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Limited effects of depth (0-80 cm) on communities of archaea, bacteria and fungi in paddy soil profiles

Running title: microbial communities in paddy soil profiles

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Abstract

Most current microbial studies in paddy soils have focused on the top (0-20 cm) layer where rice roots are concentrated. To better understand the vertical distribution of microorganisms in paddy soils, we investigated the abundances, diversities and community compositions of archaea, bacteria and fungi in six geographically and climatically distinct paddy soil profiles from 0-80 cm depth. Although microbial abundances and OTU diversities largely decreased with soil depth, only the community composition of archaea (not bacteria or fungi) was associated with soil depth, echoing that only one archaeal OTU, but no bacterial or fungal OTUs, differed significantly between depth intervals in relative abundance. Mean annual temperature, precipitation and soil iron and manganese concentrations were significantly correlated with the ordinations of microbial communities for all three domains. Besides these common environmental factors, bacterial and archaeal community structures were also influenced by soil chloride and sulphate concentrations, while the concentrations of organic matter and total nitrogen were important explanatory factors for the variation in fungal community composition. Further analyses on putative bacterial functions showed significant differences between sampling sites rather than depth intervals, and suggested that bacterial OTUs that significantly varied in relative abundance across

sampling sites might be functionally related to OM decomposition, sulphur oxidation and reduction, as well as nitrate reduction. Altogether, in the studied paddy soil profiles, the community composition and putative functions of bacteria were largely the same between different vertical layers each with a thickness of 20 cm. This study suggests that the community compositions of archaea, bacteria, and fungi are mainly driven by different soil chemical properties rather than soil depth, which could be linked to the ecological traits of the three microbial domains.

Keywords: archaea; bacteria; fungi; depth profile; paddy soil

Highlights

- Communities of archaea, bacteria and fungi were investigated in six paddy soil profiles
- Soil depth was only correlated with archaeal community structure
- Environmental factors influenced community composition differently for different domains
- Bacterial OTUs contributed to variation in community composition among sites were functionally linked to C, N and S cycling

Introduction

Paddy ecosystems compose the third largest cropland area and the largest anthropogenic wetland on Earth (Leff *et al.*, 2004; Witt & Haefele, 2005; Kögel-Knabner *et al.*, 2010). To understand the biogeochemistry of paddy soils, much attention has been paid to microbial communities in surface layer (0-20 cm) of paddy soils where rice roots are concentrated (Witt & Haefele, 2005; Kögel-Knabner *et al.*, 2010). The properties of subsurface paddy soil are, however, different from those in the ploughed surface layer, due to the leaching and enrichment effects brought about by the unique management practices in paddy fields (such as repeated ploughing, puddling, flooding and drainage) (Witt & Haefele, 2005; Kögel-Knabner *et al.*, 2010; Watanabe *et al.*, 2010). Microbial community composition in subsurface paddy soils may, therefore, be distinct from that in the surface horizon (Watanabe *et al.*, 2010). However, only a few studies have been dedicated to microbial communities in deeper paddy soil layers (> 20 cm in depth), despite their potentially important role in biogeochemical processes (e.g. the cycling of carbon) (Watanabe *et al.*, 2010). In an early study using PCR-DGGE (denaturing gradient gel electrophoresis), Watanabe *et al.* (2010) observed depth-dependent changes in the population and composition of soil bacterial and archaeal communities, which probably resulted from the distribution of rice roots and the groundwater level. A more recent survey of microbial phospholipid fatty acids

(PLFAs) in soil profiles from three paddy fields (Li *et al.*, 2017) reported that total PLFAs and their diversity decreased with soil depth. High throughput sequencing technologies enables the distributions of microbial phylotypes across soil depths to be deciphered at a higher taxonomic resolution. For example, using pyrosequencing, Lee *et al.* (2015) examined the distributions of archaea and bacteria, including various methanogens and methanotrophs, with soil depth in a rice paddy, which could be linked with soil properties such as the concentrations of oxygen, methane, and organic carbon.

Because most of previous studies employed a limited number of surveyed soil samples, environmental parameters or microbial groups, there is still a lack of knowledge about microbial ecology in paddy soil profiles. A survey concerning all archaea, bacteria and fungi will provide a more integrated view of the influence of environmental factors on microbial communities, because different microbial types may respond to environmental factors differently, as previously observed in surface upland and paddy soils (Siles & Margesin, 2016; Ma *et al.*, 2017; Yuan *et al.*, 2018; Yuan *et al.*, 2019). The latest databases (e.g., FAPROTAX (Louca *et al.*, 2016)) can help to establish linkages between microbial phylotypes and their putative functions, which may facilitate interpretation of the differentiation of microbial community composition along environmental gradients. Some soil properties may have substantial influence on

microbial community composition but have not yet been examined in paddy soil profiles. For example, we observed a strong influence of sulphate and chloride concentrations on bacterial community composition in surface paddy soils (Yuan *et al.*, 2019). Whether this phenomenon presents in paddy soil profiles is worthy of investigation. In addition, soil contains abundant iron oxides (with a median concentration of 30 g kg^{-1}) (Loeppert & Inskeep, 1996); and the dynamic redox reactions of iron oxides, which occur intensively in paddy soils, are reportedly linked with key soil biogeochemical processes such as organic matter mineralization, nitrogen and sulphur transformation, as well as the sorption of dissolved chemical species (Li *et al.*, 2012). How microbial communities change with soil iron concentration, however, remains unknown. Exploring a wider range of paddy soil profiles from different regions may help us better explain the biogeographic patterns of microbial communities.

