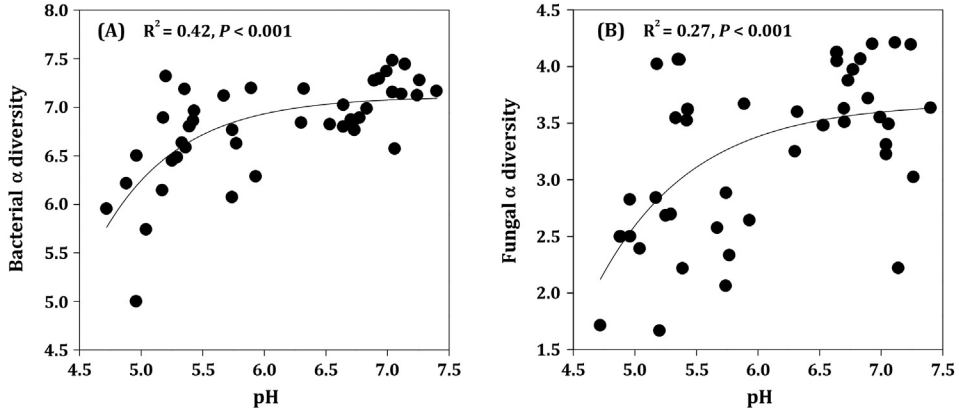

Fig. 4. Relationships between bacterial (A) and fungal (B) α diversity (Shannon index) and soil pH in paddy soils across East Asia.

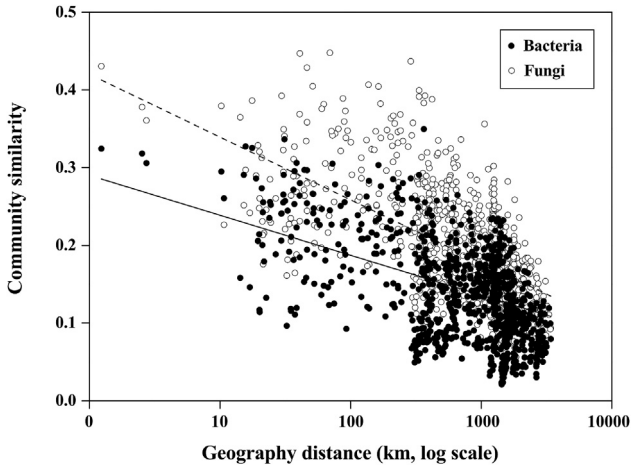

Fig. 5. Distance–decay curves for the bacterial (solid line) and fungal (dashed line) communities. The slopes of the two dashed lines were significantly less than zero. The slope of the distance–decay relationship between fungal samples was significantly ($P < 0.001$) steeper than that between bacterial samples.

We described the topological roles of each OTU and identified the keystone populations based on network topology. The shared bacteria Bradyrhizobium (order Rhizobiales) and shared fungus Hypocreales were categorized as a connector and a module hub in ecological networks, respectively (Figs. 2 and 3). The module hubs and connectors might be analogous to microbial key species to the communities as predicted from network theory (Montoya et al., 2006). Organisms associated with the phylogenetically related module hubs in the subnetworks indicated complex community interactions. For instance, the crenarchaeotal OTUs closely related to *Candidatus Nitrososphaera gargensis* (*Nitrososphaera* clusters 1.1) (Pester et al., 2012) were described as ubiquitous members in soils (Bates et al., 2010) and classified as generalists in microbial networks (Barberán et al., 2012; Jiang et al., 2015). It has been proposed that related *Crenarchaeota* may have an important role in the nitrogen cycle, such as ammonia oxidation and methane oxidation (Holmes et al., 1995; Leininger et al., 2006; Jiang et al., 2014). Keystone species are generally crucial to the entire network, and their removal from a particular ecosystem may cause catastrophic changes in it (Dunne et al., 2002). However, the effects of the loss of soil organisms depend on their specific positions in the network

Table 3
Variation partitioning analysis of the variations in bacterial and fungal community. Bold values indicated the significant effects.

