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Different responses of soil carbon chemistry to fertilization regimes in the paddy soil and upland soil were mainly reflected by the opposite shifts of OCH and alkyl C

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ABSTRACT

The supposition that cropping system might affect fertilization-induced changes in chemical composition of soil organic carbon (SOC) and microbial community composition has mostly derived from field experiments in different sites, which may have been disturbed by different climate conditions and parent materials. Here, two adjacent long-term field experiments were used, which contained similar four fertilization regimes of NPK, 2NPK, NPKOM, and an unamended control (Control), while contrasting annual cropping system of rice–rice and maize–maize, to investigate how fertilization-induced changes in SOC chemistry differ in paddy soil from upland soil, and whether they correlate with different bacterial taxa. The bacterial community composition of the paddy soil was mainly determined by NO_3^--N and total N, and that of the upland was mostly explained by SOC. The NPK, 2NPK, and NPKOM treatments from the paddy soil increased CCH_3, decreased OCH, and reduced CH/CH_2, while those from the upland soil decreased CH/CH_2, increased OCH, and raised CH/CH_2, respectively, indicating opposite shifts of OCH and alkyl C (i.e., CCH_3 and CH/CH_2) in each paired fertilizer treatment of the two soils. In the paddy soil, the CCH_3 from the NPK treatment showed no association with any bacterial taxa, the OCH from the 2NPK showed a negative association with NO_3^--related species, and the CH/CH_2 from the NPKOM showed a negative association with Proteobacteria and Bacteroidetes. In the upland soil, the CH/CH_2 from the NPK negatively associated with Thermogemmatisporales and Acidobacteriaceae subgroup 1, the OCH from the 2NPK positively associated with Thermogemmatisporales, and the CH/CH_2 from the NPKOM positively associated with copiotrophic bacterial taxa. Our results provided direct evidence that cropping system mediated the shift direction of specific functional group under specific fertilization regimes, and implied that completely opposite shifts of OCH and alkyl C in each paired fertilizer treatment of the paddy soil and upland soil can likely be attributed to different fertilization-induced shifts in the microbial community composition resulting from soil N change in the paddy soil and soil C change in the upland soil.

1. Introduction

Soil organic carbon (SOC) represents the core of soil fertility (Singh and Rengel, 2007), and can be directly improved by application of manure fertilizers or indirectly by mineral fertilizers through more residues returned to the soil from greater crop biomass (Courtier-Murias et al., 2013; Yan et al., 2013; Lin et al., 2019). The SOC is comprised of a heterogeneous mixture of chemically different organic molecules, which is critical to soil ecosystem functioning (Baldock, 2007; Lorenz et al., 2007). The SOC chemical composition is jointly regulated by a series of interactive processes, including the intrinsic properties of the added organic materials (De Deyn et al., 2008) that may replenish SOC through microbial decomposition (Li et al., 2020), microbe-mediated transformations (Wang et al., 2015; Li et al., 2018), and the selective preservation of specific biomolecules by chemical and physical stabilization (Lützow et al., 2006). In other words, soil microorganisms play a pivotal role in modulating the SOC chemical composition by processing exogenous residue-derived C into biomass and chemically distinct metabolite precursors of SOC (Gougoulias et al., 2014; Li et al., 2020) and/or by supplying microbially processed C that is stabilized in soil through chemical protection by mineral fractions and physical protection by aggregates (Six et al., 2006; Grandy and Neff, 2008; Courtier-Murias

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et al., 2013). Therefore, assessments of SOC molecular characteristics and their relationships with the microbial community composition are essential for elucidating the microbial mechanisms underlying the formation of SOC chemistry.

The responses of SOC chemical composition to different cropping systems and fertilization regimes have been relatively poorly studied compared with the amount of SOC in agricultural ecosystems (Yan et al., 2013; Xu et al., 2017), and the limited published results were not always consistent with each other. For example, paddy soil was reported to contain higher O–alkyl and alkyl C, as well as lower aromatic C levels than upland soil grown with maize (*Zea mays* L.) or wheat (*Triticum aestivum* L.) (Yan et al., 2013; Xu et al., 2017), while Wissing et al. (2013) showed that paddy soil and non-paddy soil had similar chemical compositions. As for the effects of fertilization regimes, Zhang et al. (2013a) and Li et al. (2018) reported that the chemical compositions of upland soils grown with maize and wheat were potentially modulated by long-term chemical or organic fertilizations, while this phenomenon was not found in paddy soils (Bierke et al., 2008; Yan et al., 2013). However, some other evidences have indicated that the chemical nature of paddy soils is obviously influenced by fertilization regimes, regardless the use of organic or inorganic fertilizers (Zhou et al., 2010; Xu et al., 2017; Wu et al., 2019).

Microbial community composition can be altered by fertilizations via changes in the soil properties (Ling et al., 2016; Geisseler et al., 2017). Across four upland field experiments, Ling et al. (2016) showed that long-term organic and inorganic fertilizations exerted distinct effects on soil pH, resulting in distinct distributions of key microbial populations belonging to Acidobacteria subgroup 17 and Solirubrobacter, respectively. In contrast, across six paddy field experiments, no significant differences in the microbial community composition between chemical and organic fertilization regimes were observed (Chen et al., 2017), while Dong et al. (2014) and Tian et al. (2015) reported that, in paddy soils where manure was applied, shifts in microbial community composition were more pronounced than in those mineral fertilizers were applied.

Variations in the microbial species theoretically result in changes in the SOC chemical composition. Ng et al. (2014) confirmed that variations in aromatic C showed associations with changes in Gram-negative bacteria induced by long-term organic amendments in two contrasting agricultural soils. Wang et al. (2015), Wang et al. (2016) reported that changes in the alkyl C/O–alkyl C ratio were significantly coupled with shifts in soil Gram-positive bacteria. Moreover, Li et al. (2018) found that increases in the aromatic C–O and methoxyl C groups following long-term manure application were closely associated with enrichments in Acidobacteria subgroups 6 and 5, Cytophagaceae, Chitinophagaceae, and *Bacillus* sp. species.

All the comparisons between the paddy soil and upland soil in terms of SOC chemical nature and microbial community composition were obtained from different sites, which may have been disturbed by different climate conditions and/or soil parent materials. Climate conditions strongly influence the soil microbiome (Chen et al., 2017) and regulate soil chemistry by affecting the pathway of litter decay (Parsons et al., 2014), while soil parent materials have a prolonged effect on soil chemical and microbial compositions via changes in soil pH, nutrient content, and mineralogical characteristics (Sheng et al., 2015; Angst et al., 2018). Little information, however, is available on direct comparison of how SOC chemistry relates with the microbial community composition under different crop management practices, hindering our insight into the formation mechanisms of distinct SOC chemical compositions.

Rice (*Oryza sativa* L.) and maize (*Zea mays* L.) are extensively cultivated worldwide, resulting in distinct soil properties observed in paddy soil and upland soil (Weller et al., 2015). We hypothesized that the unique SOC chemistries in the paddy soil and upland soil are associated with specific microbial assemblages, which may be governed by distinct soil properties. In the present study, to avoid any disturbance effects from climate conditions and parent materials, paddy soil and upland soil samples were collected from two adjacent experimental fields, which were subjected to similar fertilization treatments and different annual cropping systems (i.e., rice–rice and maize–maize) for >30 years. We simultaneously determined various soil properties, performed detailed analysis of the SOC chemical nature using advanced solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopy, and quantified the bacterial community composition using 16S rRNA gene sequencing. Bacteria have been previously identified as key microbial members involved in straw degradation in paddy soils (Murase et al., 2006; Zhan et al., 2018). The objectives of this study were to: (1) investigate how the cropping system affect fertilization-induced changes in SOC chemical nature and bacterial community composition, and (2) explore potential mechanisms involved in the formation of distinct SOC chemical compositions under different cropping systems, in terms of associations between the SOC chemistry and bacterial taxa.

2. Materials and methods

2.1. Long-term field experiment and soil sampling

Two adjacent long-term experimental fields were located at the Institute of Red Soil, Jiangxi Province, China (28°21′ N, 116°10′ E): one had been cultivated with paddy rice and the other with maize for >30 years. The site has a typical subtropical monsoon climate with an annually average temperature of 18.1 °C and an average precipitation of 1727 mm, 38% of which occurs from March to early July. The soil for the experimental site was developed from Quaternary red clay. The paddy soil and upland soil were classified as Typic Stagnic Anthrosols and Plinthosols, respectively, according to the World Reference Based for Soil Resources (IUSS Working Group WRB, 2006).

The rice experiment was initiated in 1981 with a cropping system of early rice (April–July), late rice (July–November), and winter fallow (November–April). The main soil properties before the experiment were: pH (H_2O), 5.4; SOC, 16.3 g kg^{-1}; total nitrogen (TN), 1.6 g kg^{-1}; total phosphorus (TP), 0.5 g kg^{-1}; and total potassium (TK), 10.4 g kg^{-1}. The maize experiment was established in 1986 with a cropping system of early maize (April–July), late maize (July–October), and winter fallow (November–April). The main soil properties before the experiment were: pH (H_2O), 6.0; SOC, 9.39 g kg^{-1}; TN, 0.98 g kg^{-1}; TP, 1.42 g kg^{-1}; and TK, 15.8 g kg^{-1}.