In this study, we report the abundances, diversities and community compositions of archaea, bacteria and fungi in six geographically and climatically distinct paddy soil profiles. This study is a part of a research project aimed at understanding microbial diversity in Chinese paddy soils, and the data for the surface (0-20 cm) soils of these six soil profiles are from our previous publications, which focused on the microbiome in surface paddy soils (Yuan *et al.*, 2018; Yuan *et al.*, 2019). We explored the

relationships of microbial distributions with soil depth as well as a wide array of environmental parameters. The objectives of this study were to examine (i) how microbial community structures change with depth in paddy soil profiles; (ii) how these changes are linked with the putative functional and physiological traits of microbial phylotypes; and (iii) how these changes differ between archaea, bacteria and fungi. To the best of our knowledge, this is the first study considering all three microbial domains in paddy soil profiles.

Materials and methods

Soil sampling and characterization

Soil profile samples were collected from six major rice cultivation areas in China with a wide range of mean annual temperature (MAT) (8-18 °C) and precipitation (MAP) (650-1500 mm) (Table S1). Samples were collected during June in the south and August in the north of China. At each of the six sites, five bulk soil cores away from rice plants were collected using an auger in an area of approximately 50 m^2. Each of the five cores were cut into depth increments of 0-20, 20-40, 40-60 and 60-80 cm (Li *et al.*, 2017), and samples of each depth section were mixed. Therefore, there were 6 soil profiles and 24 samples in total. Soil samples were sent to the laboratory on ice. A portion of the soil was frozen at -80 °C immediately upon arrival for DNA extraction. Another portion

was stored at 4 °C for the determination of multiple soil physicochemical properties (for properties, see Figure 1), as described previously (Yuan *et al.*, 2019).

DNA extraction and quantitative real-time polymerase chain reaction (qPCR)

Soil DNA was extracted from 0.5 g of soil using the Fast DNA® SPIN Kit for soil (Q BIOgene Inc., Carlsbad, CA, USA) following the manufacturer's instructions, and the DNA quality was evaluated using a NanoDrop ND-2000c UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). 16S rRNA genes of archaea and bacteria as well as 18S rRNA genes of fungi were quantified by qPCR as described previously (Yuan *et al.*, 2018; Yuan *et al.*, 2019). We also conducted qPCR for selected functional genes involved in the nitrogen cycle, including the ammonia monooxygenase gene (*amoA*) of ammonia-oxidizing archaea (AOA), *amoA* of ammonia-oxidizing bacteria (AOB), copper-containing nitrite reductase gene (*nirK*), cd_1 nitrite reductase gene (*nirS*), and nitrous oxide reductase gene (*nosZ*). The qPCR procedures for AOA and AOB *amoA* were detailed by Hu *et al.* (2013), and the procedures for *nirK*, *nirS*, and *nosZ* were described by Zhou *et al.* (2011).

Pyrosequencing and data processing

Details for pyrosequencing and data processing were described previously (Yuan *et al.*, 2018; Yuan *et al.*, 2019). Briefly, the 16S rRNA genes of archaea and bacteria as well

as the 18S rRNA gene of fungi were amplified using the primer sets A364aF/A934bR (Kemnitz *et al.*, 2005), 27F/519R (Lane, 1991) and EF4/Fung5 (Smit *et al.*, 1999), respectively. The PCR products were gel-purified using the Wizard SV Gel and PCR Clean Up Kit (Promega, SanLuis Obispo, CA, USA), combined into an equimolar mix, and sent to Macrogen Inc. (South Korea) for pyrosequencing on a Roche 454 GS FLX Titanium platform (Roche Diagnostics, Branford, CT, USA).

Pyrosequencing data was processed using the Mothur platform (Schloss *et al.*, 2009). After quality control, the sequences were classified using the Bayesian method with a bootstrap time of 1000 and a cutoff of 80%. After removing tags that did not belong to each domain (e.g., non-archaeal tags were removed for archaeal sequences), the remaining sequences were binned into operational taxonomic units (OTUs) at the 97% similarity level. The number of the remaining sequences varied substantially across samples, and, thus, we excluded some samples with a small size (< 100 sequences). Consequently, there were 21 archaeal communities, 23 bacterial communities, and 20 fungal communities remaining for further analyses. To calculate OTU-based α- and β-diversity at the same level of sampling effort, we randomly selected subsets of 212, 420, and 189 sequences per sample for the remaining archaeal, bacterial and fungal communities, respectively, based on a sample with the least number of sequences.

Rarefaction curves showed that the difference in OTU richness between communities could be detected with these sample sizes (Figure S1), and the ranking of the richness of communities with a small amount of sequences has been found to be not significantly different from that with a large amount of sequences (Shaw *et al.*, 2008). Because the Shannon diversity index became stable with these sample sizes (Figure S1), we could also compare the diversity of communities. We further annotated the functions of bacterial OTUs using FAPROTAX (http://www.zoology.ubc.ca/louca/FAPROTAX/), a database for mapping prokaryotic taxa (e.g., genera or species) to metabolic or ecological functions based on the functional descriptions of cultured strains in current literatures (Louca *et al.*, 2016).

Statistical analyses

Pearson's correlations between variables were tested using R and *P* values was adjusted by the Benjamini-Hochberg procedure. Partial least squares (PLS) regression was performed to explore the relationships between environmental factors and microbial variables using XLSTAT 2012 (Addinsoft, France). Only the environmental factors with a variable importance in the projection (VIP) score of > 1 were included in the model (Chong & Jun, 2005). The Bray-Curtis dissimilarity was used to calculate the difference in composition between two communities. The difference in investigated environmental factors between two sites was calculated with the Euclidean distance

after standardizing each environmental factor to have zero-mean and unit-variance. Canonical correspondence analysis (CCA) for community ordination was performed using the "vegan" package in R. The correlations of environmental factors with the CCA ordination were tested with the *envfit* function of "vegan" with 999 permutations, and significant environmental factors were fitted to the CCA ordination. We further performed multiple comparisons to detect OTUs that significantly differed in relative abundance between sampling sites or depth intervals using the STAMP software (Parks *et al.*, 2014). Box plots for significant OTUs were generated using R after Borcard *et al.* (2018). To compare the relative abundances of putative functional bacterial groups between sampling sites or depth intervals, one-way analysis of variance (ANOVA) with a post hoc least significant difference (LSD) test was employed according to Ge *et al.* (2018) using R after Borcard *et al.* (2018).