Affecting factors	Bacterial community		Fungal community	
	Variance (%)	*P*	Variance (%)	*P*
Crop rotations	**34.1**	**<0.001**	**28.3**	**<0.001**
Chemical variables	**27.3**		**21.4**	
pH	**11.2**	**<0.001**	**7.9**	**<0.001**
Soil organic C	2.3	0.114	2.2	0.117
Redox potential	**2.7**	**0.036**	1.8	0.206
Total N	**5.2**	**0.008**	**4.4**	**0.032**
C/N ratio	0.8	0.418	0.9	0.543
Total P	1.2	0.364	0.8	0.586
Total K	1.5	0.251	1.0	0.459
Available P	0.8	0.416	0.7	0.602
Available K	0.6	0.462	1.1	0.403
Soil texture	1.0	0.394	0.6	0.667
Climatic variables	**3.7**		**4.0**	
Mean annual temperature	2.2	0.136	2.1	0.136
Mean annual precipitation	1.5	0.242	1.9	0.174
Geographical distance (PCNMs[a])	**9.8**	**<0.001**	**22.7**	**<0.001**
Residuals	25.1		23.6	

[a] Eigenvectors from the principal coordinate analysis of the neighbor matrix (PCNM) based on great circle distance between samples.

Bradyrhizobium, related to the shared order Rhizobiales, can act as active N_2-fixing bacteria in rice systems (Chaintreuil et al., 2000). The key role of the shared bacterial and fungal communities in nitrogen cycling was coincident with the strong denitrifying activity of paddy soils (Nishimura et al., 2004).

The advent of high-throughput technique that aimed to characterize complete microbial ecosystems provided an unprecedented opportunity to delineate the network interactions in the microbial community (Raes and Bork, 2008). Generally, the networks were clearly nonrandom and exhibited higher average clustering coefficient, average geodesic distance, and modularity than the random network (Table 2). The *avgCC* ratio of empirical: random network ranged from 6.03 to 9.78, suggesting that the networks roughly matched the "scale-free, small-world" degree distribution typical of biological systems (Barabási and Oltvai, 2004). The microbial network spanned multiple environments (Barberán et al., 2012) found similar *avgCC* to our studies of microbial communities in paddy soils at continental scales (Table 2). The small world pattern of a few highly connected nodes made the networks more robust to random disruption (Albert et al., 2000).

and the critical nature of their associations with other organisms (Eiler et al., 2012). We still lacked a comprehensive understanding of how species loss propagated through complex biotic community and if this could be examined by analyzing the dynamic nature of ecological networks. Therefore, further researches need to be conducted to provide direct evidence for network analysis on complex biotic communities in a cooperative environment, and deepen our understanding of the mechanisms producing patterns of community coexistence.

Widely distributed taxa were more likely to be the most common organisms present in multiple locations (Brown, 1984). We observed that abundant microorganisms distributed widely across soil assemblages. In our dataset, the abundance of bacterial and fungal OTUs was positively correlated with the number of sites at which they were present across all paddy soil sampling sites ($r^2 = 0.27$, $P < 0.001$ and $r^2 = 0.17$, $P < 0.001$, respectively). This positive relationship has been reported within samples across continental soils (Nemergut et al., 2011) and within sewage treatment facilities, estuaries, lake water, and microbiome samples (Sloan et al., 2006). The large population sizes of the abundant