Each experiment consisted of four fertilizer treatments with three replicates of each, giving a total of 12 plots. Each replicate plot for the rice experiment measured 46.7 m^2, while those for the maize experiment measured 22.2 m^2. All the replicate plots at each treatment site were arranged in a randomized block design. The four fertilizer treatments were: (1) inorganic N, P, and K fertilizers with application rates common to the region with N at 90, P_2O_5 at 45, and K_2O at 75 kg ha^{-1} for each rice season and with N at 60, P_2O_5 at 30, and K_2O at 60 kg ha^{-1} for each maize season (NPK), (2) doubling of the NPK inputs (2NPK), (3) NPK plus organic manure (NPKOM), and (4) an unamended control (Control). The organic manure was Chinese milk vetch (*Astragalus sinicus* L.) for the early rice and pig manure for the late rice, with application rates of 22500 kg ha^{-1} (fresh weight) for each season. The organic manure for both early maize and late maize was pig manure, with an application rate of 15000 kg ha^{-1} (fresh weight) for each season. In both field trials, urea, calcium-magnesium phosphate and potassium chloride were used as N, P_2O_5, and K_2O fertilizers, respectively. All the P_2O_5 and K_2O fertilizers and the manures were applied as basal fertilizers, two-thirds of the TN fertilizer was applied as basal fertilizer, and the rest was applied as topdressing fertilizer for each rice or maize season. The basic properties of Chinese milk vetch and pig manure were described in detail in Liu et al. (2019).

Soil samples were collected on November 15, 2016, at the time when both late rice and late maize were harvested. In each replicate plot, a composite sample was obtained by mixing four random soil cores (0–20

cm), followed by sieving (<2 mm) to remove visible stones and plant residues. Each soil sample was divided into three fractions: the first fraction was stored at –80 °C for DNA extraction, the second was stored at 4 °C for measurements of the water-extractable organic C (WEOC) and available N contents, and the third was air-dried for the analysis of other soil properties.

2.2. Analyses for determining soil basic physical and chemical properties

Soil pH was measured in a 1:5 soil-deionized water suspension using a glass electrode (FE20, Mettler Toledo, Germany). The SOC and TN contents were determined using dichromate oxidation and Kjeldahl digestion (Page et al., 1982), respectively. Soil TP and TK were digested with HF-$HClO_4$ (Jackson, 1958), and then their contents were determined using the molybdenum blue method (Olsen et al., 1954) and flame photometry (FP640, Huayan, China), respectively. Soil available N was extracted with 2 M KCl (Page et al., 1982), and the nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations in the extracting solution were determined using a Skalar SAN^{plus} Segmented Flow Analyzer (Skalar Analytic B.V., De Breda, The Netherlands). Soil available P (AP) was extracted with sodium bicarbonate and the content was determined using the molybdenum blue method (Olsen et al., 1954). Soil available K (AK) was extracted with CH_3COONH_4 (Page et al., 1982) and its content was determined using flame photometry. The WEOC was extracted with deionized water at a 1:5 soil–water ratio, followed by centrifugation and filtration (<0.45 μm), and the content was determined using a total organic C analyzer (Multi N/C 3000, Analytik, Jena, Germany). Free iron oxide (free Fe_2O_3) and aluminum oxide (free Al_2O_3) were extracted using the dithionite-citrate-bicarbonate method, and amorphous iron oxide (amorphous Fe_2O_3) and aluminum oxide (amorphous Al_2O_3) were extracted using the acidic ammonium oxalate method (Pansu and Gautheyrou, 2006). Extracted iron was detected using inductively coupled plasma optical emission spectrometry (Optima 8000, PerkinElmer, USA), and extracted aluminum was measured using inductively coupled plasma mass spectrometry (NexION 300, PerkinElmer, USA).

2.3. Nuclear magnetic resonance spectroscopy

Prior to the NMR analysis, soil samples were de-ashed using successive treatments with a 2% hydrofluoric acid (HF) solution to remove paramagnetic materials and concentrate SOC (Skjemstad et al., 1994). After the final treatment, the residue was washed three times with deionized water, freeze-dried, and analyzed using NMR spectroscopy.

The NMR analyses were performed using a Bruker Avance 400 (Billerica, USA) spectrometer equipped with 4-mm sample rotors in a double-resonance probe head and operating at a ^{13}C frequency of 100 MHz. We combined samples from three field replicates into one composite. The quantitative spectra provided by ^{13}C multiple cross-polarization magic-angle spinning (multiCP/MAS) were recorded at a spinning speed of 14 kHz, a contact time of 1 ms, a relaxation delay of 0.35 s, and 90° ^{13}C pulse-length of 4 μs. All the obtained spectra exhibited good signal-to-noise ratios, with minor (<3%) spinning sidebands and limited overlap with the center bands. The ^{13}C multiCP/MAS experiments combined with 68 μs dipolar dephasing were also employed to differentiate signals from non-protonated C and mobile C from the total C signal.

The ^{13}C multiCP/MAS spectra were divided into eight chemical shift regions assigned to the following C functional groups: alkyl C (0–44 ppm), OCH_3/NCH (44–64 ppm), O–alkyl C (64–93 ppm), anomeric C (93–113 ppm), aromatic C (113–142 ppm), aromatic C–O (142–162 ppm), COO/N–C = C (162–188 ppm), and ketone/aldehyde C (C = O) (188–220 ppm). The multiCP/MAS spectra with dipolar dephasing specifically permit the separation of rotating CCH_3 and long-chained CH/CH_2 from alkyl C, of OCH_3 and NCH from OCH_3/NCH, of non-protonated O–alkyl C (OCq) and protonated O–alkyl C (OCH) from O–alkyl C, and of non-protonated aromatic C (aromatic C–C) and protonated aromatic C (aromatic C–H) from aromatic C. The percentages of the different functional groups were obtained by integrating and normalizing the spectral areas to the total signal intensity (0–220 ppm) for each spectrum. The alkyl/O–alkyl C ratio (A/O–A) represents the degree of SOC decomposition (Xu et al., 2017) and the hydrophobicity (HI) indicates the stability of SOC (Xu et al., 2019). The parameters were calculated using the following equations: A/O–A = (0–44 ppm)/(64–93 ppm) and HI = [(0–44 ppm) + (113–142 ppm)] / [(44–113 ppm) + (142–220 ppm)].

2.4. DNA extraction, 16S rRNA gene amplification and sequencing, and bioinformatics analysis

Genomic DNA was extracted from 0.5 g of fresh soil using a Fast DNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The concentration of extracted DNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher, USA) and stored at −20 °C for future use.

The primer set 519F/907R was chosen to amplify the 16S rRNA genes in the V4–V5 hypervariable region. The PCR amplification was conducted in a 50 μL reaction mixture containing 1.25 μM of dNTPs, 2 U of Taq DNA polymerase (TaKaRa, Japan), 2 μL (15 μM) of each forward/reverse primer, and 1 μL (50 ng) of genomic community DNA as a template. The PCR conditions were the following: denaturation at 94 °C for 5 min followed by 30 cycles at 94 °C for 60 s, 55 °C for 60 s, and 72 °C for 75 s, with a final extension step at 72 °C for 10 min. The reaction products were purified using a Cycle Pure Kit (OMEGA) and quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher, USA). The purified PCR products of all samples were pooled in equal amounts and sequenced using an Illumina MiSeq platform (Illumina Inc., CA, USA). The sequencing data were deposited in the NCBI Sequence Read Archive under BioProject PRJNA577759.

The 16S rRNA expression data were processed using the Qualitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010). After trimming the barcodes, primers, low-quality sequences (<Q20), and singletons, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) using a 97% identity threshold, and the most abundant sequence for each OTU was chosen as the representative sequence. Taxonomy was then assigned to each OTU by referring to a subset of the SILVA 119 database. Non-bacterial sequences (Archaea and chloroplasts) and rare taxa (defined as OTUs present in <0.001% of the total sequences) were removed and all the samples were then rarefied to the same sequencing depth (28,297 sequences per sample) for a downstream analysis.

2.5. Statistical analyses

A one-way analysis of variance (ANOVA) was performed to identify the significant effects of the fertilization regimes and cropping systems on the soil properties and bacterial taxa. Significant differences were defined as $p < 0.05$.

A principal component analysis (PCA) was conducted to visualize the effect of long-term fertilization on the chemical nature of SOC. A linear discriminant analysis (LDA) was employed to determine significant differences in bacterial community composition among treatments based on the OTUs and using the R statistical software v3.3.3 with the MASS package. The differential OTUs between treatments were identified using the "corr.test" function in the edgeR package, and the key OTUs contributing to the observed differences between treatments were revealed by performing a similarity percentage analysis (SIMPER) (Clarke, 1993). Multivariate regression tree (MRT) analyses were performed to establish the relationships between soil properties and bacterial community composition (De'ath, 2002).

Network analyses were performed to explore the co-occurrence patterns between the SOC chemical nature and bacterial communities based on SOC functional groups and OTUs using the maximal

information coefficient (MIC) in the MINE software (Reshef et al., 2011). The MIC is a very useful score that reveals the strength of linear and non-linear associations among variables (Reshef et al., 2011). Only those SOC functional groups and OTUs that exhibited significant differences between treatments were considered to reduce the network complexity. The selected OTUs and C groups with strong positive ($r > 0.8$), strong negative ($r < -0.8$), or strong non-linear (MIC-$\rho^2 > 0.8$) relationships were used to construct networks in Cytoscape v3.4.0 (Shannon et al., 2003). We calculated the topological features of the networks and the modularity using the NetworkAnalyzer tool and the MCODE plugin in Cytoscape, respectively.