Results

Soil properties

The profile PJ was from an alkaline-saline paddy field and, therefore, had high soil pH as well as high sulphate and chloride concentrations (Figure 1). The soils of profiles HY and ML were rich in free iron oxides because they were from the red soil region in the south of China (Table S1), where precipitation is high and intensive weathering and

leaching leave the soil with abundant iron oxides and a red color (Baligar *et al.*, 2004). Indeed, there were significant positive correlations of soil Fe(f) concentration with MAT and MAP (Table S2).

Soil pH (5.1-8.8) increased with depth in all but the profile PJ (Figure 1). (When samples from the profile PJ were excluded, soil pH was positively correlated with soil depth in the remaining 20 soil samples (r = 0.641, P < 0.05).) The concentrations of water (182-862 g kg^{-1}), organic matter (OM; 4.0-32.5 g kg^{-1}), total nitrogen (TN; 0.24-1.72 g kg^{-1}), sand (2-385 g kg^{-1}), ammonium-N (7.5-38.6 mg kg^{-1}), and Fe(a) (0.6-7.4 g kg^{-1}) were negatively correlated with soil depth (Figure 1; Table S2). Soil carbon to nitrogen ratio (C/N; 6.4-16.4), clay content (7-528 g kg^{-1}), as well as the concentrations of nitrate-N (0.04-5.26 mg kg^{-1}), sulphate (16.8-197.3 mg kg^{-1}), chloride (5.5-169.0 mg kg^{-1}), Mn(a) (0.04-1.03 g kg^{-1}), Mn(f) (0.08-0.69 g kg^{-1}), and Fe(f) (6.5-41.6 g kg^{-1}), however, changed with depth differently in different soil profiles (Figure 1). Mn(a) and Fe(a) are manganese and iron bound in amorphous iron oxides. Mn(f) and Fe(f) are manganese and iron bound in free iron oxides.

Gene copy numbers

The copy numbers of rRNA genes in one gram of dry soil were $10^{7.6}$-$10^{9.8}$ for archaea, $10^{7.5}$-$10^{10.2}$ for bacteria and $10^{5.4}$-$10^{7.9}$ for fungi (Figure S2). The copy numbers were

negatively correlated with soil depth and pH, but positively correlated with soil water, OM, TN, Fe(a), SO_4^{2-} and sand content (Figure 2a). In addition, one gram of dry soil contained $10^{6.7}$-$10^{8.8}$ copies of AOA *amoA*, $10^{4.2}$-$10^{6.6}$ of AOB *amoA*, $10^{5.2}$-$10^{8.0}$ of *nirK*, $10^{7.1}$-$10^{9.6}$ of *nirS* and $10^{6.8}$-$10^{9.5}$ of *nosZ* (Figure S2). The ratio of AOA *amoA* to AOB *amoA* copy number (AOA/AOB) was between 4 and 2622 with an average of 118. The abundances of AOB *amoA* genes as well as the denitrification-related genes *nirK*, *nirS* and *nosZ* generally decreased with soil depth, while AOA/AOB largely increased with soil depth (Figures 2a, S2).

Richness and diversity of OTUs

Richness and diversity of microbial OTUs within the 40-60 and 60-80 cm depth intervals were lower than those within the 0-20 and 20-40 cm intervals (Figure S3). The negative relationships of richness and diversity with soil depth were stronger for fungal communities than those were for archaeal and bacterial communities (Figures 2b, S3). Archaeal and bacterial richness and diversity were positively correlated with soil C/N ratio as well as SO_4^{2-}, Mn(a) and water concentrations. Fungal richness and diversity were positively correlated with soil OM, TN, water and sand content while negatively correlated with soil pH (Figure 2b).

Dissimilarity of community composition

Canonical correspondence analysis ordinations (Figure 3) and *envfit* analyses (Table 1) demonstrated that soil depth was only significantly correlated with archaeal community structure (Figure 3; Table 1). Mean annual temperature and precipitation were strongly correlated with the community structures for all archaea, bacteria and fungi. The community structures for different microbial domains were also influenced by different soil properties. The concentrations of soil sulphate, Mn(f), Fe(a), water and chloride were significantly correlated with archaeal community ordination (ordered by decreasing r^2); bacterial community structure was most strongly associated with soil chloride and sulphate concentrations, followed by soil pH and the concentrations of Mn(f), nitrate, TN and Fe(f); while fungal community structure was correlated with Mn(f) concentration, Fe(f) concentration, soil pH, OM content and TN content (Table 1).

The Bray-Curtis dissimilarities between community compositions across sampling sites were not significantly different between depth intervals for bacteria; while for archaea and fungi, community dissimilarities across sampling sites were smaller within the 0-20 cm depth interval than deeper horizons (Figure 4a).

To inspect which archaeal, bacterial or fungal OTU contributed to community dissimilarity between depth intervals or sampling sites, we conducted multiple comparisons using the STAMP software. Between depth intervals, only the relative abundance of the archaeal OTU 5 varied significantly. This OTU was classified as the methane-producing genus *Methanosarcina*, and it was more abundant in the surface soil than in subsurface layers (Figure S4). Ten archaeal OTUs and twenty-one bacterial OTUs were detected that significantly differed between sampling sites in relative abundance (Figures S5, S6). Many of these archaeal and bacterial OTUs were more abundant in the profile PJ than in other soil profiles (Figures S5, S6).