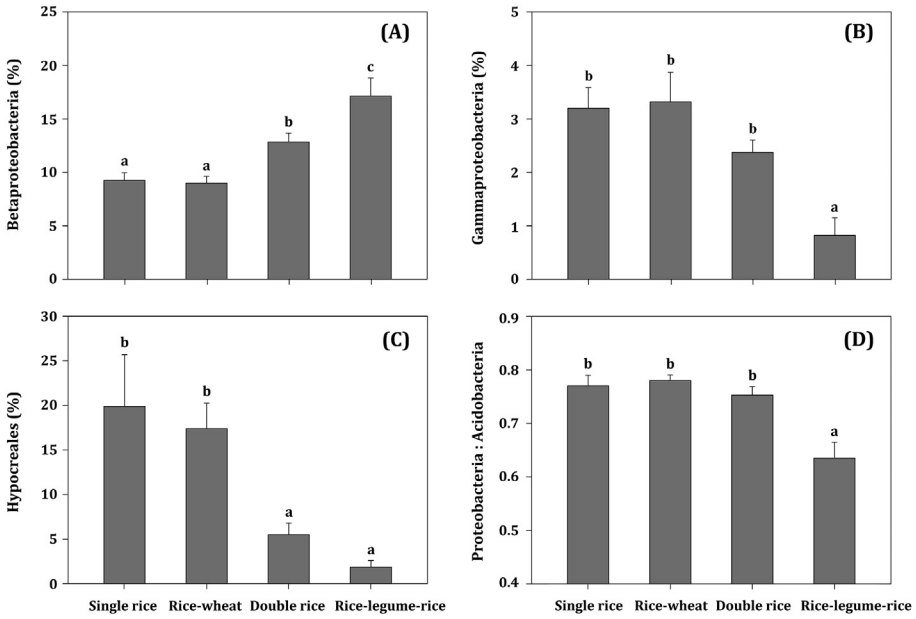

Fig. 6. Abundance of bacterial and fungal taxonomic groups in paddy soils. The abundance of Betaproteobacteria (A) was greater in rice–legume–rice rotation than in other crop rotations, in contrast to Gammaproteobacteria (B) and Hypocreales (C). Crop rotations altered the normalized ratio of all Proteobacteria to Acidobacteria (D). The letters above the bars indicated significant differences determined by Bonferroni's test ($P < 0.05$).

organisms might facilitate their dispersal and promote the positive correlation between abundance and distribution. Curtis et al. (2002) have pointed out that very rare soil organisms might be present at densities of one cell per 27 km^2, suggesting the potential restriction of their distribution. Nevertheless, most bacterial and fungal OTUs had a narrow geographical range and existed in only a single location, implying that endemism is prevalent in bacterial and fungal communities of paddy soils. Endemism is a widely recognized phenomenon in microbial ecology and has been observed between assemblages in terrestrial ecosystems (Taylor et al., 2006; Nemergut et al., 2011). The drastically different dispersal patterns of microbial species, even those of similar size, shaped their distribution range from endemic to regional to cosmopolitan (Litchman, 2010). The obvious observed endemism could be facilitated by the broad dissimilarities of soil chemical properties and climate conditions that serve as clear barriers to dispersal in many locations, suggesting that geographical location and environmental conditions exhibit a strong selection pressure on species composition.

We collected soil samples from paddy fields representing two soil groups and four crop rotations along the north–south transect across East Asia. The characteristics of paddy soils considerably varied with sampling sites within the each crop rotation (Table 1 and Table S1), which can exert significant impact on soil microbial community structure. The pivotal role of soil pH controlling soil microbial diversity and community structure has been recently confirmed in freshwater wetland (Hartman et al., 2008) and terrestrial (Fierer and Jackson, 2006) ecosystems, suggesting that this pattern may be generally applicable across habitats. In this study, the diversity and composition of bacterial and fungal communities could be largely predicted by soil pH (Figs. S2 and S3). However, the effect of pH was stronger on the bacterial community (11.2%, $P < 0.001$) than on the fungal community (7.9%, $P < 0.001$) (Table 3), which could be explained in part by the more narrow pH optima for bacterial groups (3–4 pH units) than fungal groups (5–9 pH units) (Rosso et al., 1995; Nevarez et al., 2009). The pH optima for natural bacterial communities in different soils are closely constrained to their *in situ* pH (Bååth, 1996), and the deviation of 1.5 pH units from *in situ* pH reduces consistently their activity by 50% and consequently affects their composition (Fernández-Calviño and Bååth, 2010). Although different taxa of soil fungi showed preference for certain pH values in pure culture (Domsch et al., 1980), the fungal groups had a significantly weaker direct connection with pH than bacterial groups (Beales, 2004). Our study also showed an evident influence of soil pH on the abundances of both the dominant bacterial phyla (e.g., Acidobacteria, Deltaproteobacteria and Firmicutes) and rarer phyla (e.g., Bacteroidetes, Gammaproteobacteria and Gemmatimonadetes) (Fig. S6). Analogously, soil pH broadly affected the abundance of major fungal phylum (Ascomycetes), even when fungal communities were examined at more detailed levels of taxonomic resolution (Eurotiomycetes and Sordariomycetes) (Fig. S6). The remarkable turnover in the abundance of predominant taxa appeared to cause the differences in biodiversity across the environment gradients (Fig. S1).

We observed that soil TN also significantly influenced the composition of bacterial and fungal communities, explaining 5.2% ($P = 0.008$) and 4.4% ($P = 0.032$) of the variance, respectively (Table 3). Crop rotations had a strongly interaction with TN ($P < 0.001$), suggesting that crop rotations were important in terms of how TN affected bacterial and fungal communities. Soil Eh significantly affected bacterial community composition (2.7%, $P = 0.036$) rather than fungal community composition (1.8%, $P = 0.206$) (Table 3). The magnitude and frequency of redox fluctuations in soils may be an important selective force on bacterial community composition and activity (Pett-Ridge and Firestone, 2005). Bacterial populations can be periodically activated and inactivated under cyclic fluctuating conditions (Pett-Ridge et al., 2006). However, soil indigenous bacteria were highly adapted to fluctuating redox regimens and generally possess physiological tolerance mechanisms to endure a range of redox conditions (DeAngelis et al., 2010). Under this scenario, the weak but significance effect of Eh on bacterial community composition was partly explained by the shifts in special groups that can withstand redox

periods under water logged and unsaturated conditions. Given that a large portion of the variation in the compositions of both the bacterial (25.1%) and fungal (23.6%) communities remains unexplained (Table 3), the consideration of other soil factors such as salinity would likely enhance the ability to predict shifts in bacterial and fungal communities (Lozupone and Knight, 2007).

