

Article

Microbial Communities and Soil Respiration during Rice Growth in Paddy Fields from Karst and Non-Karst Areas

Junbo Zhou ¹ , Zhenjiang Jin ^{1,2,3,*}, Wu Yuan ¹, Weijian Chen ¹, Xuesong Li ¹, Liyuan Xiong ¹ and Guanwen Cheng ^{1,2,3}

¹ Environmental Science and Engineering College, Guilin University of Technology, Guilin 541004, China; junbozhou0901@163.com (J.Z.)

² Guangxi Key Laboratory of Environmental Pollution Control Theory and Technology, Guilin University of Technology, Guilin 541004, China

³ Collaborative Innovation Center for Water Pollution Control and Water Safety in Karst Area, Guilin University of Technology, Guilin 541004, China

* Correspondence: 2004041@glut.edu.cn; Tel.: +86-773-13647737636; Fax: +86-773-5891059

Abstract: Soil microorganism and their relationships with soil respiration in paddy systems in karst areas (KA) of southern China is important for understanding the mechanisms of greenhouse gas emission reduction. Soils were collected from the tillage layer (0–20 cm) during the rice growing season from KA and non-karst areas (NKA) (red soils) from the Guilin Karst Experimental Site in China. Community structures and inferred functionalities of bacteria and fungi were analyzed using the high-throughput sequencing techniques, FAPROTAX and FUNGuild. A bacterial–fungal co-occurrence network was constructed and soil respiration was measured using dark box-gas chromatography and built their relationships. The results indicated that soil respiration was significantly lower in KA than in NKA. Principal component analysis indicated that bacterial and fungal community structures significantly differed between KA and NKA. The OTU ratio of fungi to bacteria (F/B) was positively correlated with soil respiration ($p = 0.044$). Further, the key network microorganisms were OTU69 and OTU1133 and OTU1599 in the KA. Soil respiration negatively correlated with Acidobacteria Gp6, dung saprotroph-endophyte-litter saprotroph-undefined saprotroph, aerobic nitrite oxidizers and nitrifier in KA ($p < 0.05$). Overall, this study demonstrated that soil respiration was reduced when soil microorganisms shifted from bacterial to fungal dominance during the rice growing season in KA.

Keywords: high-throughput sequencing; FAPROTAX and FUNGuild; carbon dioxide emission flux; the ratio of fungi to bacteria; functional group; co-occurrence network



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1. Introduction

Karst ecosystem is an ecosystem restricted by geological background which accounts for one-third of the national land area in China [1]. In karst area (KA) with high temperature and humidity, the dissolution of carbonate brings a large amount of calcium ion (Ca^{2+}) and HCO_3^- into the soil. HCO_3^- combines with H^+ in the water to generate Carbon dioxide (CO_2), which increases soil CO_2 concentration [2]. The carbonate weathering consumes CO_2 and represent large sinks for atmospheric CO_2 that can influence global carbon balance [3,4]. Thereby, carbon cycles during the weathering of carbonate rocks are very important [5], and biological activities catalyze and regulate this process of weathering, thus resulting in alkaline soils rich in calcium [6]. This process also alters soil microbial communities involved in carbon cycling within KA.

CO_2 is the most important greenhouse gases (GHG) [7] and the global CO_2 imbalance is one of the most critical problems for the global carbon cycle [8]. Soil respiration was an important process of CO_2 emission and initially used to describe soil metabolic processes [9], and soil aeration, temperature, moisture, and microbial communities influence respiration [10]. Microbial respiration accounts for more than 80% of all soil respiration [11],

which indicated that microbes were the main contributor to soil CO₂ emissions [12]. In general, long-term inundation slows down the degradation process of soil organic matter in paddy field, which is conducive to the accumulation of soil organic carbon (SOC). The degradable organic carbon in paddy soil increase aboveground and underground biomass of rice [13], thus enhancing the outflow of root exudates and increasing the microbial biomass [14]. Both decreasing the ratio of carbon to nitrogen [15] and increasing the exogenous organic matter [16] retard soil respiration and increase SOC accumulation in paddy soils. The soil characterized by neutral alkalinity, the metal ions (including Ca²⁺) can not only combine with inorganic carbon [17,18], but also with carboxyl group of soil organic matter, which changed the microbial community and its utilization of organic matter, thus affecting soil respiration [14,19]. Consequently, soil respiration and its relation with microbe in rice fields of KA are critical for the accumulation and stability of SOC.

Both bacteria and fungi are important microbial taxa that synergistically interact with soil respiration during rice cultivation [20,21] and determine the carbon sequestration potential of paddy fields [22]. The inter-roots of rice colonization can rapidly limit oxygen and establish anaerobic zones that lead to some microbial populations performing the anaerobic respiration of nitrates and carbonates, thereby accelerating the release of photosynthetically fixed carbon through roots into the surrounding soils [23]. The input of carbon substrates drives the structural and functional changes in soil microbial communities, making the assimilated carbon of microorganisms more stable than aboveground and root-derived carbon [24].

SOC played an important pole in shaping the pattern of soil bacteria and fungi community structures [25], which indicated that increasing biomasses of root and litter stimulated microbial growth in KA [26]. In addition, bacterial and fungal populations are able to increase the stability of calcareous SOC [27] that might reduce the decomposition of soil organic matter. For example, *Flavobacterium* and *Lysobacter* dominated the karst area communities and exhibited relative abundances of 1.24–14.73%, which *Flavobacterium* can decompose organic matter and *Lysobacter* can synthesize organic matter using CO₂ as a carbon source [25]. Further, numerous bacterial and fungal taxa (such as *Bradyrhizobium*, *Herbaspirillum*, *Cellulomonas*, *Blastococcus*, some endophytes and ectomycorrhizae) that are symbiotic with moss plants in KA are able to increase the peroxidase activity of mosses, thereby enhancing the photosynthetic efficiency of the plants [28]. Thus, bacteria and fungi in karst ecosystem play critical roles in dynamic changes of carbon fixation and CO₂ emissions. In previous studies, we reported that soil bacterial communities of rice field in KA are considerably different with those in NKA at the same latitude, which is mainly attributed to the main ecological factors such as SOC, total nitrogen, pH and so on [25]. Our study also found that there are significant differences in the bacterial community structure, key groups, and functional groups across the three particle size aggregates between KA and NKA [29]. At the same time, we monitored the soil respiration of paddy fields during fallow and found that the soil respiration in KA was far lower than that in NKA (Supplementary Figure S1). During rice growth, soil microbial community structure may be more variable due to the influence of aboveground litter, roots and their secretions, which will affect soil respiration [30–32]. However, if there was difference in the soil respiration of paddy field during rice growth between KA and NKA? Which specific microorganisms are closely related to soil respiration? and which are the top microorganisms in the two different areas? The above problems are rarely reported.

In this study, the karst experimental site in Maocun village of Guangxi Zhuang Autonomous Region, China was as research site. The paddy fields across the entire rice growing period in KA and NKA were selected to investigate: (1) dynamic changes in bacterial and fungal abundances during rice growth using real-time PCR and high-throughput sequencing; (2) in situ soil respiration in rice fields across the growth season using static box-meteorological chromatography; (3) predicted functional groups of bacteria and fungi; and (4) co-occurrence networks of fungal and bacterial communities. These studies were used to analyze the relationship between major microbial groups and soil respiration and

to help us understand the process and mechanism of microbial community affecting soil respiration in KA during rice growth.

2. Materials and Methods

2.1. Soil Collection and Gas Sampling

2.1.1. Soil Sample Collection and Soil Physicochemical Properties Analysis

From June to September 2019, rice fields from a typical karst area (KA) (25°08′30″ N, 110°31′28″ E) and a non-karst area (NKA) (25°10′51″ N, 110°31′35″ E) were selected as studying fields within the karst experimental site in Maocun village of Guangxi Zhuang Autonomous Region, China. The soil type in the KA is a limestone brown soil, whereas the soil type in NKA is a silicate red soil (Soil forming parent material are mainly sandstone and granite). Each field was divided into three replicate plots with the same area (KA: 42.20 m² and NKA: 46.67 m²) using by ridge. Each plot was subjected to uniform irrigation, fertilization, and management practices. Three random sampling points were used in each plot. Paddy fields were planted with single-season rice, and the rice growing period was 94 d. Compound fertilizers (N-P₂O₅-K₂O) containing 18% each of nitrogen, phosphorus and potassium were applied as base fertilizer to the fields at 7.56 kg (KA) and 8.53 kg (NKA) before transplanting seedlings. In addition, 5.70 kg (KA) and 6.40 kg (NKA) of compound fertilizer were applied in each plot as follow-up fertilizer on 14 July. A total of 1 kg of soil was collected from each sample point at a sampling depth of 0–20 cm; the soil samples from the three sampling points were equally and uniformly mixed into a single 3 kg sample. A total of 48 soil samples were collected on eight dates with intervals of about 14 d (with the exception of the first sampling interval of 7 d). The sample from 4 June represented the rice planting date, 12 June was from the seedling stage, 26 June and 10 July were from the tillering stage, 24 July was from the nodulation stage, 7 August was from the gestation stage, 21 August was from the tasseling stage, and 4 September was from the maturity stage.