Putative functions of bacterial OTUs

We further explored the putative metabolic or ecological functions of bacterial OTUs with the FAPROTAX database. Only 8% of bacterial OTUs could be annotated. The main functional features were associated with trophic types (e.g. photoautotrophy and chemoheterotrophy) and metabolic capacities related to the biogeochemical cycling of carbon (e.g. methylotrophy, hydrocarbon degradation and fermentation), nitrogen (e.g. ureolysis, nitrogen fixation, nitrate respiration and nitrate reduction), sulphur (e.g. sulphate respiration and sulphide oxidation) and iron (e.g. iron respiration) (Figure 5). Eight functional features (aerobic chemoheterotrophy, chemoheterotrophy, chitinolysis, dark hydrogen oxidation, dark sulphide oxidation, fermentation, nitrate reduction and

sulphur respiration) significantly differed in relative abundance between sampling sites, and their relative abundances were higher in the PJ profile than other five profiles (Figure 6). No functional features significantly differed in relative abundance between depth intervals.

Discussion

Variation in soil properties with depth

Soil OM and TN content decreased with depth (Figure 1, Table S2) because surface soil receives the majority of plant residues, root exudates and fertilizer inputs (Li *et al.*, 2017). The decomposition of OM releases carbon dioxide (CO_2) and organic acids (Weil & Brady, 2016), and, therefore, consistent with the observations at many other locations (Will *et al.*, 2010; Eilers *et al.*, 2012; Li *et al.*, 2017), the pH values of top soils were lower than those of deeper layers (Figure 1). Li *et al.* (2017) proposed an alternative explanation for the lower pH in surface soil; soil pH was measured with air-dried soil, and during the drying process, ammonium can be oxidized to nitrate, resulting in the release of protons ($NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$). Indeed, in this study, surface soils contained more ammonium than subsoils (Figure 1, Table S2). (We followed the standard methods and determined soil pH using air-dried soil and ammonium concentration using fresh wet soil.) Nevertheless, soil pH was correlated

more strongly with OM content than with ammonium concentration (Table S2). The concentration of amorphous iron oxides also tended to decrease with soil depth (Figure 1, Table S2); this is probably due to the smaller OM content in the subsoil (Figure 1, Table S2), considering that organic matter can inhibit the crystallization of amorphous iron oxides (Schwertmann, 1966).

Microbial abundance and OTU diversity largely decreased with soil depth

The abundances of microbial rRNA genes in surface soils (Figure S2) were comparable to previous observations. In a greenhouse pot experiment, 10^7-10^9 copies of archaeal 16S rRNA gene per gram of soil were detected in non-rhizosphere paddy soils with a depth of 12 cm (Zhai *et al.*, 2017). The copy numbers of bacterial and fungal rRNA genes were found to be 10^8-10^{11} and 10^5-10^7 per gram of soil in the top 0-5 cm depth of non-rhizosphere paddy soil (Lee *et al.*, 2011). These results suggest that archaea, bacteria and fungi are abundant in surface bulk paddy soil.

Due to the decrease in soil OM and TN (sources of microbial nutrients and energy), microbial abundances largely decreased with soil depth (Figure 2a). Most ammonia-oxidizing bacteria are chemolithotrophic (Zhang *et al.*, 2012), but AOB abundance also tended to decline with soil depth (r = -0.563, P < 0.05), probably because TN and especially ammonium-N was less abundant in deep soils (Figure 1). On the other hand,

AOA are more tolerant to a low ammonia concentration than AOB (Zhang *et al.*, 2012). Therefore, the correlation between AOA abundance and soil depth was weak ($r = 0.262$, $P > 0.05$), and AOA/AOB largely increased with soil depth (Figures 2a, S2).

Fungi cannot fix carbon and nitrogen and thus require organic matter and nitrogenous compounds for growth and energy (Walker & White, 2011), while archaea and bacteria are trophically diverse. This may account for the observation that the decrease in richness and diversity with soil depth was greater for fungi than that was for archaea and bacteria (Figure S3). Indeed, PLS regression showed that fungal α-diversity was more strongly correlated with soil depth, OM content and TN content compared to archaeal and bacterial α-diversities (Figure 2b).

Soil depth had limited influence on microbial community compositions

Soil depth was significantly correlated only with the ordination of archaeal communities; further, the correlation of soil depth with archaeal community structure was much weaker than those of climatic and soil properties (Figure 3, Table 1). The limited influence of soil depth on microbial community structures could be linked to the observation that only one archaeal OTU, but no bacterial or fungal OTUs, differed significantly between depth intervals in relative abundance. Also, no relative abundances of putative bacterial functional groups differed significantly between depth

intervals. By contrast, a study in a flooded rice paddy in South Korea found that archaeal and bacterial community structures, as well as functional microbial groups (methanotrophs and methanogens), changed significantly with soil depth (Lee *et al.*, 2015). The fact that the influence of soil depth on community composition depends on sampling sites and microbial domains has been observed before. For instance, Watanabe *et al.* (2010) reported that community structure differed between surface soil layers (< 20 cm depth) and deeper layers for both archaea and bacteria in two well-drained paddy fields, but only for archaea (not bacteria) in a wet paddy field. Also, the distributions of microbial phylotypes along a depth gradient are site- and phylotype-specific and dependent on the environmental characteristics of the profiles and the ecological traits of the phylotypes (Eilers *et al.*, 2012) (see also Figures 5, S2). Our results suggest that, in the studied paddy soil profiles, microbial community compositions and putative bacterial functions were largely the same between different layers, each with a thickness of 20 cm.

A study in forest soils by Eilers *et al.* (2012) showed that microbial communities across sites were more variable in the near-surface horizons than in the layers between 20 and 80 cm in depth; the authors ascribed these results to the greater variability in environmental factors in the surface soils. In this study, however, the variation in

community composition across sites in the 0-20 cm depth interval was less than (for archaea and fungi) or equal to (for bacteria) that in the subsurface horizons (Figure 4a). We hypothesize that the same type of land use (rice cropping) creates some common environmental filters (e.g., soil moisture regime) on microbial distributions in surface paddy soils at different sites. Indeed, the variation in the investigated environmental parameters across sites was not larger in the surface soils than in the subsurface soils in this study (Figure 4b). Nevertheless, why archaea and fungi communities were more variable in the subsurface horizons requires further investigation.