The changes in microbial taxa over space and time were fundamental to understand their ecology and evolution. The distance–decay relationship described how the similarity between two communities varied with geographical distance (Green and Bohannan, 2006). We found that the similarity declined significantly with increasing geographical distance in both bacterial and fungal communities of paddy soils (Table S2). Furthermore, the slope of the distance–decay relationship between fungal samples was significantly ($P < 0.001$) steeper than that of the bacterial samples (Fig. 4, Fig. S4), implying a faster turnover in fungal OTU composition across a landscape and hence a steeper distance–decay relationship. Soil variables overwhelmed the effect of geographical factors for bacterial communities, whereas geographical factors predicted fungal communities better than soil variables (Table 3), reflecting differences between the two groups of microorganisms in the relative importance of the dispersal mechanisms for determining distribution. The findings suggest that different underlying processes contribute to shaping the spatial distribution patterns of different types of microorganisms (van der Gast et al., 2011).

Our results demonstrated the significant responses of bacterial and fungal communities to crop rotations in paddy soils. Crop rotations contributed the most to changes in bacterial and fungal community structures, respectively (Fig. S6 and Table 3). Intensive irrigated rice cropping could substantially dampen microbial abundance and functional diversity (Reichardt et al., 1997). The highest value of *avgK* in rice–legume–rice rotation indicated the most complex network (West, 2001). However, we observed a higher abundance of Betaproteobacteria in rice–legume–rice rotation, in contrast to Gammaproteobacteria and Hypocreales (Fig. 5), and the shifts in their abundance along cropping intensity gradients suggest an important response to crop rotations. Betaproteobacteria and Gammaproteobacteria are capable of denitrification (Morgan-Sagastume et al., 2008), ammonium oxidation (Martens-Habbena et al., 2009), and N_2O reduction (Ishii et al., 2011), implying a critical role for these groups in nutrient cycling in paddy soils. In our study, the genus Herbaspirillum bacteria closely related to the order Burkholderiales were dominated in Betaproteobacteria. Herbaspirillum strains were previously shown to be involved in nitrate reduction of rice paddy soil (Ishii et al., 2009). Herbaspirillum strains obtained by functional single-cell isolation carried *nosZ* and reduced exogenous N_2O to N_2, suggesting that they are also important players in N_2O reduction (Ishii et al., 2011). Furthermore, some Herbaspirillum species (for example, *Herbaspirillum seropedicae* in our study) could colonize rice roots and stems and fix atmospheric N_2 (Baldani et al., 1986; Elbertagy et al., 2001). The changes in the abundance of bacterial phyla might be readily indexed, as we found that crop rotations significantly altered the ratio of Proteobacteria to Acidobacteria (Fig. 5, $P < 0.001$). The ratio was significantly correlated with SOC ($r = 0.351$, $P = 0.021$), TN ($r = 0.327$, $P = 0.032$), and TP ($r = 0.340$, $P = 0.026$), respectively. The ratio has been suggested as an evaluating parameter of trophic status across a range of terrestrial soils (Smit et al., 2001), with values of 0.16 in an oligotrophic soil (Dunbar et al., 1999), 0.34 in a low-input agricultural soil (Borneman et al., 1996), and 0.87 in a high-input agricultural soil (McCaig et al., 1999).

5. Conclusions

In conclusion, ubiquitous taxa were extremely rare in bacterial and fungal communities. The shared cosmopolitan OTUs Rhizobiales (bacterium) and Hypocreales (fungus) were likely to act as the connector and module hub in microbial networks and play keystone-species roles. Crop rotations strongly altered the diversity and composition of bacterial and fungal communities. Soil variables exhibited a stronger effect on bacterial communities than geographical distance, whereas geographical factors played a more important role in fungal community structure. We provided reliable baseline ecological knowledge with which to pursue further research on soil microbial function in paddy soils.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.01.007.

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