3. Results

3.1. Basic physical and chemical properties

Table 1 shows that the paddy soil consistently exhibited higher SOC content than the upland soil, regardless of the fertilization treatments ($p < 0.05$, Table S1). Within the paddy soil, the SOC from the NPKOM treatment increased by 24.2%, while that from the NPK and 2NPK treatments was similar, as compared with Control; within the upland soil, the SOC from the NPKOM, 2NPK, and NPK treatments increased by 44.4%, 19.7%, and 20.6%, respectively. The 2NPK treatment in the paddy soil had the lowest NO_3^--N content, while the same treatment in the upland soil had the highest NO_3^--N content. The NH_4^+-N content was significantly higher in the NPKOM treatment than in the other three treatments in the paddy soil, but it was not affected by fertilization in the upland soil. As expected, the NPKOM treatment had the highest WEOC content among the treatments in both soils. In addition, the NPK treatment and Control of the paddy soil shared similar nutritional contents, but the NPK treatment had higher free Fe_2O_3 and lower amorphous Fe_2O_3 and Al_2O_3 contents than the Control ($p < 0.05$, Table 1).

3.2. Chemical nature of the SOC

Fig. 1 shows that the SOC chemical structure completely differed among the four fertilizer treatments of each cropping system. The fertilization-induced changes in SOC chemistry were mainly reflected by shifts in OCH, alkyl C (i.e., CCH_3 and CH/CH_2), and aromatic C (i.e., aromatic C–C and aromatic C–H) to different degrees and directions in both soils. The OCH and alkyl C accounted for 55.2% and 47.1% of the total C groups in the paddy soil and upland soil, respectively (Fig. 1, Table 2). Within the paddy soil, the NPK treatment raised CCH_3 and aromatic C–C while lowered aromatic C–H content, and the 2NPK and NPKOM treatment decreased OCH and CH/CH_2 contents, respectively. Within the upland soil, the NPK treatment lowered CH/CH_2 content, the 2NPK treatment raised OCH while reduced aromatic C–C content, and the NPKOM treatment raised CH/CH_2 while reduced aromatic C–C and aromatic C–H contents (Table 2, Fig. 1). It can be seen that the fertilization-induced changes in SOC chemistry differed between the

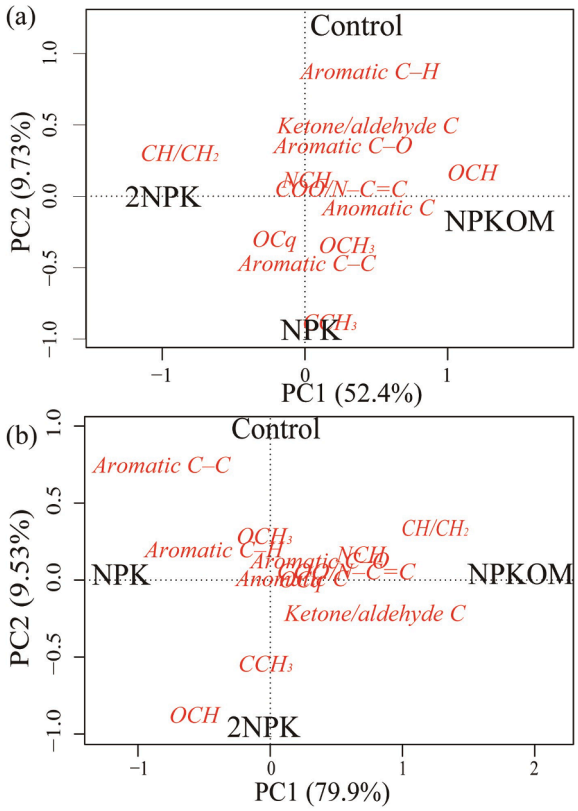

Fig. 1. Principal component analysis (PCA) combined ordination plot performed on relative contents of 12 SOC functional groups from the paddy soil (a) and upland soil (b).

Table 1
Soil pH and contents of nutrients and free/amorphous Fe- or Al-oxides.

	Paddy soil				Upland soil			
	Control	NPK	2NPK	NPKOM	Control	NPK	2NPK	NPKOM
pH	5.43 ± 0.22 a	5.39 ± 0.08 a	5.31 ± 0.26 a	5.49 ± 0.08 a	5.10 ± 0.08b	4.71 ± 0.10c	4.55 ± 0.06 d	6.01 ± 0.08 a
SOC (g/kg)	20.00 ± 0.28b	19.88 ± 1.19b	19.08 ± 1.99b	24.84 ± 0.01 a	7.82 ± 0.63c	9.43 ± 0.61b	9.36 ± 0.63b	11.29 ± 0.24 a
TN (g/kg)	2.08 ± 0.05b	2.05 ± 0.14b	2.14 ± 0.07b	2.72 ± 0.02 a	0.90 ± 0.04c	1.03 ± 0.03b	1.07 ± 0.03b	1.27 ± 0.02 a
TP (g/kg)	0.44 ± 0.02 d	0.57 ± 0.04c	0.85 ± 0.10b	1.34 ± 0.00 a	0.54 ± 0.03c	0.68 ± 0.12c	0.88 ± 0.07b	1.78 ± 0.08 a
TK (g/kg)	10.87 ± 0.70 a	11.61 ± 0.41 a	11.69 ± 0.37 a	11.19 ± 0.17 a	14.44 ± 0.14 a	13.84 ± 0.15b	14.51 ± 0.01 a	13.76 ± 0.48b
NO_3^--N (mg/kg)	3.52 ± 0.20 a	3.32 ± 0.23 a	0.66 ± 0.20b	3.50 ± 0.06 a	0.63 ± 0.01 d	7.24 ± 0.64b	14.00 ± 1.03 a	4.52 ± 0.53c
NH_4^+-N (mg/kg)	13.14 ± 3.12b	15.42 ± 4.73b	16.60 ± 1.17b	31.83 ± 3.08 a	1.82 ± 0.41 a	2.40 ± 0.07 a	2.79 ± 0.25 a	2.44 ± 0.91 a
AP (mg/kg)	2.78 ± 0.17c	3.51 ± 0.07c	27.49 ± 0.19b	35.03 ± 1.13 a	1.36 ± 0.24 d	8.89 ± 0.47c	19.79 ± 0.52b	29.04 ± 0.85 a
AK (mg/kg)	46.04 ± 3.25b	46.66 ± 7.04b	44.03 ± 6.56b	76.71 ± 0.07 a	72.47 ± 10.07c	202.17 ± 10.86b	329.89 ± 20.82 a	233.14 ± 28.47b
WEOC (mg/kg)	64.10 ± 3.87 bc	75.40 ± 14.28b	55.00 ± 7.84c	136.91 ± 6.99 a	4.13 ± 0.91b	6.71 ± 2.13b	5.75 ± 0.80b	54.15 ± 4.51 a
Free Fe_2O_3 (g/kg)	27.34 ± 2.56 d	34.53 ± 0.63b	37.83 ± 0.41 a	30.05 ± 0.69c	45.39 ± 0.62 a	45.82 ± 0.62 a	46.05 ± 1.34 a	44.68 ± 0.35 a
Amorphous Fe_2O_3 (g/kg)	0.86 ± 0.06b	0.61 ± 0.03c	0.70 ± 0.06c	2.40 ± 0.04 a	6.10 ± 0.25c	7.03 ± 0.37b	9.52 ± 0.31 a	9.06 ± 0.30 a
Free Al_2O_3 (g/kg)	5.76 ± 0.87b	6.45 ± 0.77 ab	7.23 ± 0.44 a	5.85 ± 0.60b	11.06 ± 1.35 a	11.97 ± 1.04 a	11.00 ± 0.13 a	10.85 ± 0.51 a
Amorphous Al_2O_3 (g/kg)	1.88 ± 0.17b	1.35 ± 0.17c	1.15 ± 0.03c	2.12 ± 0.07 a	1.83 ± 0.12b	1.82 ± 0.11b	2.29 ± 0.05 a	2.01 ± 0.18b

Lowercase letters refer to the comparison within the four treatments of each soil at $p < 0.05$.

Table 2
Percentages of total spectral area (%) assigned to different functional groups resolved by ^{13}C multiCP/MAS nuclear magnetic resonance and dipolar dephased multiCP/MAS.