Environmental factors influencing microbial community compositions and bacterial functions

The community ordinations for all three domains were significantly correlated with climatic factors (MAT and MAP) (Table 1), which have been frequently observed to shape microbial community structures (Siles & Margesin, 2016; Li *et al.*, 2017; Ma *et al.*, 2017; Yuan *et al.*, 2019). The community compositions of different domains were influenced, however, by different soil parameters (Table 1). Archaea can thrive in a wide range of pH (Valentine, 2007). Therefore, the community structure of archaea, unlike those of bacteria and fungi, did not vary significantly along a pH gradient (Table 1). Chloride and sulphate concentrations, which were highly correlated with each other (Table S2), was associated with bacterial community composition more strongly than

with archaeal community composition (Table 1), probably because the cycling of sulphur is driven by bacteria rather than archaea (Muyzer & Stams, 2008; Rabus *et al.*, 2013). Another explanation is that chloride and sulphate concentrations reflect soil salinity, and archaea are more tolerant to high salinity than bacteria (Jarrell *et al.*, 2011). Bacterial community composition was additionally associated with soil TN and nitrate concentration (Table 1), implying the stronger requirement for nitrogen of bacteria compared to archaea, considering that, for example, ammonia-oxidizing archaea can outcompete their bacterial counterparts at low ammonia concentrations (Zhang *et al.*, 2012). On the other hand, both TN and OM content were significant factors for fungal community ordination (Table 1), suggesting that nutrient availability is the key factor influencing fungal distribution. The interesting association of soil iron and manganese concentrations with microbial β-diversities was possibly indirect because iron and manganese concentrations were correlated with some significant environment variables, such as MAT and MAP (Table S2). Nevertheless, considering that the ecology of many microbial taxa remains unknown, we cannot exclude the possibility that iron and manganese oxides can exert direct impacts on microbial distribution and community composition.

Intriguingly, we observed many bacterial OTUs with higher relative abundances in the

profile PJ than other soil profiles (Figure S6), highlighting the differentiation in bacterial community composition across sampling sites (Fig 3b). Further analyses on putative functional bacterial groups suggest that the OTUs with higher relative abundances in the profile PJ than other soil profiles might be related to certain functions involved in the cycling of carbon (chemoheterotrophy, chitinolysis and fermentation), nitrogen (nitrate reduction), and sulphur (dark sulphide oxidation and sulphur respiration), since these functions were also more abundant in the PJ profile than other profiles (Figure 6). The greater percentages of bacterial groups related to chemoheterotrophy, chitinolysis and fermentation may account for the smaller soil OM content in the PJ profile than other soil profiles (Figure 1). The greater percentages of functional groups related to dark sulphide oxidation, sulphur respiration and nitrate reduction in the profile PJ than other soil profiles (Figure 6) could be linked to the higher soil sulphate and nitrate concentrations in this profile (Figure 1).

Conclusions

This study provides an overview of the variations in abundance, α-diversity and community composition of archaea, bacteria and fungi with soil depth in paddy soil profiles. Microbial abundance and OTU diversity largely decreased with soil depth, but the influence of soil depth on microbial community compositions was limited. Instead,

the widely varied mean annual temperature and precipitation, as well as different soil properties, were significantly correlated with the variations in community composition for different microbial domains. The relationships of iron and manganese concentration with microbial β-diversity were possibly indirect but require further investigation. Further functional analyses showed that the bacterial OTUs accounting for community composition dissimilarity between sampling sites were possibly associated with the cycling of carbon, sulphur and nitrogen. These results suggest that microbial community compositions in paddy soil profiles are driven mainly by different soil chemical properties rather than soil depth, which may help better understanding the microbe-meditated biogeochemical processes in paddy soils.

Conflict of interest

All authors have declared no conflict of interest.

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Data Availability Statement

Raw sequences were submitted to NCBI (SRP116099).