Soil physicochemical properties were analyzed by routine methods [33]. Briefly, soil moisture content was measured using the drying method. pH was measured using CO₂-free distilled water as the leaching agent after mixing soil with water at a 1:2.5 ratio, followed by measurement with a precision pH meter (model: IS128C). SOC was measured using the sulfuric acid potassium dichromate external heating method. Soil total nitrogen (TN) and hydrolyzed nitrogen (AN) measured using the concentrated sulfuric acid digestion-Kjeldahl method and the alkali hydrolysis diffusion method. Total phosphorus (TP) and available phosphorus (AP) determined by the sodium carbonate melting method and the hydrochloric acid-ammonium fluoride method. Soluble organic carbon (DOC) was extracted by shaking a soil-water mix (at a ratio of 10:1), filtering through a 0.45 µm fiber membrane, and analysis on a multi N/C3100 total organic carbon analyzer (Analytik Jena, Jena, Germany). Cation exchange capacity (CEC) was measured using the EDTA-ammonium fast method. An optima 7000 DV inductively coupled plasma emission spectrometer (Perkin Elmer, Waltham, MA, USA) (ICP-OES) was used to measure the exchangeable calcium (Ca²⁺) and magnesium (Mg²⁺) ion concentrations.

2.1.2. Measurement of Soil Respiration

Static dark boxes with 50 cm × 50 cm × 50 cm were used as a field gas collection device, with the boxes raised as plant heights changed. The box was constructed polyethylene (PVC) covered with foam and reflective material and had a fan for mixing gases inside the box installed on the top along with a gas collection port. The gas was collected in a vacuum bag using a syringe from 9:00 to 9:30 on the above-mentioned soil sample collection date. Each sample collection point needs to be separated by 10 min for continuous gas collection. Temperature was measured with a thermometer (JM624, Guangzhou, China) at 5 cm depth. Three rice plants were randomly collected from each sample site during rice harvesting and then dried in oven at 105 °C until achieving. Gas samples were immediately brought to the laboratory for quantification of CO₂ using an Agilent 7890 B gas chromatograph-mass

spectrometer (Agilent, Santa Clara, CA, USA) with a hydrogen flame ion detector (FID). An external standard working curve was plotted after running every 48 samples. The gas emission rate (dc/dt) was derived from the slope of four consecutive sample concentration values using linear regression analysis. Greenhouse gas emission flux was calculated using the following equation:

$$F = H \frac{MP}{R(273 + T)} \frac{dc}{dt} \quad (1)$$

where F is the gas emission flux (units of $\text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$); H is the height of the sampling box (m); M is the molar mass of gas ($\text{g} \cdot \text{mol}^{-1}$); P is the air pressure at the sampling point (Pa); R is the universal gas constant ($8.314, \text{Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$); T is the average temperature inside the box at the time of sampling ($^{\circ}\text{C}$) and dc/dt is the gas emission rate ($\text{uL} \cdot \text{L}^{-1} \cdot \text{min}^{-1}$).

Heterotrophic respiration (R_h) was then calculated using the following equations:

$$NPP = NPP_{\text{seeds}} + NPP_{\text{straw}} + NPP_{\text{roots}} + NPP_{\text{apoplast}} + NPP_{\text{root sediment}} \quad (2)$$

$$GPP = NPP + R_a \quad (3)$$

$$R_h = R_e - R_a \quad (4)$$

where NPP is the total carbon added to the above- and below-ground portions of the plant during the entire growth cycle ($\text{kg} \cdot \text{hm}^{-2}$); NPP_{seeds} and NPP_{straw} are the estimated biomass from the harvested plants after drying, while NPP_{root} , NPP_{apoplast} and $NPP_{\text{root sediment}}$ were estimated by referencing previous studies [30–32]. The equation assumed an above-ground/root system ratio for rice of 1.0/0.1, and apoplankton coverage of 5% and 8% of the upper and root dry biomass, respectively, and root sediment accounted for 15% of the total plant biomass. In Equation (3), GPP is total primary productivity, R_a is autotrophic respiration (unit: $\text{kg} \cdot \text{hm}^{-2}$), and the NPP/GPP ratio is 0.58 [34]. In Equation (4), R_e is ecosystem respiration and R_h is heterotrophic respiration ($\text{kg} \cdot \text{hm}^{-2}$).

The cumulative greenhouse gas (single-season rice) emissions were calculated as follows:

$$E_c = \left[\frac{F_1 + F_n}{2} + \sum_{i=1}^n \left(\frac{F_i + F_{i+1}}{2} \right) \times (t_{i+1} - t_i) \right] \times 24 \times 0.01 \times a \quad (5)$$

where E_c is cumulative greenhouse gas (single-season rice) emissions ($\text{kg} \cdot \text{hm}^{-2}$); n is the number of observations during a single rice season; i is the sampling interval, F_i and F_{i+1} are the GHG emission fluxes ($\text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) at the i th and $i + 1$ th sampling, respectively; F_1 and F_n are the GHG emission fluxes at the first and last sampling times, respectively; t_{i+1} and t_i are the time intervals (d) between the $i + 1$ th and i th sampling; and a is a conversion factor of 98/94.

The cumulative net CO_2 emission, F_{CO_2} , was calculated as follows:

$$F_{\text{CO}_2} = E_c - R_a \quad (6)$$

where F_{CO_2} is the cumulative CO_2 emission minus plant autotrophic respiration ($\text{kg} \cdot \text{hm}^{-2}$).

2.2. High-Throughput DNA Sequencing

The genomic DNA of total 48 soil samples was extracted using an EZNA Soil DNA extraction kit (Omega, Norcross, GA, USA) according to the manufacturer's instructions (0.5 g per sample). The V3-V4 and ITS3-ITS4 amplicons of 16S rRNA genes and an internal transcribed spacer between ribosomal genes were amplified using a KAPA HiFi Hot Start Ready Mix (2x, TaKaRa Bio Inc., Kusatsu City, Japan). Universal PCR primers (PAGE purified) were used including the bacterial PCR forward primer (CCTACGGGNG-GCWGCAG) and reverse primer (GACTACHVGGGTATCTAATCC) in addition to the fungal forward primer (GCATCGATGAAGAACGCAGC) and fungal reverse primer (TC-CTCCGCTTATTGATATGC) [35,36] (primer sequences provided by Sangon Biotech company (Shanghai, China)). Reactions included 2 μL of DNA ($10 \text{ ng } \mu\text{L}^{-1}$); 1 μL each of PCR

forward and reserve primers (10 μ mol each); 15 μ L of 2 $\times$ KAPA HiFi Hot Start Ready Mix; and total reaction 30 μ L. PCR plates were sealed and PCRs were performed in a thermal cycler (Applied Biosystems 9700, Waltham, MA, USA) using the following steps: pre-denaturation at 94 $^{\circ}$ C for 3 min, followed by denaturation at 95 $^{\circ}$ C for 20 s, 20 s of annealing at 55 $^{\circ}$ C, and a final extension at 72 $^{\circ}$ C for 30 s. The above denaturation, annealing, and extension steps were repeated for 20 cycles. The PCR products were detected gel electrophoresis using a 1% agarose gel in TBE (Tris- H^3BO^3 -EDTA) buffer and stain with ethidium bromide (EB), followed by visualization with UV light. Samples were used to construct libraries using the universal Illumina adapters and indices. Sequencing was then performed on an Illumina MiSeq system (Illumina MiSeq, San Diego, CA, USA). The paired-end (PE) reads were obtained via paired-end sequencing and first combined using the fast length adjustment of short reads (FLASH) software package, which was also used to quality filter the datasets and obtain high-quality sequences for subsequent analysis. Using PRINSEQ to cut off the bases with quality value below 20 in the tail of reads, and set a 10 bp window. If the average quality value in the window is lower than 20, cut off the back-end bases from the window, filter the N-containing sequences and short sequences after quality control and finally filtered out the sequences with low complexity. After sequencing, chimeras were removed using the UCHIME programs. Quality-filtered sequences from each sample were taxonomically classified using the RDP classifier accessed on 28 June 2020. (RDP 16S database: <http://rdp.cme.msu.edu/misc/resources.jsp> and RDP ITS database: <http://rdp.cme.msu.edu/misc/resources.jsp>). A total of 211,930 bacterial sequences with 211,696 valid sequences and 67,106 fungal sequences with 66,988 valid sequences were detected. The datasets generated during the current study have been uploaded to the Sequence Read Archive (SRA), and had the accession number PRJNA763299.