	Treatment	220–188 ppm	188–162 ppm	162–142 ppm	142–113 ppm		113–93 ppm	93–64 ppm			64–44 ppm		44–0 ppm			
		Ketone/ aldehyde C	COO/N–C = C	Aromatic C–O	Aromatic C		Anomeric C	O–alkyl C			OCH_3/ NCH		Alkyl C			
					Total	Arom. C–C	Arom. C–H	Total	OC_q	OCH	Total	OCH_3	NCH	Total	CCH_3	CH/CH_2
Paddy soil	Control	1.81 ± 0.47	9.25 ± 0.15	3.04 ± 0.29	8.19 ± 0.40	3.40 ± 0.37	4.79 ± 0.59	7.73 ± 0.17	28.50 ± 0.27	1.50 ± 0.16	27.00 ± 0.33	13.70 ± 0.23	1.04 ± 0.23	12.66 ± 0.35	27.78 ± 0.32	5.23 ± 0.27	22.56 ± 0.45
	NPK	0.80 ± 0.23	9.14 ± 0.28	2.32 ± 0.26	7.42 ± 0.43	4.58 ± 0.44	2.84 ± 0.67	7.94 ± 0.24	28.77 ± 0.22	2.23 ± 0.13	26.54 ± 0.27	14.27 ± 0.22	1.77 ± 0.14	12.49 ± 0.28	29.35 ± 0.28	7.17 ± 0.14	22.17 ± 0.32
	2NPK	1.20 ± 0.37	9.42 ± 0.23	2.65 ± 0.31	7.91 ± 0.36	4.19 ± 0.56	3.72 ± 0.71	7.28 ± 0.31	27.71 ± 0.18	2.42 ± 0.09	25.28 ± 0.21	14.09 ± 0.44	1.33 ± 0.16	12.76 ± 0.46	29.73 ± 0.41	6.37 ± 0.35	23.36 ± 0.58
	NPKOM	0.99 ± 0.25	9.49 ± 0.17	2.48 ± 0.35	7.45 ± 0.14	3.57 ± 0.69	3.88 ± 0.64	8.02 ± 0.36	29.59 ± 0.31	1.82 ± 0.07	27.77 ± 0.29	14.46 ± 0.21	1.88 ± 0.09	12.58 ± 0.23	27.52 ± 0.23	6.67 ± 0.29	20.85 ± 0.40
Upland soil	Control	1.33 ± 0.46	9.61 ± 0.35	4.54 ± 0.39	15.26 ± 0.67	8.58 ± 0.67	6.68 ± 1.03	8.27 ± 0.44	26.99 ± 0.45	2.10 ± 0.24	24.90 ± 0.53	13.30 ± 0.42	2.78 ± 0.31	10.52 ± 0.56	20.69 ± 0.17	5.25 ± 0.38	15.44 ± 0.42
	NPK	1.59 ± 0.67	8.82 ± 0.29	3.67 ± 0.52	16.61 ± 0.49	9.57 ± 0.73	7.04 ± 0.94	8.42 ± 0.56	28.32 ± 0.65	1.91 ± 0.35	26.41 ± 0.77	12.00 ± 0.25	2.37 ± 0.36	9.63 ± 0.47	20.57 ± 0.22	6.25 ± 0.75	14.31 ± 0.38
	2NPK	1.80 ± 0.74	9.56 ± 0.34	4.33 ± 0.41	13.46 ± 0.54	7.14 ± 0.38	6.32 ± 0.71	8.29 ± 0.66	28.8 ± 0.27	2.12 ± 0.26	26.68 ± 0.41	12.57 ± 0.34	2.33 ± 0.47	10.24 ± 0.62	21.19 ± 0.49	6.36 ± 0.64	14.82 ± 0.87
	NPKOM	2.74 ± 0.75	10.19 ± 0.27	3.95 ± 0.45	11.12 ± 0.31	5.92 ± 0.43	5.20 ± 0.57	8.37 ± 0.41	26.73 ± 0.44	2.21 ± 0.39	24.52 ± 0.64	13.54 ± 0.20	2.07 ± 0.33	11.47 ± 0.41	23.37 ± 0.27	5.81 ± 0.39	17.55 ± 0.51

The error value is the noise level of the chemical shift region beyond 0–220 ppm in each NMR spectrum.

paddy soil and the upland soil, which were mainly reflected by the completely opposite shifts in OCH and alkyl C for each paired fertilizer treatment of the two soils.

The results of the calculated A/O–A ratio and HI index showed that in the paddy soil they were consistently larger in the NPK and 2NPK treatments while lower in the NPKOM treatment than in the Control. In the upland soil, the A/O–A ratio from the NPK and 2NPK treatments was lower and that from the NPKOM was larger than the Control, while for the HI index, the value from the NPK treatment was larger and values from the 2NPK and NPKOM were lower than the Control (Fig. 2).

3.3. Bacterial community composition and its determinants

Chloroflexi (13.5–33.7%), Proteobacteria (11.9–25.8%), and Acidobacteria (13.3–20.9%) were the dominant phyla in both soils across the four treatments (Fig. S1). As shown by the LDA analyses, the response of bacterial community composition to fertilizations differed between the paddy soil and upland soil (Fig. 3). Specifically, three clusters were identified in the paddy soil. In these, the NPK treatment and Control shared a similar community composition, which significantly differed from those in the 2NPK and NPKOM treatments. Meanwhile, in the upland soil, the community compositions of the four treatments were distinctly separated from each other.

The edgeR and SIMPER analyses were performed separately for the paddy soil and upland soil to identify the key OTUs that contributed to the differences induced by long-term fertilizations in each soil (Tables 3 and 4, Datasets S1 and S2). Within the paddy soil, the differences between the Control plus NPK and 2NPK treatments were mainly attributed to the occurrence of Chloroflexi and Nitrospirales members in the 2NPK treatment, and between the Control plus NPK and NPKOM treatment to the higher abundances of members of Proteobacteria, Bacteroidetes, and Anaerolineaceae in the NPKOM treatment (Table 3, Dataset S1). Within the upland soil, the differences between the Control and the chemical fertilizer treatments (i.e., NPK and 2NPK) were attributed to the enrichment of Thermogemmatisporales and Acidobacteriaceae subgroup 1 species in the NPK and 2NPK treatments, between the Control and excessive fertilization treatments (i.e., 2NPK and NPKOM) to the depletion of Acidobacteria subgroup 2 and Solirubrobacterales in the 2NPK and NPKOM treatments. Meanwhile, the NPKOM treatment greatly enhanced the abundances of *Bacillus* sp., *Nitrospira* sp., *Roseiflexus* sp., and unclassified Betaproteobacteria (Table 4, Dataset S2).

Fig. 2. The ratio of alkyl C/O–alkyl C (A/O–A) and hydrophobicity (HI) index of SOC from the paddy soil and upland soil.

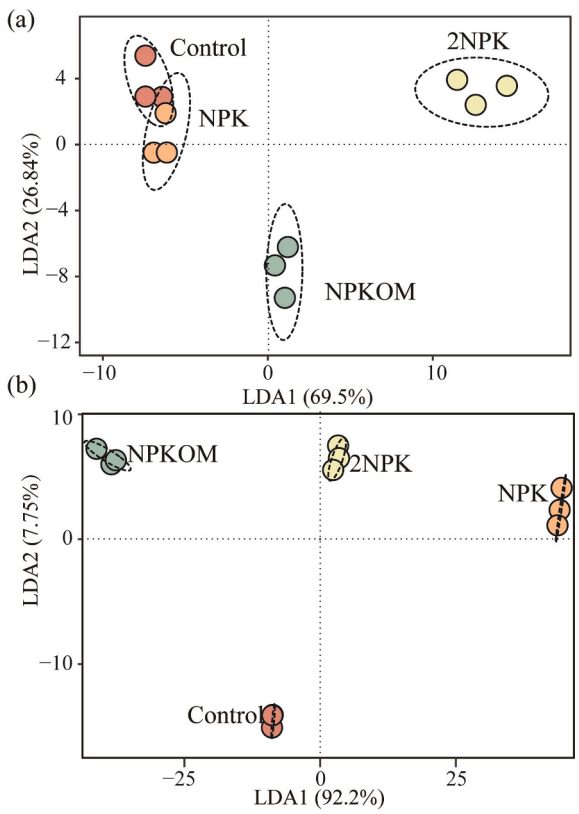

Fig. 3. Linear discriminant analysis (LDA) plot depicting the bacterial communities in the paddy soil (a) and upland soil (b). Dashed circles represent 95% confidence ellipse.

The MRT analyses revealed that NO_3^--N and TN emerged as the primary factors regulating the bacterial community composition in the paddy soil, splitting them into three groups of Control plus NPK, 2NPK, and NPKOM, in which NO_3^--N explained 54.2% of the variation (Fig. 4a). Within the upland soil, separation of four distinct groups were observed under the dominant control of SOC, which explained 75.4% of the variation (Fig. 4b).

3.4. Relationships between the SOC chemical nature and bacterial species

Networks for the paddy soil and upland soil were constructed separately using the OTUs and C groups that differed significantly ($p < 0.05$) among fertilizer treatments, to explore the potential interactions between the bacterial taxa and C functional groups (Fig. 5). Within the paddy soil, the OCH showed negative correlations with unclassified members of Chloroflexi and Nitrospirales, and the CH/CH_2 showed negative correlations with unclassified Proteobacteria and Bacteroidetes members (Fig. 5a). Within the upland soil, the unclassified Thermogemmatisporales showed a negative correlation with CH/CH_2 and a positive correlation with OCH. The unclassified Acidobacteriaceae subgroup 1 showed a negative association with CH/CH_2. The unclassified Acidobacteria subgroup 2 and Solirubrobacterales exhibited positive associations with aromatic C–C. In addition, *Nitrospira* sp., *Roseiflexus* sp., and unclassified Betaproteobacteria presented negative correlations with aromatic C–H and positive correlations with CH/CH_2 (Fig. 5b).

4. Discussion

4.1. Separation of the bacterial community composition among fertilizations and its determinants

The NO_3^--N and TN were identified as the most influential factors modifying the bacterial community composition of the paddy soil (Fig. 4a). The succession of soil bacterial communities was strongly influenced by soil conditions and agricultural management (Wu et al., 2011). Moreover, N availability tended to be more important in paddy soils, and NO_3^--N has been reported as one of the important determinants in many publications (e.g. Chen et al., 2016; Yi et al., 2019). The 2NPK treatment was significantly separated from other treatments under the dominant control of NO_3^--N, and unclassified members of Nitrospirales and Chloroflexi selectively harbored in this treatment (Table 3). Nitrospirales have been reported to be involved in soil nitrification (Starke et al., 2017), promoting the transformation of NH_4^+-N to NO_3^--N. Chloroflexi have been shown to be capable of denitrification (Long et al., 2018), facilitating the reduction of NO_3^--N. The 2NPK treatment had lower NO_3^--N than the other treatments and similar NH_4^+-N to the NPK treatment and Control, while the NPKOM treatment had a higher NH_4^+-N content than the other treatments (Table 1), despite the 2NPK treatment had received more NH_4^+-N from the fast hydrolysis of urea than the other three treatments. These results indicated that the 2NPK treatment had a

Table 3
Results of SIMPER analyses indicating the contribution of specific OTUs to observed dissimilarities of bacterial community between treatments in the paddy soil.