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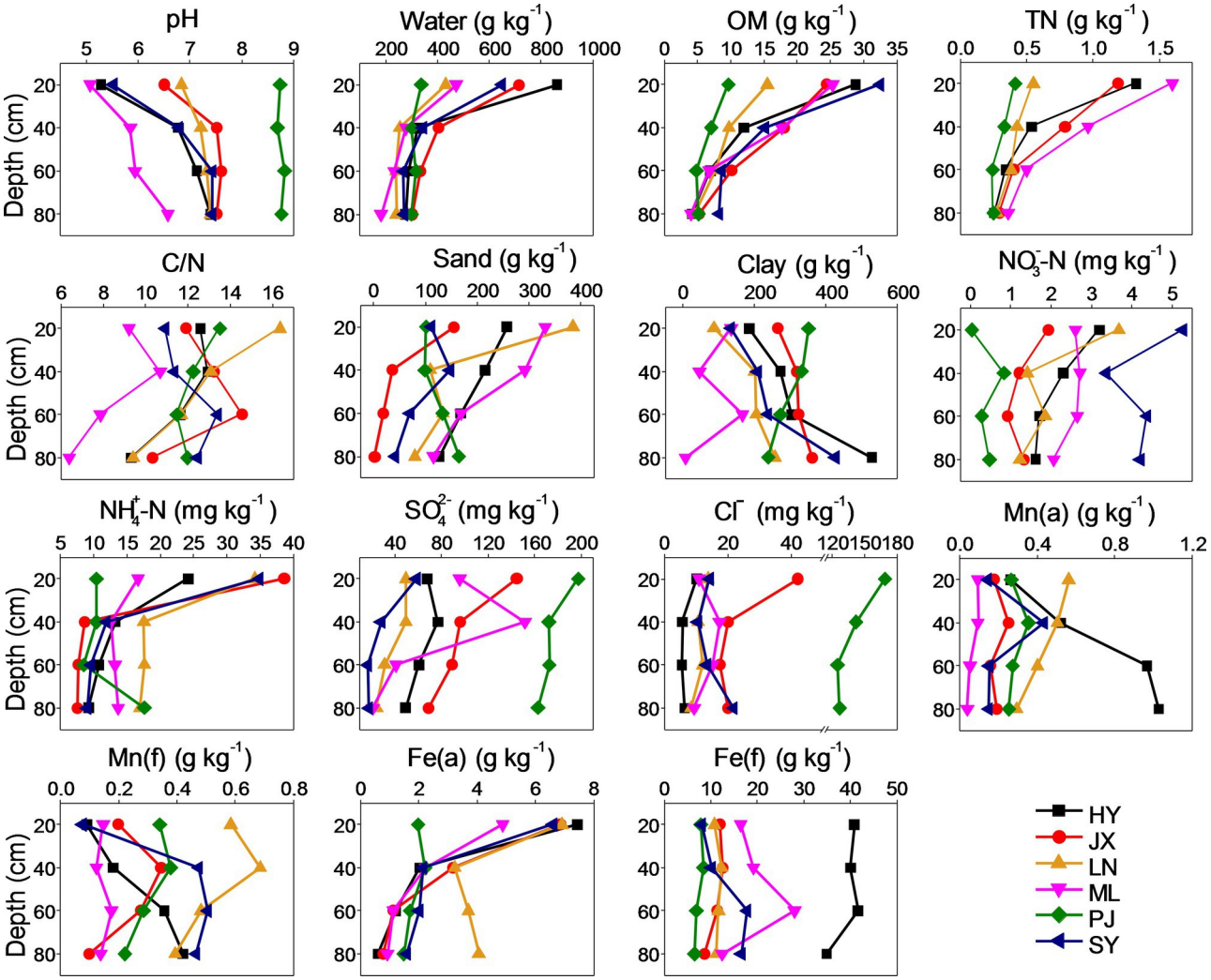

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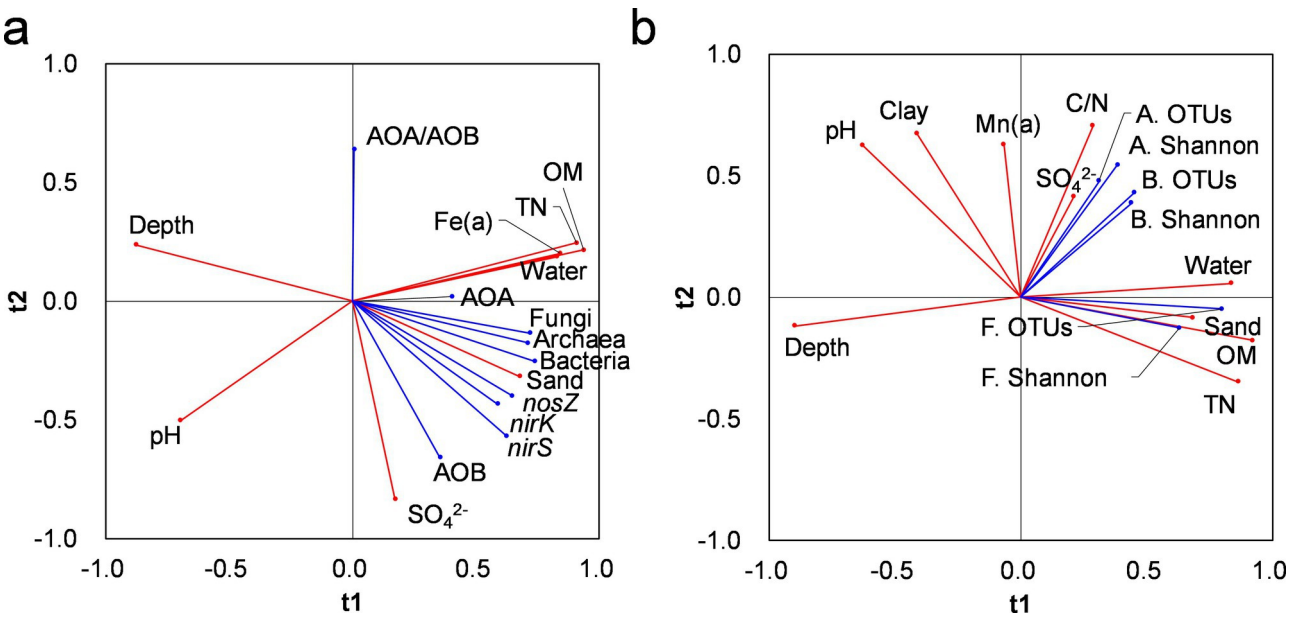

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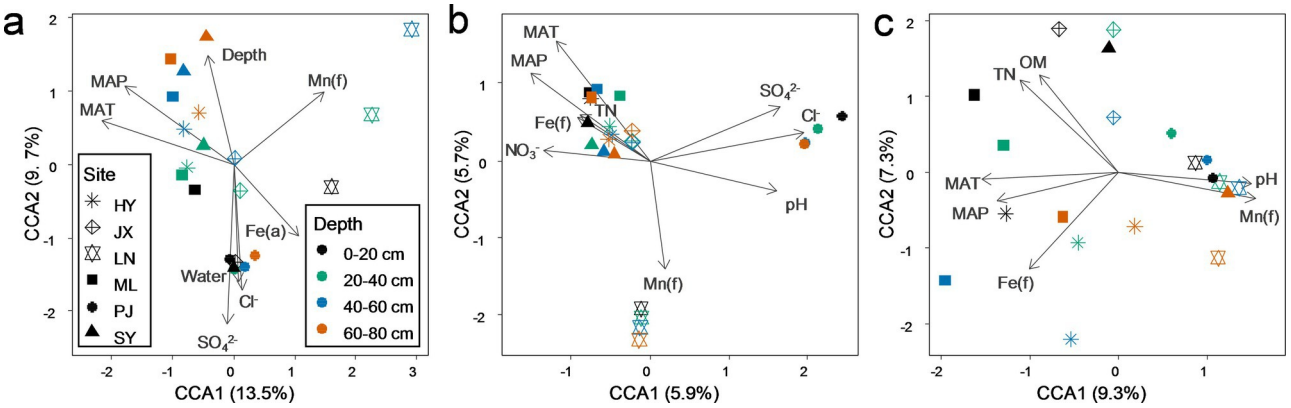