2.3. Data Processing

Data processing was performed using the Excel 2016 software program. Correlation line plots were produced from fungal and bacterial OTUs in addition to fungal/bacterial OTU ratios in association with in situ CO_2 fluxes for the 8 sampling dates. Histograms of fungal and bacterial OTUs numbers, in addition to soil respiration rates, were also established for the 8 and 14 sampling dates, respectively. The above were all using Origin 2017 software. Classes with greater than 1% relative abundance were designated as dominant classes, and the Origin 2017 software established relative abundance histogram. OTUs with greater than 1% relative abundance were designated as dominant OTUs, and the CoNet plug-in for the Cytoscape 3.7.1 software was used to construct a bacterial-fungal co-occurrence network [37]. Four statistical algorithms were used: Pearson's correlation, Spearman correlation, Bray-Curtis dissimilarity and Kullback-Leibler dissimilarity. The Brown method was used to integrate the p values. The data with significant correlation ($p < 0.05$) were selected for subsequent analysis. The Benjamin-Hochberg method was used as the multiple test correction. The MCODE plug-in for Cytoscape 3.7.1 was used to analyze network modularity using default criteria, whereas the Network Analyzer for Cytoscape 3.7.1 was used to identify the number of nodes, number of connected edges, and the connectivity within the network. Functional predictions based on the dominant fungal and bacterial OTUs were inferred using the FUNGuild and FAPROTAX databases, respectively, and the resulting functional profiles were visualized as heat maps using the R Studio 5.3.1 program. All statistical analyses were carried out with SPSS 24.0. Correlation analysis was conducted using Pearson correlation (trigonometric function converted to normal distribution) and Spearman correlation [38]. The abundance difference was conducted with independent sample t -test [39].

3. Results

3.1. Physicochemical Properties of Paddy Soils in Karst and Non-Karst Areas

The 11 physicochemical indicators of paddy soils in KA were significantly higher than those in NKA [Table 1], which indicated that there was significantly difference between KA and NKA.

Table 1. Physicochemical properties of paddy soils in KAs and NKAs.

Site	pH (H ₂ O)	SOC /g·kg ^{−1}	DOC /μg·L ^{−1}	TN /g·kg ^{−1}	AN /mg·kg ^{−1}	TP /g·kg ^{−1}	AP /mg·kg ^{−1}	C/N /g·kg ^{−1}	CEC /cmol·kg ^{−1}	Ca ²⁺ /cmol·kg ^{−1}	Mg ²⁺ /cmol·kg ^{−1}
Karst area	7.40 ± 0.18 a	25.15 ± 1.03 a	261.62 ± 9.22 a	1.74 ± 0.09 a	90.21 ± 1.24 a	1.24 ± 0.02 a	22.04 ± 1.63 a	12.46 ± 0.11 a	13.79 ± 0.42 a	3.89 ± 0.04 a	1.20 ± 0.01 a
Non- karst area	5.76 ± 0.15 b	13.86 ± 1.61 b	202.78 ± 20.46 b	1.50 ± 0.04 b	85.24 ± 0.09 b	0.49 ± 0.01 b	18.63 ± 0.57 b	9.23 ± 0.47 b	6.09 ± 0.17 b	2.36 ± 0.01 b	0.48 ± 0.03 b

Note: Different lowercase letters in the same column indicate statistically significant differences between KA and NKA ($p < 0.05$). Data represent means ± standard deviation. KA, karst areas; NKA, non-karst areas; SOC, soil organic carbon; DOC, dissolved organic carbon; TN, total nitrogen; TP, total phosphorus; AP, Available phosphorus; C/N, ratio of SOC to TN; CEC, Cation exchange capacity; Ca²⁺, exchangeable calcium; magnesium Mg²⁺, exchangeable magnesium.

3.2. In Situ Soil Respiration and Cumulative CO₂ Emissions of Paddy Fields during Rice Growth in KA and NKA

Variation of in situ soil respiration in KA and NKA ranged from 26.86 to 1293.59 mg·m^{−2}·h^{−1} and 62.99 to 1501.21 mg·m^{−2}·h^{−1}, respectively, during rice growing where the temperature ranged from 27 to 36 °C [Figure 1]. Among the 14 sampling times evaluated across the growing period, eight of the in situ CO₂ flux values in KA were significantly lower than those in NKA, while one sample measurement was significantly higher than those in NKA, and five were not significantly different. The total cumulative CO₂ emissions across the entire growth period were 7632.82 kg·hm^{−2} and 9637.66 kg·hm^{−2} in KA and NKA, respectively. Thus, soil respiration of paddy field in KA was generally lower than that in NKA.

3.3. The Abundance of Bacteria and Fungi and the Ratio of Fungi to Bacteria (F/B) in Paddy Field Soils of KA and NKA

Among the 14 sampling times evaluated across the growing period, the bacterial abundance in the KA which was $(87.52 \pm 15.54) \times 10^{10} \sim (242.79 \pm 96.20) \times 10^{10}$ copies·g^{−1} was significantly higher than that in the NKA which had an abundance of $(2.51 \pm 0.54) \times 10^{10}$ copies·g^{−1} $\sim (5.52 \pm 0.87) \times 10^{10}$ copies·g^{−1} [Figure 2a]. The fungal abundance in the KA which was $(17.26 \pm 1.32) \times 10^9$ copies·g^{−1} $\sim (27.73 \pm 0.61) \times 10^9$ copies·g^{−1} was significantly higher than that in the NKA which had an abundance of $(1.90 \pm 0.55) \times 10^9 \sim (13.26 \pm 1.47) \times 10^9$ copies g^{−1} on Jun-26 (tillering stage) [Figure 2b].

Across the entire rice growing period, the F/B was $5.76 \times 10^{-2} \sim 31.1 \times 10^{-2}$ and $1.26 \times 10^{-2} \sim 2.51 \times 10^{-2}$ in KA and NKA, respectively [Figure 3a]. The F/B in KA exhibited significantly higher than those in NKA. The F/B was also significantly negatively correlated with soil respiration when combining the data of both areas ($p = 0.044$) [Figure 3b]. Thus, soil respiration decreases with reducing F/B during the rice growing period.

3.4. Variation in Fungal and Bacterial Communities in Paddy Fields of KA and NKA

PCoA analysis based on Bray-Curtis distances revealed that 50.56% and 13.56% of the fungal community variation was explained by axes 1 and 2, respectively [Figure 4a], while 66.78% and 18.79% of bacterial community variation was explained by axes 1 and 2 [Figure 4b]. Bacterial and fungal communities generally segregated across the ordinations, indicating clear microbial differences between the KA and the NKA.