Group A *vs.* B	Ten most influential OTUs	Percent contribution to similarity	Average abundance in group A	Average abundance in group B
Control plus NPK *vs.* 2NPK	Uncl. Anaerolineaceae	1.767	829.0	223.3
	Others	1.463	0.0	501.3
	Uncl. Chloroflexi	1.381	0.0	473.3
	Uncl. Acidobacteriaceae subgroup 1	1.299	0.0	445.3
	Uncl. Acidobacteriaceae subgroup 1	1.186	831.3	424.7
	Uncl. Acidobacteria subgroup 2	0.800	377.0	103.0
	Uncl. Nitrospirales	0.788	0.0	270.3
	Uncl. Ktedonobacterales	0.765	314.3	52.3
	Uncl. Anaerolineaceae	0.718	0.0	246.3
	Uncl. Nitrospirales	0.640	0.0	219.3
Control plus NPK *vs.*NPKOM	Uncl. Acidobacteriaceae subgroup 1	2.327	831.3	296.0
	Uncl. Anaerolineaceae	1.676	829.0	442.3
	Uncl. Acidobacteria subgroup 2	1.270	377.0	84.0
	Uncl. Anaerolineaceae	1.089	81.7	333.0
	Uncl. Ktedonobacterales	0.656	314.3	163.0
	Uncl. Ktedonobacterales	0.585	142.7	7.7
	Uncl. Nitrospiraceae	0.546	377.0	486.7
	Uncl. Proteobacteria	0.542	143.7	268.7
	Uncl. Bacteroidetes	0.537	10.7	134.7
	Uncl. Chloroflexi	0.519	196.3	76.7

Table 4
Results of SIMPER analyses indicating the contribution of specific OTUs to observed dissimilarities of bacterial community between treatments in the upland soil.

Group A *vs.* B	Ten most influential OTUs	Percent contribution to dissimilarity	Average abundance in group A	Average abundance in group B
Control *vs.* NPK	Uncl. Chloroflexi	4.381	1525.0	606.7
	Uncl. Thermogemmatisporales	3.742	159.3	943.7
	Uncl. Chloroflexi	3.632	1380.3	619.0
	Uncl. Holophagae subgroup 7	1.797	445.3	68.7
	Uncl. Acidobacteriaceae subgroup 1	1.546	135.0	459.0
	Uncl. Gemmatimonadaceae	1.482	410.0	99.3
	Uncl. Acidobacteria	1.342	161.3	442.7
	Uncl. Acidobacteriaceae subgroup 1	1.178	171.3	418.3
	Others	1.104	100.0	331.3
	Uncl. Acidobacteria subgroup 2	0.968	1124.3	1209.3
Control *vs.* 2NPK	Uncl. Chloroflexi	3.604	1525.0	348.3
	Uncl. Chloroflexi	2.92	1380.3	426.7
	Uncl. Chloroflexi	1.342	0.0	438.0
	Uncl. Holophagae subgroup 7	1.201	445.3	53.0
	Uncl. Chloroflexi	1.187	0.0	387.3
	Uncl. Acidobacteria subgroup 2	1.100	1124.3	765.0
	Uncl. Gemmatimonadaceae	0.992	410.0	86.3
	Uncl. Solirubrobacterales	0.868	475.3	191.7
	Uncl. Thermogemmatisporales	0.866	159.3	442.0
	Uncl. Acidobacteriaceae subgroup 1	0.721	0.0	235.3
Control *vs.* NPKOM	Uncl. Chloroflexi	3.443	1525.0	76.7
	Uncl. Chloroflexi	3.218	1380.3	26.3
	Uncl. Acidobacteria subgroup 2	2.433	1124.3	101.0
	Bacillus sp.	1.265	126.0	658.3
	Uncl. Solirubrobacterales	1.066	475.3	27.0
	Uncl. Holophagae subgroup 7	0.960	445.3	41.3
	Uncl. Thermogemmatisporales	0.954	405.0	3.7
	Nitrospira sp.	0.923	269.0	657.3
	Uncl. Betaproteobacteria	0.862	18.3	380.7
	Roseiflexus sp.	0.809	0.7	341.0

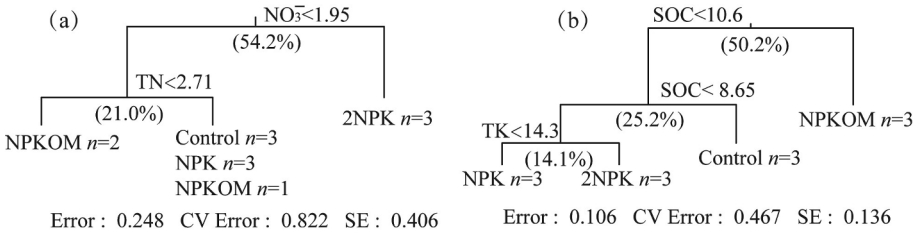

Fig. 4. Multivariate regression tree (MRT) analysis of bacterial communities in the paddy soil (a) and upland soil (b). Numbers under the crosses of each split indicate percentages of variation explained by the split. CV Error represent cross-validation error, SE represent standard error of the tree. Specific treatment regime and the numbers of samples included in the analysis are shown in each split.

greater potential for transforming NH_4^+-N and subsequently NO_3^--N loss through various ways than the other three treatments. The NPKOM treatment was characterized by a significantly higher abundance of Proteobacteria and Bacteroidetes species (Table 3), both of them exhibiting copiotrophic attributes and preferring higher C and N availability conditions (Fierer et al. 2007). The growth of both taxa was reported to be stimulated by manure application (Zhang et al., 2013b; Li et al., 2018).

In the upland soil, SOC was identified as the determinant factor shaping the bacterial community composition (Fig. 4b), consistently with many previous publications (e.g. Marschner et al., 2003; Ng et al., 2014). Both the quantity and quality of SOC played essential roles in establishing the soil bacterial community and metabolic profile (Li et al., 2018). In the present study, the bacterial community composition of the NPKOM treatment distinctly differed from that in the other treatments under the dominant influence of SOC, being enriched with *Nitrospira* sp., *Roseiflexus* sp., *Bacillus* sp., and unclassified Betaproteobacteria (Table 4). It is likely that long-term fertilization with organic manure effectively stimulated the growth of these species, as many studies have addressed their copiotrophic lifestyle that prefer to SOC-rich conditions (Wang et al., 2017, 2019; Chen et al., 2018; Li et al., 2018, 2019). Meanwhile, the NPKOM and 2NPK treatments simultaneously decreased the abundance of unclassified Acidobacteria subgroup 2 and Solirubrobacterales species, which may relate to their oligotrophic lifestyles that flourish in soils with depleted nutrients (Fierer et al., 2007; Shange et al., 2012). Moreover, the chemical fertilizer treatments (i.e., NPK and 2NPK) were enriched with Thermogemmatisporales and Acidobacteriaceae subgroup 1 (Table 4), which have been reported to be selectively enriched in soils treated with mineral fertilizers (Lin et al., 2019; Wang et al., 2019).

4.2. Fertilization-induced changes in SOC chemistry and their associations with the bacterial community composition

Fertilization-induced changes in SOC chemistry differed between the paddy soil and the upland soil, which were mainly reflected by the completely opposite shifts of OCH and alkyl C in each paired fertilizer treatment (Table 2, Fig. 1). The OCH group primarily originates from polysaccharides and represents the most easily degradable C fraction (Kögel-Knabner 2002), while alkyl C is mainly comprised of terminal methyl (CCH_3) and long-chained (ploy)-methylene (CH/CH_2) from lipids, suberins, cutins, or waxes, and represents the recalcitrant C