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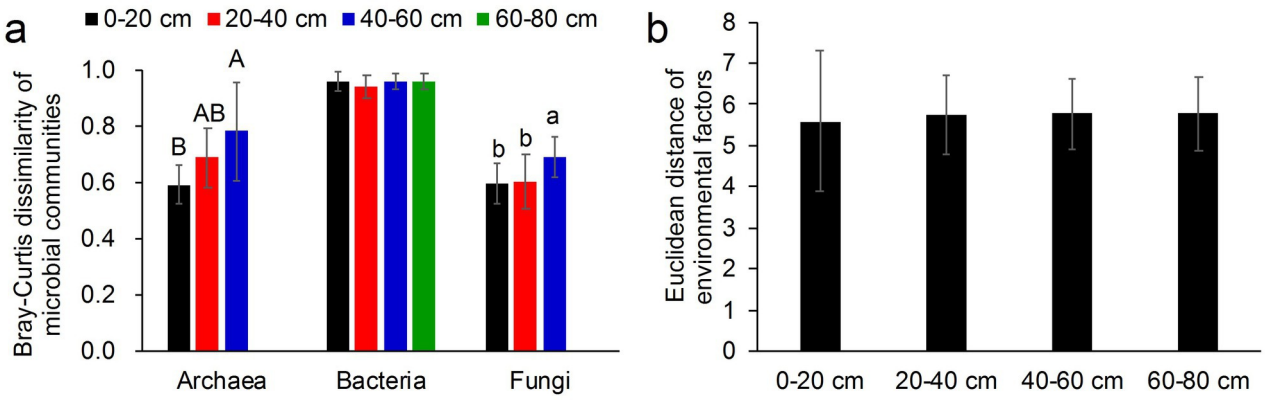

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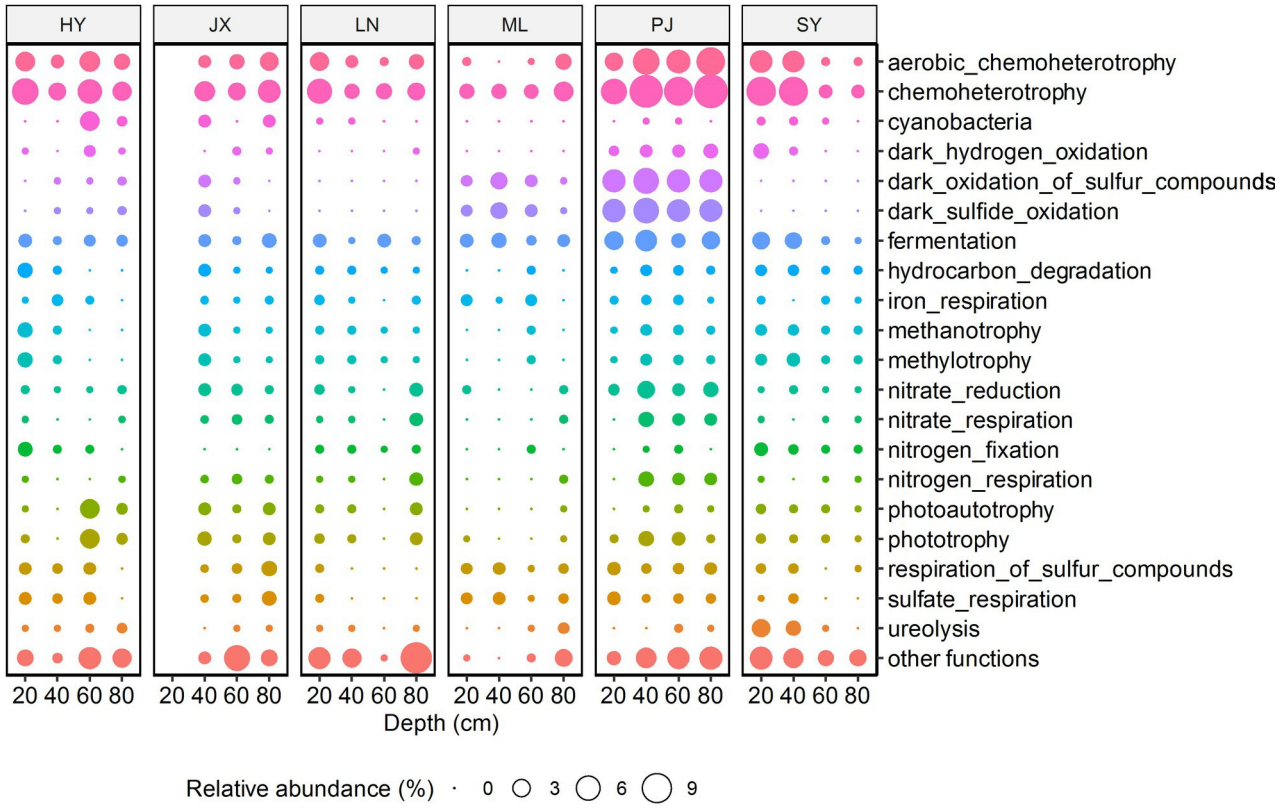