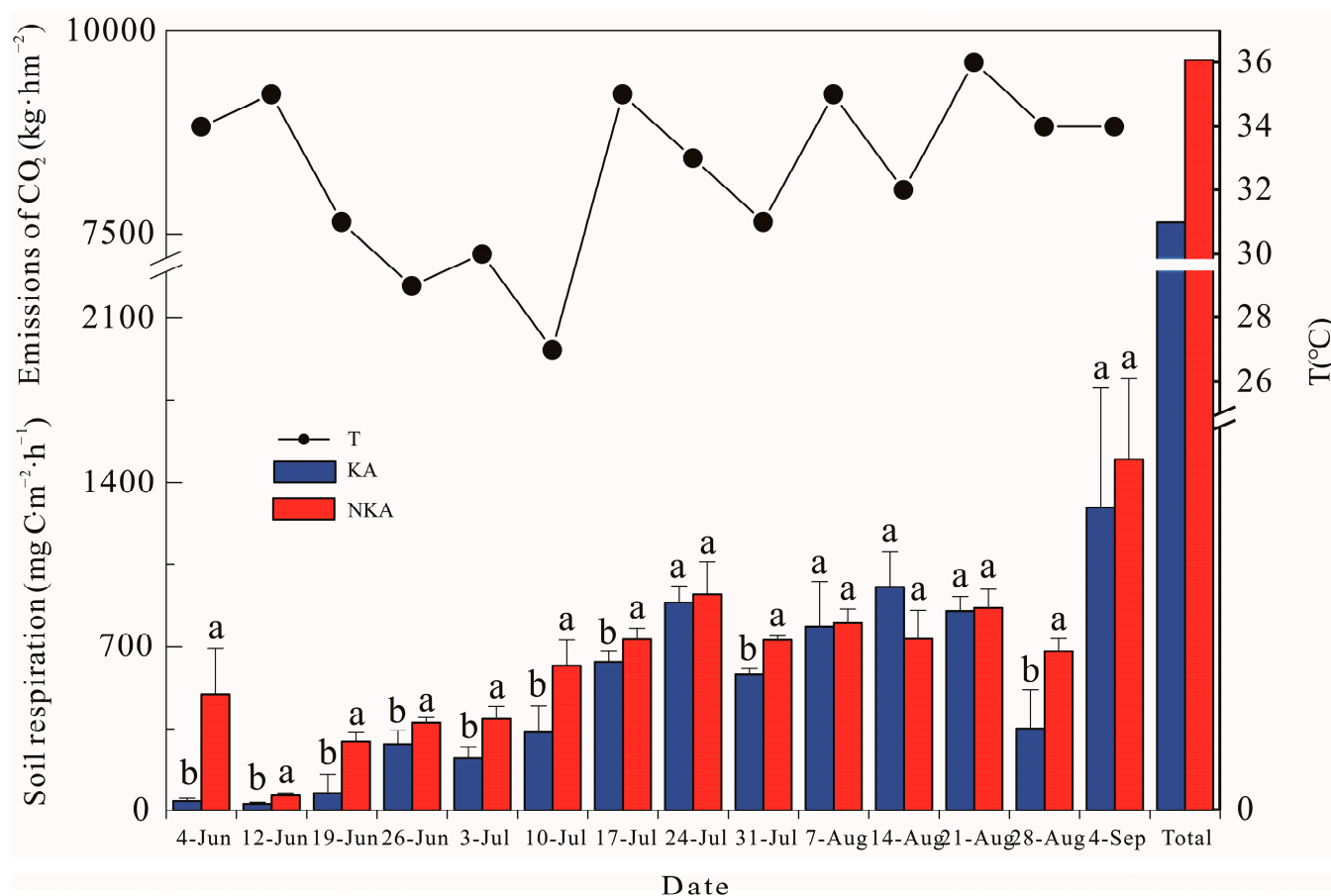


Figure 1. Situ soil respiration and cumulative CO₂ emissions of paddy fields. Note: Different lowercase letters in the same date indicate statistically significant differences between the KA and NKA ($p < 0.05$); KA, karst areas; NKA, non-karst areas.

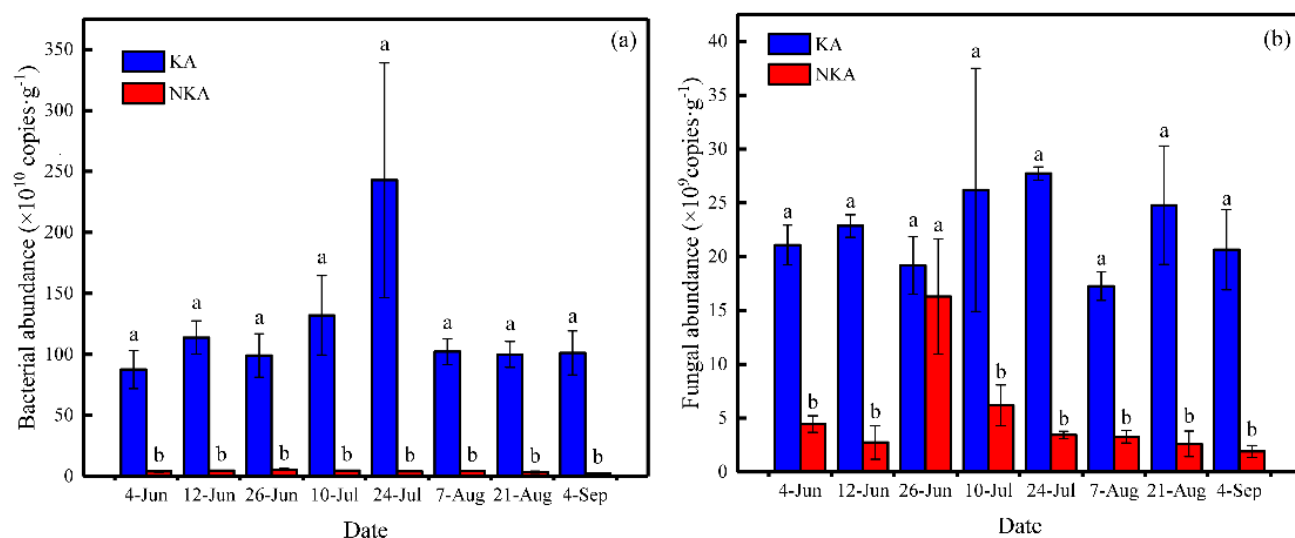


Figure 2. The abundance of soil bacteria (a) and fungi (b) based on real-time PCR during rice growth in the KA and NKA. Note: Different lowercase letters in the same date indicate statistically significant differences between KA and NKA ($p < 0.05$); KA, karst areas; NKA, non-karst areas.

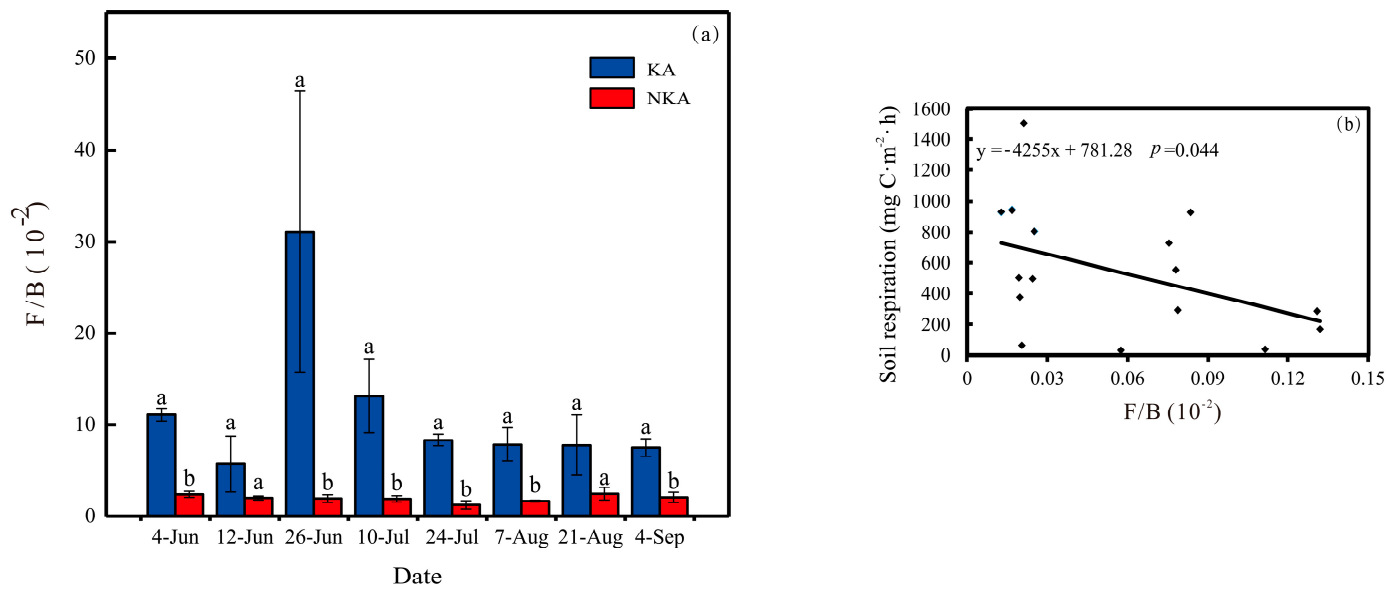


Figure 3. (a) The F/B during rice growth; (b) Correlation between soil respiration and the F/B. Note: Different lowercase letters in the same date indicate statistically significant differences between KA and NKA ($p < 0.05$); KA, karst areas; NKA, non-karst areas; F/B, ratio of fungi to bacteria.