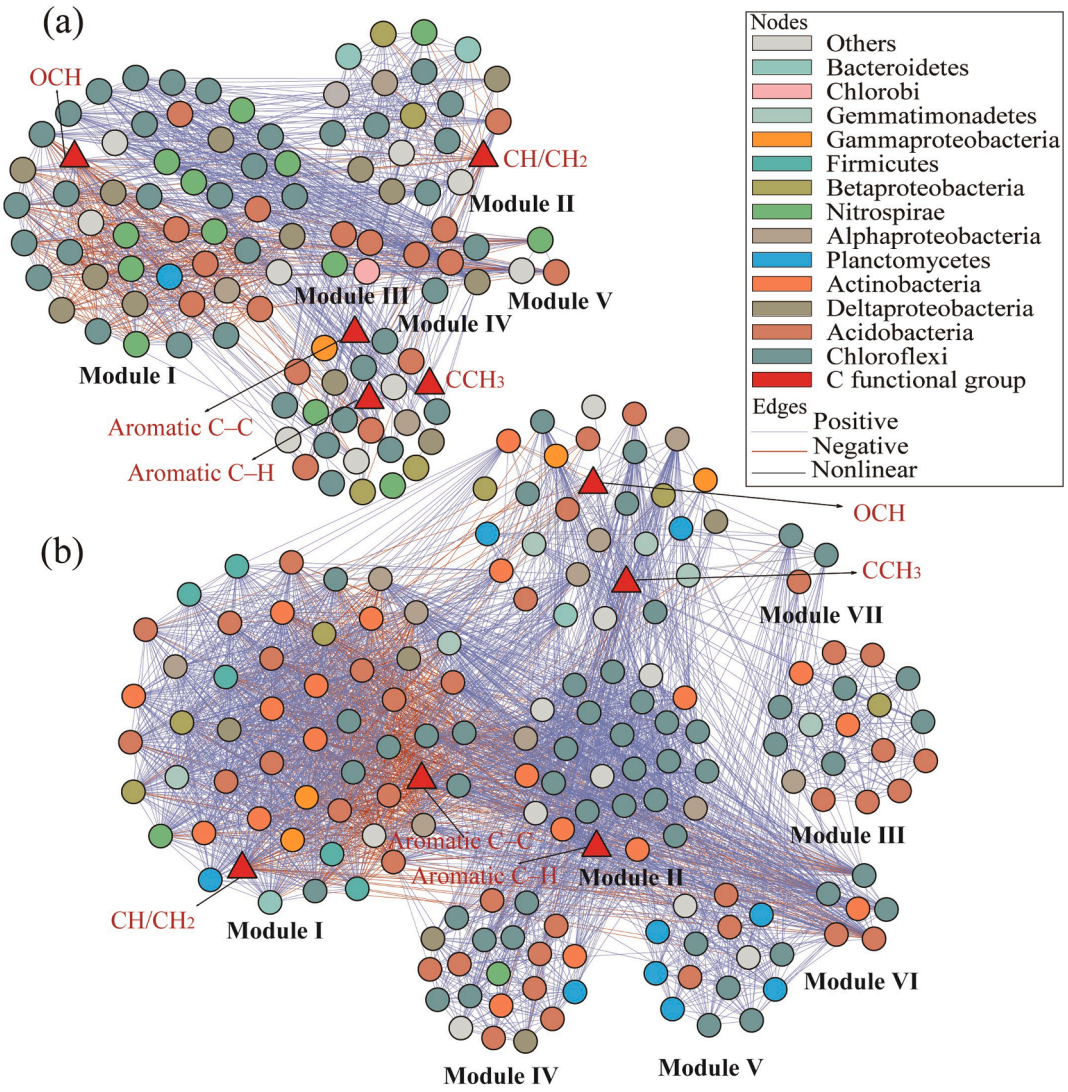

Fig. 5. Network analyses revealing the associations between bacterial taxa and SOC chemical groups in the paddy soil (a) and upland soil (b) under long-term different fertilization regimes. Green line, red line, and gray line represent strong positive linear ($r > 0.8$), strong negative linear ($r < -0.8$), and strong nonlinear (MIC-ρ2 > 0.8) relationships, respectively. Colored nodes signify corresponding OTUs assigned to major phyla and classes. SOC functional groups are indicated with triangle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fraction (Baldock et al., 1992). In the present study, the NPK, 2NPK, and NPKOM treatments in the paddy soil increased CCH_3, decreased OCH, and decreased CH/CH_2 contents, respectively, while in the upland soil, the NPK, 2NPK, and NPKOM treatments decreased CH/CH_2, increased OCH, and increased CH/CH_2 contents, respectively.

4.2.1. Paddy soil

The NPK and 2NPK treatments in the paddy soil showed larger A/O–A ratios and HI indexes than the Control (Fig. 2), indicating a greater degree of SOC decomposition (Xu et al., 2017). A higher N availability enhanced the microbial degradation of SOC by improving soil microbial biomass and activity in the paddy soil (Liu et al., 2011; Su et al., 2015). With the proceeding of decomposition, alkyl C increased and O–alkyl C decreased (Baldock et al., 1992). Liu et al. (2018) demonstrated that chemical fertilizer application can enhance the microbial degradation of SOC and promote its chemical stability (Baldock and Preston, 1995), resulting in the selective preservation of recalcitrant C components or the preferential utilization of labile C fractions (Wang et al., 2015).

In the present study, the NPK treatment had similar bacterial community composition, SOC content, and nutritional levels as the Control (Table 1, Fig. 3), resulting in no associations between the NPK-induced changes in the C functional groups and bacterial taxa. However, the NPK treatment exhibited a higher free Fe_2O_3 content and a lower amorphous Fe_2O_3 content than the Control (Table 1), with the former displaying a preferential association with microbial-derived C and the latter tending to associate with plant-derived C (Hall et al., 2018). The CCH_3 and aromatic C–C were considered as microbially originated products (Zhang et al., 2015). We thus inferred that the higher CCH_3 and aromatic C–C contents observed in the NPK treatment of the paddy soil may mainly be attributed to the higher free Fe_2O_3 content, that is, the CCH_3 and aromatic C–C were chemically protected by free Fe_2O_3 and could be selectively preserved during SOC decomposition. Meanwhile, the aromatic C–H was probably a plant-derived compound and appeared to be more decomposable than aromatic C–C (Zhang et al., 2015). The lower aromatic C–H content in the NPK treatment may have derived from an efficient utilization during enhanced decomposition.

The N-cycle related species that were selectively enriched in the 2NPK treatment, including unclassified Chloroflexi and Nitrospirales members, showed negative correlations with the OCH content (Fig. 5a). These species have all been shown to be able to degrade polysaccharides under anaerobic conditions (Ahn et al., 2012; Siniscalchi et al., 2017), likely resulting in the decreased OCH content (Table 2), since OCH

represents the most easily degradable fraction in polysaccharides (Kögel-Knabner, 2002). This suggested that the decreased abundance of OCH observed in the 2NPK treatment may be attributed to the preferential mineralization of the easily degradable OCH fraction by Chloroflexi and Nitrospirales species.

On the contrary, the NPKOM treatment had lower A/O–A ratio and HI index than the Control (Fig. 2), and the recalcitrant CH/CH_2 content decreased in the NPKOM treatment (Table 2), perhaps mainly due to the incorporation of exogenous organic manure. The NPKOM treatment had higher SOC than the Control (Table 1), which may result in the relative depletion of the recalcitrant compounds, as Sun et al. (2013) found that the increased SOC after organic amendment in the paddy soil was primarily stored in the labile C fractions. Moreover, the labile C contained in the fresh manure might have promoted the mineralization of inherent recalcitrant SOC fraction (Blagodatskaya and Kuzyakov, 2008), probably resulting in the depletion of recalcitrant C components. The CH/CH_2 content in the NPKOM treatment was negatively correlated with the Proteobacteria and Bacteroidetes species (Fig. 5a), which were enriched in the NPKOM treatment (Table 3). Although Proteobacteria and Bacteroidetes responded sensitively to labile C addition, their capabilities to degrade recalcitrant SOC biopolymers have also been reported (Nemergut et al., 2008; Ai et al., 2015; Zhan et al., 2018). This suggests that the Proteobacteria and Bacteroidetes species, stimulated by organic manure, may have accelerated the decomposition of refractory alkyl structures in the paddy soil.

4.2.2. *Upland soil*

The NPK and 2NPK treatments in the upland soil had lower A/O–A ratios than the Control (Fig. 2), indicating a lower degree of SOC degradation, and less accumulation of recalcitrant C fraction or greater preservation of labile C fraction, which likely resulted in the lower CH/CH_2 content observed in the NPK treatment and the higher OCH content observed in the 2NPK treatment (Fig. 1, Table 2). However, the CH/CH_2 and OCH contents were correlated with different microbes. Specifically, the CH/CH_2 content showed a negative correlation with Thermogemmatisporales and Acidobacteriaceae subgroup 1 (Fig. 5b), which were enriched in the NPK treatment (Table 4), since they are capable of decomposing recalcitrant plant biopolymers (Barton et al., 2014; Bárta et al., 2017; Zhu et al., 2019). Meanwhile, the 2NPK treatment was enriched with Thermogemmatisporales (Table 4), which had a positive correlation with OCH (Fig. 5b), presumably due to the enhanced degradation of recalcitrant biopolymers, followed by the production of more small organic molecules (e.g., OCH groups) (Li et al., 2020). Moreover, unclassified Solirubrobacterales and Acidobacteria subgroup 2 species, which were significantly depleted in the 2NPK and NPKOM treatments, had positive correlations with the aromatic C–C content (Fig. 5b). As shown in the study of Oh et al. (2017), Solirubrobacterales can selectively degrade hemicellulose within lignocellulose. This degradation process may partially result in the simultaneous decrease of Solirubrobacterales and aromatic C–C, an indicator of lignin (Carvalho et al., 2009), in the 2NPK and NPKOM treatments (Table 2).

The NPKOM treatment had a higher A/O–A ratio than the Control (Fig. 2), and higher CH/CH_2 and lower aromatic C–C and aromatic C–H contents were observed in the NPKOM treatment (Fig. 1, Table 2). Meanwhile, the enriched *Nitrospira* sp., *Roseiflexus* sp., *Bacillus* sp., and unclassified Betaproteobacteria species in the NPKOM treatment exhibited positive correlations with the CH/CH_2 content and negative correlations with the aromatic C–H content (Table 4, Fig. 5b). These taxa have potential roles in the degradation of lignin, phenolic compounds, and/or aromatic hydrocarbons (Martin et al., 2012; Huang et al., 2013; Li et al., 2019), and their enrichment might have contributed to the decrease of aromatic C–H and the accumulation of intermediate product-CH/CH_2 compounds (Geng and Li, 2002).

5. Conclusions

Field experimental evidence, obtained from direct comparisons and without any disturbance from soil parent materials and climate conditions, showed that fertilization-induced changes in the SOC chemical composition in the paddy soil significantly differed from those in the upland soil. This was mainly reflected by the completely opposite shifts of OCH and alkyl C (i.e., CCH_3 and CH/CH_2) in each paired fertilizer treatment of the two soils. We further found that the shifted functional groups in the paddy soil and upland soil were correlated with different bacterial taxa, which were mainly governed by soil N and C, respectively, in the paddy soil and upland soil. Our findings have important implications for improving our ability to regulate SOC chemistry in rice paddies and maize fields through N and C management, respectively.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114876.