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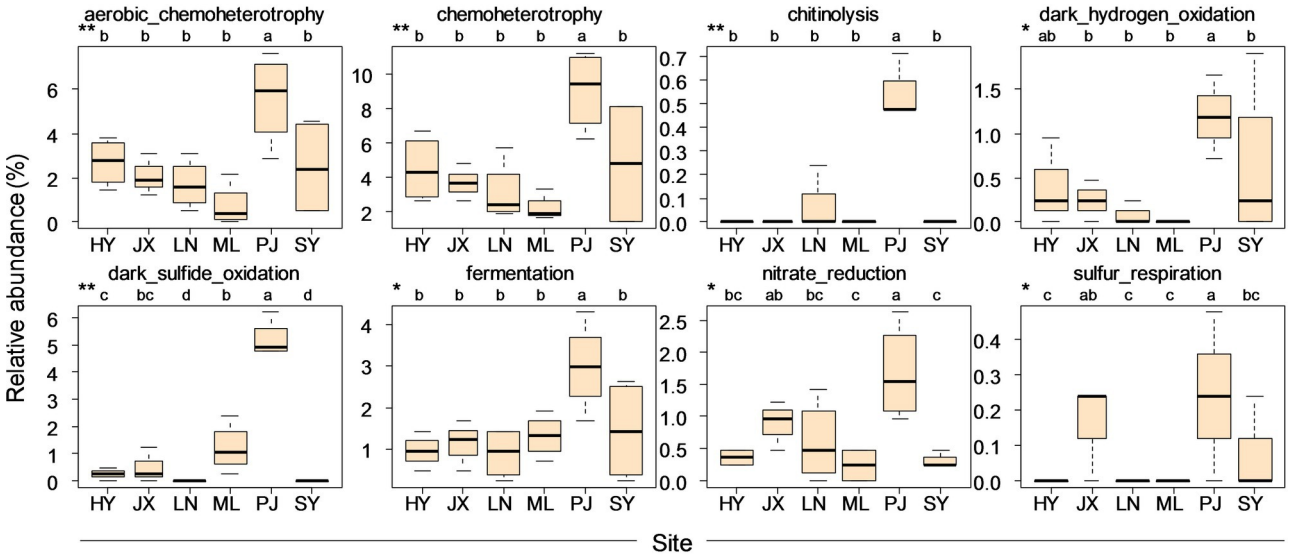

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Figure 1 Selected soil properties in six paddy soil profiles. Values are averages of measurement replicates. OM is organic matter. TN is total nitrogen. C/N is carbon to nitrogen ratio. Fe(f) and Mn(f) are iron and manganese bound in free iron oxides. Fe(a) and Mn(a) are iron and manganese bound in amorphous iron oxides.

Figure 2 Partial least squares regression analyses for the relationships of (a) microbial gene copy numbers (blue lines) and (b) OTU richness and diversity (blue lines) with environmental factors (red lines). For panel b, A., B. and F. indicate archaea, bacteria and fungi, respectively. For abbreviations of soil properties, see Figure 1. Variables pointing in opposite directions are negatively related, while those pointing in the same direction are positively related. Variables perpendicular to each other have weak correlations. If the line of a variable is short, its correlations with other variables are weak.

Figure 3 Canonical correspondence analysis (CCA) for (a) archaeal, (b) bacterial and (c) fungal communities. Colours indicate different depths, and symbols indicate different sites. Only environmental variables that were significantly correlated with the CCA ordination in *envfit* analysis were included in the figures. For abbreviations of environmental factors, see Figure 1.

Figure 4 (a) Variations in community structure across the same sampling sites as measured by the Bray-Curtis dissimilarity (n = 10) within each depth interval. There

were only 6 archaeal and fungal community dissimilarities at the 60-80 cm interval, so this interval was not included for archaea and fungi. (b) Variations in environmental factors across sites as measured by the Euclidean distance (n = 15) within each depth interval. Error bars show the standard deviation. Values that do not share the same letter are significantly different at $P < 0.05$, with upper-case letters for archaeal communities and lower-case letters for fungal communities. The Bray-Curtis dissimilarities of bacterial communities and the Euclidean distance of environmental factors across sites were not significantly different between depth intervals.

Figure 5 Relative abundances of functional bacterial groups in six paddy soil profiles.

Figure 6 Functional bacterial groups that significantly differed in relative abundance between sampling sites. Statistical significance: * for $P < 0.1$, ** for $P < 0.01$ and *** for $P < 0.001$. Values that do not share the same letter are significantly different.

Table 1 Correlations between environmental variables and the canonical correspondence analysis (CCA) ordination using *envfit* analysis for archaeal, bacterial and fungal communities. Only significant correlations ($P < 0.05$) are displayed.

Environmental	Archaea		Bacteria		Fungi	
variables	r^2	*P*	r^2	*P*	r^2	*P*
MAT	0.719	**0.001**	0.901	**0.001**	0.553	**0.001**
MAP	0.611	**0.002**	0.862	**0.001**	0.464	**0.004**
Depth	0.377	**0.010**				
pH			0.675	**0.001**	0.481	**0.003**
Water	0.308	**0.045**				
OM					0.413	**0.012**
TN			0.316	**0.030**	0.476	**0.004**
C/N						
Sand						
Clay						
NO_3^--N			0.442	**0.005**		
NH_4^+-N						
SO_4^{2-}	0.559	**0.002**	0.776	**0.001**		
Cl^-	0.302	**0.043**	0.972	**0.001**		
Mn(a)						
Mn(f)	0.412	**0.008**	0.530	**0.001**	0.535	**0.001**
Fe(a)	0.322	**0.041**				
Fe(f)			0.280	**0.045**	0.486	**0.002**

MAT and MAP are mean annual temperature and precipitation. OM is organic matter. TN is total nitrogen. C/N is carbon to nitrogen ratio. Fe(f) and Mn(f) are iron and manganese bound in free iron oxides. Fe(a) and Mn(a) are iron and manganese bound in amorphous iron oxides.