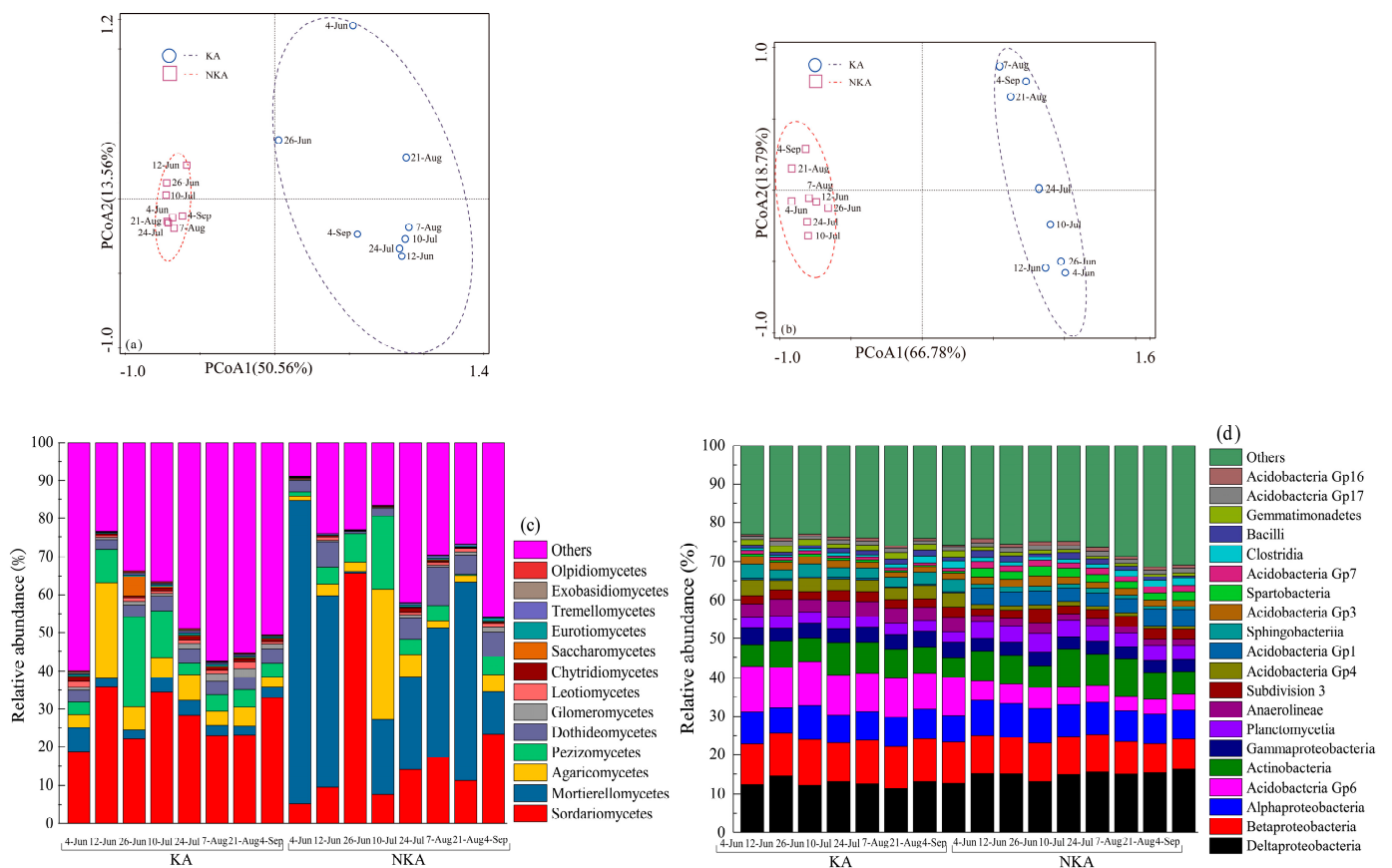


Figure 4. Principal coordinates analysis (PCoA) ordination of fungal (a) and bacterial (b) community variation based on Bray-Curtis distances; The relative abundances of the soil fungal (c) and bacterial (d) community at Class level. Note: KA, karst areas; NKA, non-karst areas.

At the class level, fungi and bacteria had a total of 13 and 20 dominant classes, respectively [Figure 4c,d]. The average relative abundances of fungal Glomeromycetes, Chytridiomycetes and Exobasidiomycetes (0.91%, 0.98% and 0.23%) in KA were significantly higher than those in NKA (0.47%, 0.28% and 0.04%). The average relative abundances of bacterial β -proteobacteria, Acidobacteria Gp6, γ -proteobacteria, Anaerolineae, Acidobacteria Gp4, Sphingobacteriia and Gemmatimonadetes (10.32%, 9.88%, 3.74%, 3.54%, 3.13%, 2.76% and 1.38%) in KA were significantly higher than those (9.07%, 4.54%, 3.18%, 1.78%, 1.01%, 1.10% and 0.73%). The above results showed that there was a significant difference of microorganisms between KA and NKA.

3.5. Co-Occurrence Network Analysis of Fungal and Bacterial Taxa in Rice Field Soils

The distributions of dominant fungal (19 total) and bacterial (34 total) OTUs were used to construct fungal and bacterial co-occurrence networks [Figure 5a]. With bacterial and fungal networks analyzed separately in [Figure 5b,c]. The characteristic path length (CPL) of the fungal-bacterial co-occurrence network was 1.59 cm (Figure 5), with a diameter of 5.00 cm, and a clustering coefficient (CC) of 0.35. The bacterial-only co-occurrence network also contained 53 nodes that were significantly correlated, comprising a total of 502 edges representing 293 and 209 positive and negative correlations, respectively. Thus, putative mutualistic or cooperative relationships between bacterial and fungal taxa (58.36%) were more apparent than putative competitive associations (41.64%). The OTUs with the highest connectivity in the fungal and bacterial networks were identified as top network taxa. The top taxa in KA soil networks included OTU69 (*Emericellopsis*) (connectivity of 31) in addition to OTUs 1599 (Acidobacteria Gp6) and 1133 (Acidobacteria Gp6) (both with a connectivity of 24), which were mutually exclusive. The top fungal and bacterial taxa in NKA were OTU139 (Sordariales) and OTU9 (*Nitrospira*) (connectivity of 26), respectively, which exhibited a positive correlation with one another.

The CPL of the bacterial correlation network was 1.38 cm [Figure 5b], while the diameter was 3.00 cm, and the clustering coefficient (CC) was 0.41. The network consisted of 34 nodes and 329 edges representing 229 positive and 100 negative correlations. The CPL of the network in module 1 was 1.254 cm, and the diameter was 3.00 cm, while the CC was 0.43, and the module comprised 29 nodes and 296 edges (196 positive and 100 negative correlations). The top bacterial OTUs, OTU1599 (Acidobacteria Gp6) and OTU9 (*Nitrospira*), were present in module 1. The top bacteria of KA were OTU1599 (Acidobacteria Gp6), which exhibited positive correlations with OTU1603 (Burkholderiales), OTU3528 (*Phycoccus*), and OTU835 (β -proteobacteria). The top bacteria in NKA included OTU9 (*Nitrospira*), which exhibited mutually exclusive relationships with OTU835 (β -proteobacteria), OTU3528 (*Phycoccus*), and OTU1603 (Burkholderiales).

The internal fungal correlation network CPL was 1.39 cm, its diameter was 3.00 cm, and its CC was 0.28 [Figure 5c]. The network consisted of 19 nodes and 38 edges representing 30 positive and 8 negative correlations. The network CPL for module 1 was 1.23 cm, its diameter was 2.00 cm, and its CC was 0.41. The network consisted of 9 nodes and 27 edges, with 22 positive and 5 negative correlations. The network CPL for module 2 was 1.17 cm, while its diameter was 2.00 cm, and its CC was 0.42. The network consisted of four nodes and four positively correlated edges. The top fungal taxa, OTU69 (*Emericellopsis*) and OTU139 (Sordariales), in KA were present in module 1. Both the OTUs exhibited positive correlations with OTU3379 (*Gaeumannomyces*), OTU81 (Sordariaceae), and OTU63 (*Fusarium*).