References

Ahn, J.H., Song, J., Kim, B.Y., Kim, M.S., Joa, J.H., Weon, H.Y., 2012. Characterization of the bacterial and archaeal communities in rice field soils subjected to long-term fertilization practices. J. Microbiol. 50, 754–765. https://doi.org/10.1007/s12275-012-2409-6.

Ai, C., Liang, G., Sun, J., Wang, X., He, P., Zhou, W., He, X., 2015. Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. Soil Biol. Biochem. 80, 70–78. https://doi.org/10.1016/j.soilbio.2014.09.028.

Angst, G., Messinger, J., Greiner, M., Häusler, W., Hertel, D., Kirfel, K., Kögel-Knabner, I., Leuschner, C., Rethemeyer, J., Mueller, C.W., 2018. Soil organic carbon stocks in topsoil and subsoil controlled by parent material, carbon input in the rhizosphere, and microbial-derived compounds. Soil Biol. Biochem. 122, 19–30. https://doi.org/10.1016/j.soilbio.2018.03.026.

Baldock, J.A., 2007. Composition and cycling of organic carbon in soil. In: Marschner, P., Rengel, Z. (Eds.), Nutrient Cycling in Terrestrial Ecosystems. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 1–35.

Baldock, J.A., Oades, J.M., Waters, A.G., Peng, X., Vassallo, A.M., Wilson, M.A., 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state13C NMR spectroscopy. Biogeochemistry 16, 1–42. https://doi.org/10.1007/BF02402261.

Baldock, J.A., Preston, C.M., 1995. Chemistry of carbon decomposition processes in forests as revealed by solid-state carbon 13 nuclear magnetic resonance. W.W. McFee, J.M. Kelly (Eds.), Carbon Forms and Functions in Forest Soils, Soil Sci. Soc. Am., Madison, WI, pp. 89–117.

Bárta, J., Tahovská, K., Šantrůčková, H., Oulehle, F., 2017. Microbial communities with distinct denitrification potential in spruce and beech soils differing in nitrate leaching. Sci. Rep. 7 https://doi.org/10.1038/s41598-017-08554-1.

Barton, H.A., Giarrizzo, J.G., Suarez, P., Robertson, C.E., Broering, M.J., Banks, E.D., Vaishampayan, P.A., Venkateswaran, K., 2014. Microbial diversity in a Venezuelan orthoquartzite cave is dominated by the *Chloroflexi* (Class Ktedonobacterales) and *Thaumarchaeota* Group I.1c. Front. Microbiol. 5, 615.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45, 115–131. https://doi.org/10.1007/s00374-008-0334-y.

Bierke, A., Kaiser, K., Guggenberger, G., 2008. Crop residue management effects on organic matter in paddy soils — The lignin component. Geoderma 146, 48–57. https://doi.org/10.1016/j.geoderma.2008.05.004.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
Carvalho, A.M., Bustamante, M.M.C., Alcântara, F.A., Resck, I.S., Lemos, S.S., 2009. Characterization by solid-state CPMAS ^{13}C NMR spectroscopy of decomposing plant residues in conventional and no-tillage systems in Central Brazil. Soil Till. Res. 102, 144–150.
Chen, C., Zhang, J.N., Lu, M., Qin, C., Chen, Y.H., Yang, L., Huang, Q.W., Wang, J.C., Shen, Z.G., Shen, Q.R., 2016. Microbial communities of an arable soil treated for 8 years with organic and inorganic fertilizers. Biol. Fert. Soils 52, 455–467.
Chen, R.R., Zhong, L.H., Jing, Z.W., Guo, Z.Y., Li, Z.P., Lin, X.G., Feng, Y.Z., 2017. Fertilization decreases compositional variation of paddy bacterial community across geographical gradient. Soil Biol. Biochem. 114, 181–188.
Chen, W., Teng, Y., Li, Z.G., Liu, W.X., Ren, W.J., Luo, Y.M., Christie, P., 2018. Mechanisms by which organic fertilizer and effective microbes mitigate peanut continuous cropping yield constraints in a red soil of south China. Appl. Soil Ecol. 128, 23–34.
Clarke, K.R., 1993. Nonparametric multivariate analyses of changes in community structure. Australian J. Ecol. 18, 117–143.
Courtier-Murias, D., Simpson, A.J., Marzadori, C., Baldoni, G., Ciavatta, C., Fernández, J. M., López-De-Sá, E.G., Plaza, C., 2013. Unraveling the long-term stabilization mechanisms of organic materials in soils by physical fractionation and NMR spectroscopy. Agric. Ecosyst. Environ. 171, 9–18.
De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecol. Lett. 11, 516–531.
De'ath, G., 2002. Multivariate regression trees: a new technique for modeling species–environment relationships. Ecology 83, 1105–1117.
Dong, W.Y., Zhang, X.Y., Dai, X.Q., Fu, X.L., Yang, F.T., Liu, X.Y., Sun, X.M., Wen, X.F., Schaeffer, S., 2014. Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. Appl. Soil Ecol. 84, 140–147.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Geisseler, D., Linguist, B.A., Lazicki, P.A., 2017. Effect of fertilization on soil microorganisms in paddy rice systems – a meta-analysis. Soil Biol. Biochem. 115, 452–460.
Geng, X., Li, K., 2002. Degradation of non-phenolic lignin by the white-rot fungus *Pycnoporus cinnabarinus*. Appl. Microbiol. Biot. 60, 342–346.
Gougoulias, C., Clark, J.M., Shaw, L.J., 2014. The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. J. Sci. Food Agric. 94, 2362–2371.
Grandy, A.S., Neff, J.C., 2008. Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Sci. Total Environ. 404, 297–307.
Hall, S.J., Berhe, A.A., Thompson, A., 2018. Order from disorder: do soil organic matter composition and turnover co-vary with iron phase crystallinity? Biogeochemistry 140, 93–110.
Huang, X.F., Santhanam, N., Badri, D.V., Hunter, W.J., Manter, D.K., Decker, S.R., Vivanco, J.M., Reardon, K.F., 2013. Isolation and characterization of lignin-degrading bacteria from rainforest soils. Biotechnol. Bioeng. 110, 1616–1626.
IUSS Working Group WRB, 2006. World Reference Base for Soil Resources 2006. FAO, Rome. World Soil Resources Reports No.103.
Jackson, M.L., 1958. Soil Chemical Analysis. Prentice Hall, Englewood Cliffs, NJ.
Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem. 34, 139–162.
Li, D.D., Li, Z.Q., Zhao, B.Z., Zhang, J.B., 2020. Relationship between the chemical structure of straw and composition of main microbial groups during the decomposition of wheat and maize straws as affected by soil texture. Biol. Fert. Soils 54, 999–1012.
Li, D.D., Chen, L., Xu, J.S., Ma, L., Olk, D.C., Zhao, B.Z., Zhang, J.B., Xin, X.L., 2018. Chemical nature of soil organic carbon under different long-term fertilization regimes is coupled with changes in the bacterial community composition in a Calcaric Fluvisol. Biol. Fert. Soils 54, 999–1012.
Li, X.M., Chen, Q.L., He, C., Shi, Q., Chen, S.C., Reid, B.J., Zhu, Y.G., Sun, G.X., 2019. Organic carbon amendments affect the chemodiversity of soil dissolved organic matter and its associations with soil microbial communities. Environ. Sci. Technol. 53, 50–59.
Lin, Y.X., Ye, G.P., Kuzyakov, Y., Liu, D.Y., Fan, J.B., Ding, W.X., 2019. Long-term manure application increases soil organic matter and aggregation, and alters microbial community structure and keystone taxa. Soil Biol. Biochem. 134, 187–196.
Liu, M., Ekschmitt, K., Zhang, B., Holzhauer, S.I.J., Li, Z.P., Zhang, T.L., Rauch, S., 2011. Effect of intensive inorganic fertilizer application on microbial properties in a paddy soil of subtropical China. Agric. Sci. China 10, 1758–1764.
Liu, Y., Hu, C., Hu, W., Wang, L., Li, Z.G., Pan, J.F., Chen, F., 2018. Stable isotope fractionation provides information on carbon dynamics in soil aggregates subjected to different long-term fertilization practices. Soil Till. Res. 177, 54–60.
Ling, N., Zhu, C., Xue, C., Chen, H., Duan, Y.H., Peng, C., Guo, S.W., Shen, Q.R., 2016. Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. Soil Biol. Biochem. 99, 137–149.
Liu, K.L., Huang, J., Li, D.M., Yu, X.C., Ye, H.C., Hu, H.W., Hu, Z.H., 2019. Comparison of carbon sequestration efficiency in soil aggregates between upland and paddy soils in a red soil region of China. J. Integr. Agr. 18, 1348–1359.
Long, X.E., Huang, Y., Chi, H.F., Li, Y.Y., Ahmad, N., Yao, H.Y., 2018. Nitrous oxide flux, ammonia oxidizer and denitrifier abundance and activity across three different landfill cover soils in Ningbo, China. J. Clean. Prod. 170, 288–297.
Lorenz, K., Lal, R., Preston, C.M., Nierop, K.G.J., 2007. Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro) molecules. Geoderma 142, 1–10.
Lützow, M.V., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. Eur. J. Soil Sci. 57, 426–445.
Martin, F., Torelli, S., Le Paslier, D., Barbance, A., Martin-Laurent, F., Bru, D., Geremia, R., Blake, G., Jouanneau, Y., 2012. Betaproteobacteria dominance and diversity shifts in the bacterial community of a PAH-contaminated soil exposed to phenanthrene. Environ. Pollut. 162, 345–353.
Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biol. Biochem. 35, 453–461.
Murase, J., Matsui, Y., Katoh, M., Sugimoto, A., Kimura, M., 2006. Incorporation of ^{13}C-labeled rice-straw-derived carbon into microbial communities in submerged rice field soil and percolating water. Soil Biol. Biochem. 38, 3483–3491.
Nemergut, D.R., Townsend, A.R., Sattin, S.R., Freeman, K.R., Fierer, N., Neff, J.C., Bowman, W.D., Schadt, C.W., Weintraub, M.N., Schmidt, S.K., 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. Environ. Microbiol. 10, 3093–3105.
Ng, E.L., Patti, A.F., Rose, M.T., Schefe, C.R., Wilkinson, K., Smernik, R.J., Cavagnaro, T. R., 2014. Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? Soil Biol. Biochem. 70, 54–61.
Oh, H.N., Lee, T.K., Park, J.W., No, J.H., Kim, D., Sul, W.J., 2017. Metagenomic SMRT sequencing-based exploration of novel lignocellulose-degrading capability in wood detritus from *Torreya nucifera* in Bija forest on Jeju Island. J. Microbiol. Biotechn. 27, 1670–1680.
Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. US Department of Agriculture Circular, Washington, DC, USA.
Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), 1982. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Soil Science Society of America, Madison, WI.
Pansu, M., Gautheyrou, J., 2006. Handbook of Soil Analysis: Mineralogical. Organic and Inorganic Methods, Springer, Berlin, Heidelberg, New York.
Parsons, S.A., Congdon, R.A., Lawler, I.R., 2014. Determinants of the pathways of litter chemical decomposition in a tropical region. New Phytol. 203, 873–882.
Reshef, D.N., Reshef, Y.A., Finucane, H.K., Grossman, S.R., McVean, G., Turnbaugh, P.J., Lander, E.S., Mitzenmacher, M., Sabeti, P.C., 2011. Detecting novel associations in large data sets. Science 334, 1518–1524.
Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504.
Sheng, R., Qin, H., O'Donnell, A.G., Huang, S., Wu, J., Wei, W., 2015. Bacterial succession in paddy soils derived from different parent materials. J. Soil. Sediment. 15, 982–992.
Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., Newman, R.H., 1994. The removal of magnetic-materials from surface soils – a solid-state 13C CP/MAS NMR Study. Aust. J. Soil Res. 32, 1215–1229.
Singh, B., Rengel, Z., 2007. The role of crop residues in improving soil fertility. In: Marschner, P., Rengel, Z. (Eds.), Nutrient Cycling in Terrestrial Ecosystems. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 183–214.
Siniscalchi, L.A.B., Leite, L., Oliveira, G., Chernicharo, C.A.L., de Araujo, J.C., 2017. Illumina sequencing-based analysis of a microbial community enriched under anaerobic methane oxidation condition coupled to denitrification revealed coexistence of aerobic and anaerobic methanotrophs. Environ. Sci. Pollut. R. 24, 16751–16764.
Shange, R.S., Ankumah, R.O., Ibekwe, A.M., Zabawa, R., Dowd, S.E., 2012. Distinct soil bacterial communities revealed under a diversely managed agroecosystem. PLoS ONE 7, e40338.
Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555–569.
Starke, R., Müller, M., Gaspar, M., Marz, M., Küesel, K., Totsche, K.U., von Bergen, M., Jehmlich, N., 2017. Candidate Brocadiales dominates C, N and S cycling in anoxic groundwater of a pristine limestone-fracture aquifer. J. Proteomics 152, 153–160.
Su, J.Q., Ding, L.J., Xue, K., Yao, H.Y., Quensen, J., Bai, S.J., Wei, W.X., Wu, J.S., Zhou, J. Z., Tiedje, J.M., Zhu, Y.G., 2015. Long-term balanced fertilization increases the soil microbial functional diversity in a phosphorus-limited paddy soil. Mol. Ecol. 24, 136–150.
Sun, Y.N., Huang, S., Yu, X.C., Zhang, W.J., 2013. Stability and saturation of soil organic carbon in rice fields: evidence from a long-term fertilization experiment in subtropical China. J. Soil. Sediment. 13, 1327–1334.
Tian, W., Wang, L., Li, Y., Zhuang, K.M., Li, G., Zhang, J.B., Xiao, X.J., Xi, Y.G., 2015. Responses of microbial activity, abundance, and community in wheat soil after three years of heavy fertilization with manure-based compost and inorganic nitrogen. Agr. Ecosyst. Environ. 213, 219–227.
Wang, H., Liu, S.R., Chang, S.X., Wang, J.X., Shi, Z.M., Huang, X.M., Wen, Y., Lu, L.H., Cai, D.X., 2015. Soil microbial community composition rather than litter quality is linked with soil organic carbon chemical composition in plantations in subtropical China. J. Soil. Sediment. 15, 1094–1103.
Wang, H., Liu, S.R., Wang, J.X., Shi, Z.M., Xu, J., Hong, P.Z., Ming, A.G., Yu, H.L., Chen, L., Lu, L.H., Cai, D.X., 2016. Differential effects of conifer and broadleaf litter

inputs on soil organic carbon chemical composition through altered soil microbial community composition. Sci. Rep.-UK 6, 27097.
Wang, J.C., Song, Y., Ma, T.F., Raza, W., Li, J., Howland, J.G., Huang, Q.W., Shen, Q.R., 2017. Impacts of inorganic and organic fertilization treatments on bacterial and fungal communities in a paddy soil. Appl. Soil Ecol. 112, 42–50.
Wang, L.M., Huang, D.F., Wang, F., Li, Q.H., He, C.M., Liu, C.L., Huang, Y.B., 2019. Bacterial communities in acid tea soils treated for 10 years with chemical *vs.* integrated fertilizers. Commun. Soil Sci. Plan. 50, 307–320.
Weller, S., Kraus, D., Ayag, K., Wassmann, R., Alberto, M., Butterbach-Bahl, K., Kiese, R., 2015. Methane and nitrous oxide emissions from rice and maize production in diversified rice cropping systems. Nutr. Cycl. Agroecosys. 101, 37–53.
Wissing, L., Kölbl, A., Häusler, W., Schad, P., Cao, Z.H., Kögel-Knabner, I., 2013. Management-induced organic carbon accumulation in paddy soils: the role of organo-mineral associations. Soil Till. Res. 126, 60–71.
Wu, M., Wei, S.P., Liu, J., Liu, M., Jiang, C.Y., Li, Z.P., 2019. Long-term mineral fertilization in paddy soil alters the chemical structures and decreases the fungistatic activities of humic acids. Eur. J. Soil Sci. 70, 776–785.
Wu, M.N., Qin, H.L., Chen, Z., Wu, J.S., Wei, W.X., 2011. Effect of long-term fertilization on bacterial composition in rice paddy soil. Biol. Fert. Soils 47, 397–405.
Xu, J.S., Zhao, B.Z., Chu, W.Y., Mao, J.D., Olk, D.C., Zhang, J.B., Wei, W.X., 2017. Evidence from nuclear magnetic resonance spectroscopy of the processes of soil organic carbon accumulation under long-term fertilizer management. Eur. J. Soil Sci. 68, 703–715.
Xu, J.S., Zhao, B.Z., Li, Z.Q., Chu, W.Y., Mao, J.D., Olk, D.C., Zhang, J.B., Xin, X.L., Wei, W.X., 2019. Demonstration of chemical distinction among soil humic fractions using quantitative solid-state ^{13}C NMR. J. Agr. Food Chem. 67, 8107–8118.
Yan, X., Zhou, H., Zhu, Q.H., Wang, X.F., Zhang, Y.Z., Yu, X.C., Peng, X., 2013. Carbon sequestration efficiency in paddy soil and upland soil under long-term fertilization in southern China. Soil Till. Res. 130, 42–51.
Yi, X.M., Yi, K., Fang, K.K., Gao, H., Dai, W., Cao, L.K., 2019. Microbial community structures and important associations between soil nutrients and the responses of specific taxa to rice-frog cultivation. Front. Microbiol. 10, 2206.
Zhan, Y.S., Liu, W.J., Bao, Y.Y., Zhang, J.W., Petropoulos, E., Li, Z.P., Lin, X.G., Feng, Y. Z., 2018. Fertilization shapes a well-organized community of bacterial decomposers for accelerated paddy straw degradation. Sci. Rep.-UK 8, 7981.
Zhang, J.C., Zhang, L., Wang, P., Huang, Q.W., Yu, G.H., Li, D.C., Shen, Q.R., Ran, W., 2013a. The role of non-crystalline Fe in the increase of SOC after long-term organic manure application to the red soil of southern China. Eur. J. Soil Sci. 64, 797–804.
Zhang, Q., Sun, J., Liu, S.Z., Wei, Q.P., 2013b. Manure refinement affects apple rhizosphere bacterial community structure: a study in sandy soil. PLoS ONE 8, e76937.
Zhang, Y.L., Yao, S.H., Mao, J.D., Olk, D.C., Cao, X.Y., Zhang, B., 2015. Chemical composition of organic matter in a deep soil changed with a positive priming effect due to glucose addition as investigated by ^{13}C NMR spectroscopy. Soil Biol. Biochem. 85, 137–144.
Zhou, P., Pan, G.X., Spaccini, R., Piccolo, A., 2010. Molecular changes in particulate organic matter (POM) in a typical Chinese paddy soil under different long-term fertilizer treatments. Eur. J. Soil Sci. 61, 231–242.
Zhu, F.X., Zhu, C.Y., Zhou, D.M., Gao, J., 2019. Fate of di (2-ethylhexyl) phthalate and its impact on soil bacterial community under aerobic and anaerobic conditions. Chemosphere 216, 84–93.