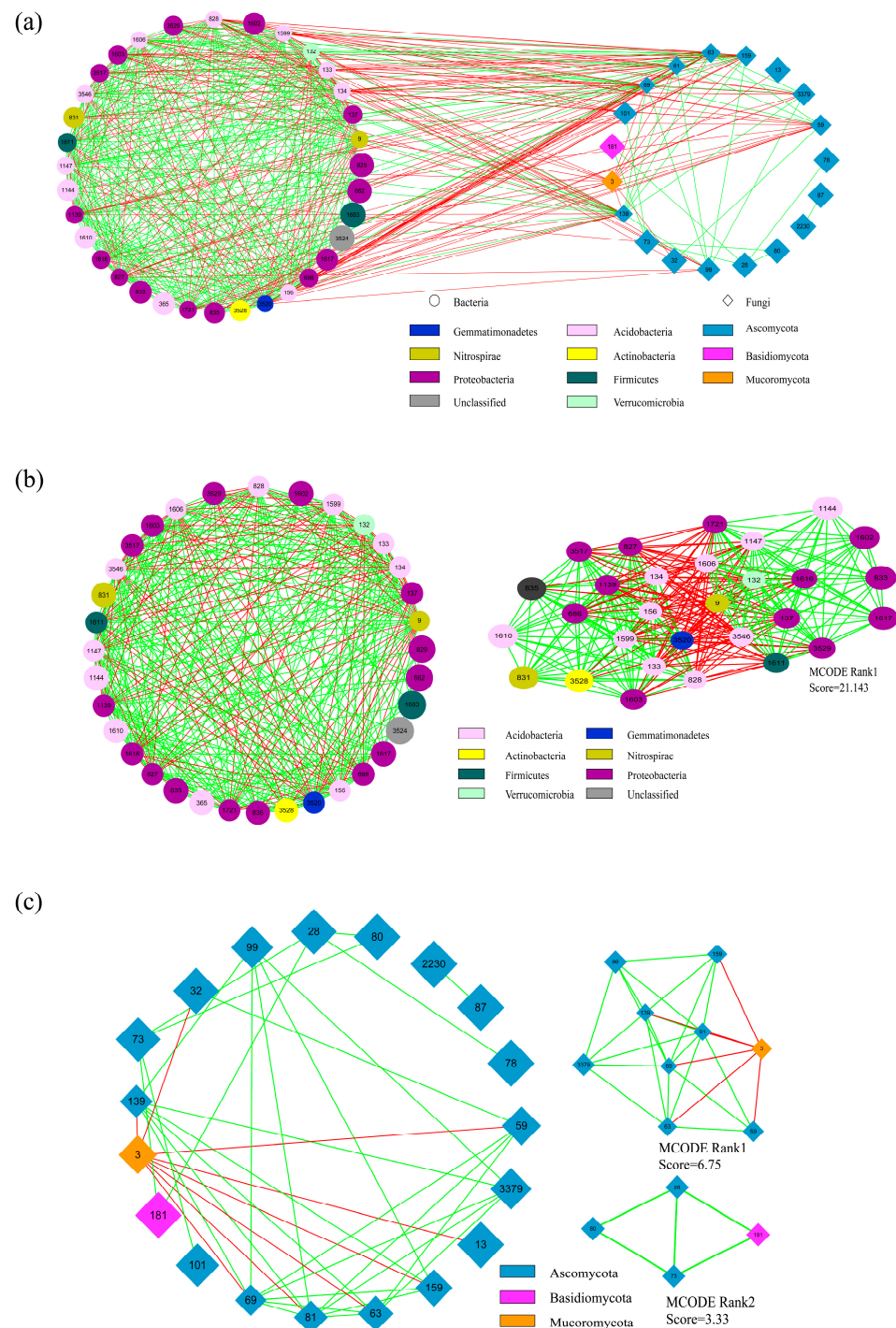


Figure 5. (a): Co-occurrence network analysis of dominant fungal and bacterial taxa; (b): Co-occurrence network and modules comprising dominant bacterial OTUs among KA and NKA soils; (c): Co-occurrence network and modules of dominant fungal OTUs among KA and NKA soils. Note: KA, karst areas; NKA, non-karst areas. Different colored nodes in [Figure 5a] represent different clades of bacteria and fungi, with the value on the node indicating the OTU number for the particular taxa, and node size representing the abundance of the taxa. Different colored nodes in [Figure 5b] represent the different phylum classifications for bacterial OTUs; and the value on the node represents the number of each bacterial OTU, while the size of the node indicates abundance. Different colored nodes [Figure 5c] represent different phylum classifications for fungal OTUs; and the value on the node represents the number of each OTU, while the size of the node indicates abundance. Green edges indicate positive correlations, while red edges indicate negative correlations.

3.6. Correlational Analysis of Soil Respiration with Fungal and Bacterial Functional Groups

The abundances of the dominant fungal and bacterial OTUs were subjected to functional group prediction using FAPROTAX and FUNGuild, respectively [Figure 6a,b], and their correlations with in situ CO₂ fluxes were analyzed [Table 2]. The mean abundance of *Stellatospora* in NKA accounted for 0.07% of that of all fungi and its abundances were positively correlated with in situ CO₂ fluxes. The mean abundance of Acidobacteria Gp6 in KA accounted for 7.05% of that of all bacteria and was negatively correlated with in situ CO₂ fluxes. Among the predicted functional groups, the average abundance of fungal plant pathogens in KA and endomycorrhizal-plant pathogen-undefined saprotrophs in NKA accounted for 2.61% and 0.26% of the average abundance of functional taxa, respectively, and the abundances of both were positively correlated with in situ CO₂ fluxes. In addition, the average abundance of dung saprotroph-endophyte-litter saprotroph-undefined saprotrophs in KA accounted for 0.88% of the total fungal functional groups and was negatively correlated with in situ CO₂ fluxes. The average abundance of aerobic nitrite oxidizers (nitrifiers) were 2.13% of all bacterial functional groups and negatively correlated with in situ CO₂ fluxes.

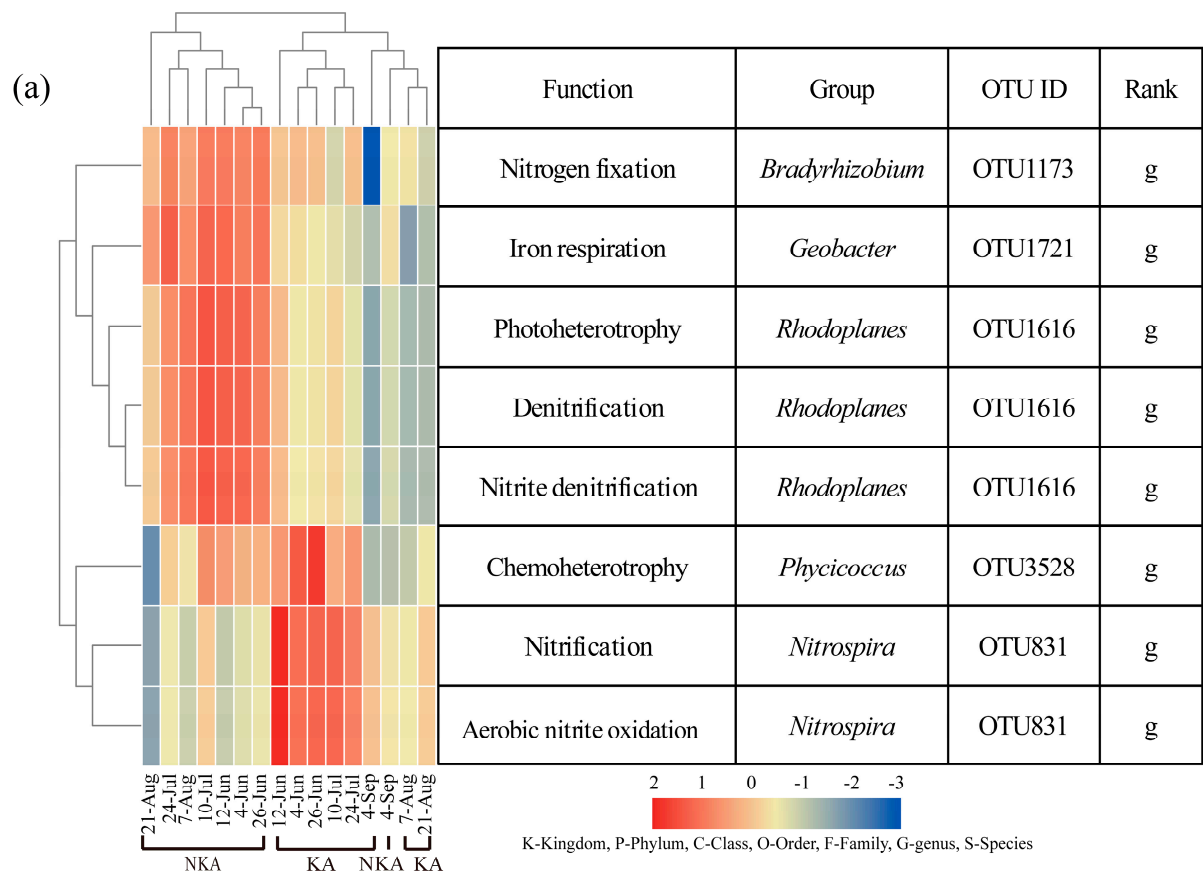


Figure 6.
 Cont.

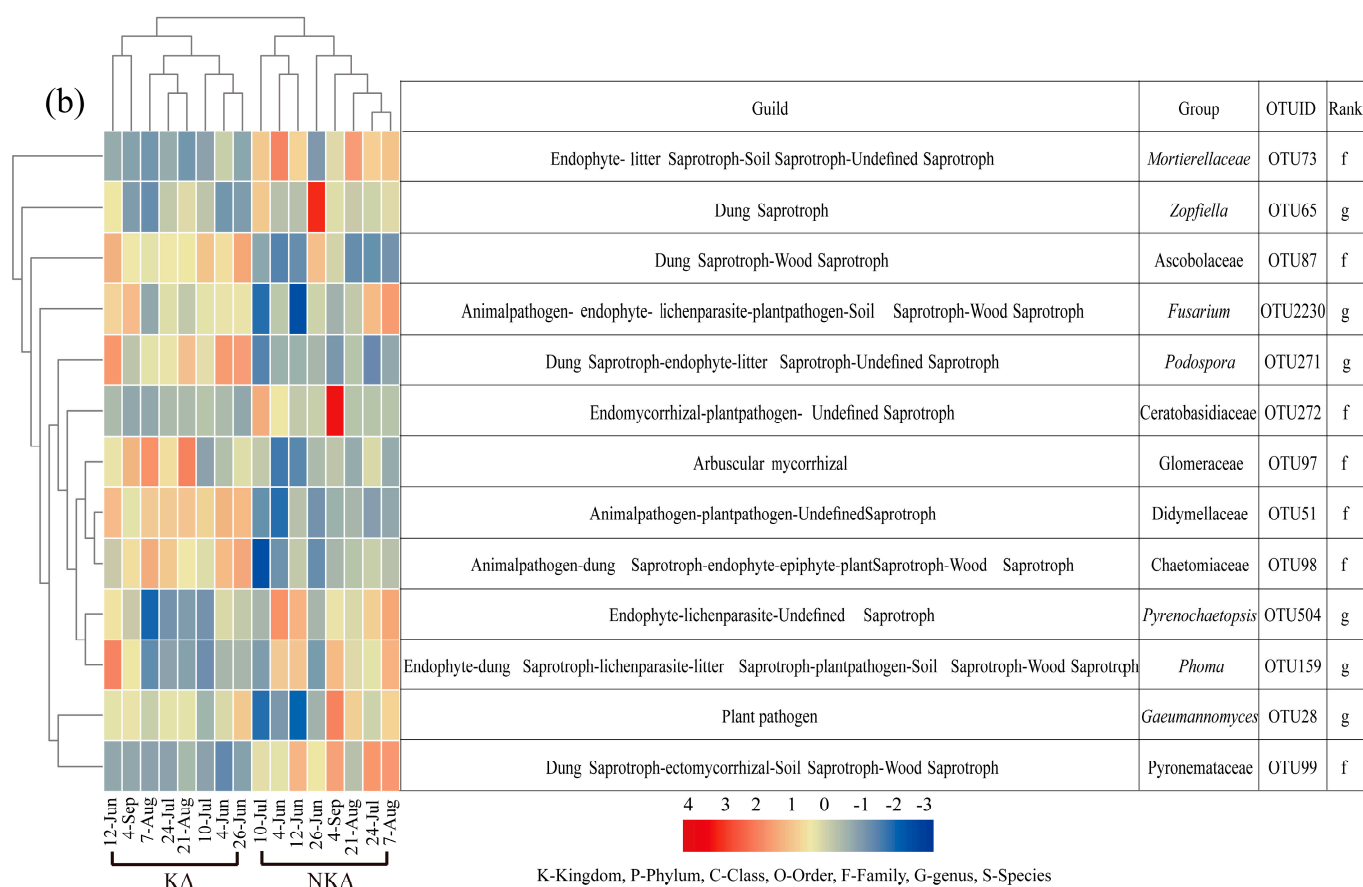


Figure 6. The heatmap clustering and distribution of bacterial (a) and fungal (b) functional groups. Note: KA, karst areas; NKA, non-karst areas.

Table 2. Correlation between soil respiration and bacterial and fungal functional.

Microbial Taxa	Groups	Region	Percentage	Correlation Coefficient
Bacteria	Acidobacteria Gp6	KA	7.05%	−0.565 *
Fungi	<i>Stellatospora</i>	NKA	0.07%	0.571 *
Functional groups of fungi	Plant pathogen	KA	2.61%	0.582 *
	Endomycorrhizal-plant pathogen-undefined saprotroph	NKA	0.26%	0.530 *
	Dung saprotroph-endophyte-litter saprotroph-undefined saprotroph	KA	0.88%	−0.568 *
Functional groups of bacteria	Aerobic nitrite oxidizers (Nitrifiers)	KA	2.13%	−0.545 *

Note: * Significant at the 0.05 level (two-tailed); KA, karst areas; NKA, non-karst areas.

4. Discussion

4.1. Effects of the F/B on In Situ CO₂ Fluxes

In present study, the abundance of bacteria and fungi in KA was higher than that in NKA, which indicated that higher soil nutrients shaped higher abundance of microorganisms. In KA, high levels of cations (such as Ca²⁺ and Mg²⁺) tend to combine with easily decomposed organics to form more stable large aggregates and organic carbon

pools [40,41]. Therefore, compared with fungi, bacteria are more susceptible to partly inhibition in KA environment.

The higher contents of chitin and chitosan in fungi cell wall could not be easily degraded, so that the total biomass of fungi was significantly higher than that of bacteria, which indicated that refractory organic matter from fungi is an important source of SOC accumulation [42]. Long-term combination of organic and inorganic fertilization increased SOC accumulation of paddy fields in South China. The numbers of fungi and bacteria both also increased significantly, but fungi increased more than bacteria, thereby increasing the F/B [14]. Thiet argued that fungi didn't have greater growth efficiency than bacteria in greater C storage and slower C turnover in fungal-dominated soils [43]. Luo et al. [40] reported that there was a significant correlation between the SOC and F/B with the reason of increased fungal necromass resulting in a change in the SOC composition. The above result is in accordance with the findings of higher SOC content and F/B in KA in present study.

The KA with high temperature and humidity, the dissolution of carbonate brings a large amount of Ca^{2+} and HCO_3^- into the soil. HCO_3^- combines with H^+ in the water to generate CO_2 , which increases soil CO_2 concentration [44]. Though some CO_2 in KA soil was consumed through reversibly combining with H_2O to form H_2CO_3 to a certain [3], CO_2 concentration of surface soil in KA is still higher than that in NKA. The higher concentration of soil CO_2 also change the quantity and quality of soluble sugars, organic acids, amino acids and other compounds secreted by rice roots. The bacterial abundance was significantly decreased and the fungal abundance and biomass were significantly increased, resulting in a significant increase in the F/B as previously reported [45,46].

More studies in the past 20 years have shown that the primary producers of CO_2 in soils are microorganism [13,14,47]. In present study, soil respiration in KA was lower than that in NKA, and the communities of both bacteria and fungi in KA were also significantly different with those in the NKA. Moreover, the F/B was higher in the KA than in the NKA. We also found that there was a significant negative correlation between soil respiration and the F/B across KA and NKA. In KA, soil carbon turnover rate would increase due to the changes on soil physical properties and the effect of fungi on physiology [43]. Keiblinger reported that more CO_2 will be released in bacterium-dominated soil because of lower carbon utilization efficient of bacteria [48]. Other researches have showed that fungal-derived soil processes and fungal biomass increased in the presence of elevated atmospheric CO_2 concentrations, which leading to increased organic C inputs into soils [46]. These results showed that fungi played an important role in the allocation of higher carbon and nitrogen nutrients in paddy soil, thus affecting soil respiration.

In our study, arbuscular mycorrhizal fungi were dominant in KA (showed in Figure 6b). The contribution of mycorrhizal fungi to the high carbon content of soil is reflected the conversion of floor C into mineral soil C [49] and the acceleration of microbial residues into soil [50]. Studies had shown that inoculation with arbuscular mycorrhizal fungi could promote the formation of large aggregates and ease their decomposition into aggregates with smaller particle sizes [51], which enhanced the formation of macroaggregates caused by sequestration of SOC [52]. Fungal hyphae and their cell wall residues act as binders by adsorption, cross-linking and adhesion of primary mineral particles, organic matter, and microaggregates, thereby enhancing the formation and stability of large aggregates [52]. Therefore, the distribution difference of mycorrhizal fungi in aggregates with different particle sizes in paddy soil between KA and NKA deserves further study.

4.2. Effect of Fungal and Background Community Composition On Paddy Field In Situ CO_2

Fungal-bacterial co-occurrence network analysis indicated a greater degree of putative mutualistic symbiotic relationships between fungi and bacteria (58.36%) compared with putative competitive relationships (41.64%) of KA and NKA. The presence of a typical "core" plant-microbe microbiota has been documented in several environmental studies [20,53]. These microbiomes may interact through direct or indirect mechanisms of carbon sequestration and can promote plant development [53,54]. Significantly higher relative abundance of

Acidobacteria Gp6 in KA of this study (average relative abundance of 7.05%) was negatively correlated with in situ CO₂ fluxes. Acidobacterium Gp6 may be the top microbial species in paddy soil in karst areas, as it is present in aggregate networks of different particle size grades [29]. Previous studies have demonstrated that the average relative abundance of Acidobacteria Gp6 was positively correlated with SOC and pH [55] in addition to amylase activity [56]. These results suggest the presence of coordinated activities of bacteria in KA with respect to SOC and pH. In addition, elevated CO₂ treatments have been shown to increase the relative abundance of Acidobacteria Gp6 [57], presumably because some Acidobacteria exhibit photosynthetic capacity. Some acidobacterial genomes contain *pscA* genes that encode the Fenna-Matthews-Olson (FMO) protein that binds to the bacterial chlorophyll molecule (BCh1) in photoresponse I, enabling chlorophyll-based photosynthetic capacity [58]. In this study, some strains of Acidobacteria Gp6 who absorb CO₂ for photosynthesis might have led to reduced CO₂ emissions in KA soils. Nevertheless, the carbon sequestration capacity of Acidobacteria Gp6 taxa in KA soils deserves our attention.

Nitrospira (OTU9) was also a top taxon in the internal bacterial correlation network and was dominant in NKA. *Nitrospira* abundance were negatively correlated with pH and TOC [59], and are highly resistant to high ammonia, high pH, salt, and SOC environments. In particular, the *Nitrospira* was most likely represented by the single-step nitrifying bacteria comammox *Nitrospira* that drives full nitrification [60]. Comammox *Nitrospira* encode a single gene cluster containing both *amo* and *hao* genes, but do not use Ca²⁺ (e.g., calcium nitrate and calcium nitrate) in nitrification reactions [61]. Instead, comammox *Nitrospira* require oxygen molecules to activate ammonia during nitrification [62], which are key adaptations for red soil environments with low carbon, nitrogen, and Ca²⁺ concentrations. Thus, OTU9 (comammox *Nitrospira*) is probably an important indicator in NKA rather than KA.

The internal fungal correlation network indicated that OTU69 (*Emericellopsis*) and OTU139 (Sordariales) as the core groups within the soil fungal networks in KA and NKA, respectively. *Emericellopsis* is an alkalophilic endophytic fungus with the ability to resist pathogenic peptide synthesis [63] and it enhances the plant host's ability to cope with environmental stresses, while also contributing to healthy host-plant growth and reproduction [64]. The neutral-alkaline pH in KA resulted in increased relative abundances of *Emericellopsis* that may then intermittently raise the quantity and quality of algae during the flooding periods within rice fields. Further, most water column algae in KA can use CO₂ and free HCO₃[−] to conduct photosynthesis and produce organic carbon. The synergistic interaction between algae and their endophytic fungi in photosynthesis of KA therefore requires further investigation [65]. *Stellatospora* belongs to the Sordariales group, whose abundances were positively correlated with CO₂ emission. Some studies have shown that Sordariales are highly adapted to tropical soil environments that experience warming due to elevated atmospheric CO₂ [66]. Thus, *Stellatospora* might be regarded as a biomarker indicator to distinguish NKA from KA.

4.3. Effects of Fungal and Bacterial Functional Groups on Paddy Field In Situ CO₂ Fluxes

Among the predicted fungal functional groups, the endomycorrhizal-plant pathogen-undefined saprotroph functional group was largely represented by Ceratobasidiaceae, with dominant relative abundances in NKA. Ceratobasidiaceae are able to rice blight, where in stems and leaves become yellow and wilt, leading to weakened photosynthetic ability [67]. The plant pathogenic fungal functional group was largely represented by the genus *Gaeumannomyces*, which also exhibited higher abundances in NKA. Some fungi of this genus are capable of infesting wheat and resulted in wheat allozyme diseases. The plant pathogenic and endomycorrhizal-plant pathogen-undefined saprotroph abundances were positively correlated with CO₂ in situ emission. The infected plants transmit infection information to soil through root exudates, causing the bacterial quorum sensing [68] leading to compensatory increase in soil respiration. In addition, Ca²⁺ affect activity of the antifungal proteins released by bacteria [69], which in turn influences the resistance

of crops to allozyme disease. Therefore, the interaction between pathogenic microbes and antagonistic microbes in KA and NKA should be further studied.

The abundance of dung saprotroph-endophyte-litter saprotroph-undefined saprotroph group were negatively correlated with in situ CO₂ emission. This functional group was mostly represented by *Podospira* that are dominant in KA. van Erven reported that *Podospira anserine*, a late colonizer of herbivorous dung, has high NADPH oxidase activity [70]. The oxidase promotes the formation of H₂O₂ and acts specifically on the more recalcitrant fraction of lignocellulose, which may promote the degradation of organic matter. Malagnac reported that NADPH oxidase can also promote the sexual reproduction and ascospore germination of the filamentous fungus *Podospira anserina* [71]. The products of lignocellulose degradation by fungi are likely to be used by bacteria, which improved utilization efficiency of organic matter and increased thermally stable SOC [72]. These results suggest that the relationship between soil microorganisms including *Podospira* and the thermal stability of SOC in KA is worth further study.

CO₂ in soils not only changed general microbial taxa, but also can be assimilated by autotrophic bacteria and turned into microbial biomass [73]. In the present study, autotrophic bacteria (such as aerobic nitrite oxidizers and nitrifiers) using CO₂ as the only carbon source were dominant in KA (as shown in Figure 6a). This was similar to the results of our previous investigation, which showed that high abundance of carbon fixing bacteria was in KA [25]. Among the predicted bacterial functional groups, the abundances of aerobic nitrite oxidizers and nitrifiers were negatively correlated with in situ CO₂ fluxes. These functional groups derive from the aerobic nitrite oxidation and nitrification activities associated with OTU831 (*Nitrospira*), which were dominant in KA. Ammonia-oxidizing bacteria (AOB) are autotrophic microorganisms using CO₂ as a carbon source in autotrophy [74]. Nitrification substrates are NH₃ molecules rather than NH₄⁺ ions, and a neutral alkaline karst environment might make the chemical equilibrium of NH₃ and NH₄⁺ tend towards NH₃ molecule production. Consequently, the abundances of AOB and their ability to fix CO₂ might be improved by increasing the abundances of NH₄⁺-N [75,76]. Therefore, the lower in situ CO₂ emission in KA might also contribute to autotrophic nitrification by *Nitrospira* [77].

5. Conclusions

In this study, we observed higher abundance of bacteria and fungi and higher F/B but lower soil respiration in karst area compared with those in non-karst area. We found that there was a significant difference of bacterial and fungi community between karst area and non-karst area. We also found that there was a significantly negative correlation between soil respiration and ratio of fungi to bacteria. On the one hand, higher abundances of fungi and their associated functional activities might enable better use of recalcitrance. On the other hand, bacterial abundances were decreased to adapt to environmental stresses from reduced easily available carbon supplies. Further, in response to the unique karst environments, microbial community structures and their associated functional groups were altered. In addition, the abundances of some autotrophic carbon-fixing bacteria and arbuscular mycorrhizal fungi increased and the abundance of pathogenic fungi decreased, which thereby also probably improved the utilization of SOC and reduces soil CO₂ emissions. In the future research, combining these microbial characteristics associated with soil respiration and through C and N isotopic labeling tracking technology and indoor incubation experiments can be provided more accurate soil carbon and nitrogen dynamics in KA.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13082001/s1>, Figure S1: The soil respiration of paddy fields during fallow.

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Data Availability Statement: The datasets generated during the current study were uploaded to the Sequence Read Archive (SRA) and have the accession number PRJNA763299.

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

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