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The effects of diverse microbial community structures, driven by arbuscular mycorrhizal fungi inoculation, on carbon release from a paddy field

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Abstract: Arbuscular mycorrhizal fungi (AMF) play a key role in regulating the carbon cycle in terrestrial ecosystems. However, there is little information on how AMF inoculation affects the carbon fluxes of paddy fields, which are major sources of global carbon emissions. We, therefore, designed an experiment to study the effects of AMF inoculation on methane and carbon dioxide emissions from a paddy field. Results showed that: (1) Among the tested factors, the C/N ratio was the main environmental determinant of microbial community structure in the investigated soil; (2) compared with traditional fertilisation (control), the soil C/N ratio increased by 2.1~15.2% and 1.4~10.5% as a result of AMF application alone (M) or in combination with mineral fertiliser (FM) throughout the growing season, respectively. This change shifted microbial community composition to higher G^+/G^- bacterial and fungal/bacterial ratios; (3) the microbial community change favoured soil carbon retention. Methane (CH_4) emission peaks were reduced by 59.4% and 76.0% *versus* control in the M treatment and by 52.5% and 29.4% in the FM treatment in the midseason and end-of-season drainage periods, and CO_2 emission peaks were reduced by 70.1% and 52.3% in the M plots and by 55.4% and 66.4% in the FM plots.

Keywords: *Rhizophagus irregularis*; global warming; gram-positive bacteria; chemoautotroph; decomposition

The carbon cycle in wetland systems has an important influence on the global carbon budget. Wetlands contain large carbon reserves due to low decomposition rates under anaerobic soil conditions (Valach et al. 2021). The total carbon stored in wetlands accounted for 20~30% of the estimated 1 500 Pg of global soil carbon (Nahlik and Fennessy 2016). The carbon pool poses a potential threat to global warming because methane (CH_4) is released during anaerobic decomposition (Peng et al. 2022). It is reported that wetlands contribute 30~40% to the total CH_4 emissions, and current emissions may increase by 50~80% by 2100 (Koffi et al. 2020). Paddy fields, special manmade wetlands for food production, cover approximately one-fifth of the global wetland area (Balasooriya et al. 2016). The long-term fertilisation and flooding practices have accelerated carbon loss from paddy fields to the atmosphere. According to IPCC, there was a trend of 10% growth in agriculture, forestry, and other land uses (AFOLU) CH_4 emissions between 1990 and 2019, and rice production was the most important contributor to overall growth trends in atmospheric CH_4 (Nabuurs et al. 2022).

Carbon fluxes between paddy soils and the atmosphere are strongly associated with microbial

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community structure and function. It is well known that chemoautotrophs, the majority of which are gram-negative (G^-) bacteria, contribute to the sequestration of carbon dioxide (CO_2) through the Calvin-Benson-Bassham pathway in flooded paddy soil (Yuan et al. 2012, Long et al. 2015). Recent studies demonstrated that G^- bacteria, specifically Proteobacteria, were more strongly correlated with inorganic carbon fixation than gram-positive (G^+) bacteria in the paddy soil of South China (Long et al. 2015). However, gram-positive bacteria could incorporate rhizosphere-derived labile carbon into microbial biomass, producing recalcitrant organic carbon (Balasooriya et al. 2016). Thus, a higher G^+/G^- bacterial ratio in the soil microbial community favours carbon stability and accumulation. Fungal abundance is generally lower than bacterial abundance in soils; however, fungi play a key role in carbon cycling in terrestrial ecosystems (Juan-Ovejero et al. 2020). Fungi have a good capacity to decompose recalcitrant organic matter, but at the same time, their products are resistant and are preferentially protected from decomposition through their interactions with clay minerals or soil aggregates (Klink et al. 2022, Tunlid et al. 2022).

The fertilisation mode significantly impacts the microbial community and, thus, the carbon balance. A shift in microbial community composition to G^+ bacteria has been found in agricultural soil with long-term application of organic or balanced inorganic fertiliser, which promoted soil organic carbon and aggregation (Zhang et al. 2015b). In a paddy field in subtropical China, Dong et al. (2014) found that adding organic fertiliser was more effective than mineral fertiliser in promoting the growth of bacteria, and there were opposite effects on components of fungal growth. However, Wang et al. (2022) reported that the application of organic fertilisers significantly reduced G^+/G^- bacterial ratio but significantly increased the fungi/bacteria ratio in a continuous tobacco field. Arbuscular mycorrhizal fungi (AMF), which are ubiquitous fungi in terrestrial ecosystems (Wang et al. 2008), have recently been regarded as potential green bio-fertilisers for sustainable agriculture (Sun and Shahrajabian 2023) due to their ability to improve soil structure and nutrient conditions (Veresoglou et al. 2012). AMFs are important regulators of the carbon cycle between the biosphere and the atmosphere because they use the fixed photosynthates from their host plants (Singh et al. 2013, Basu et al. 2018). However, we know little about the effects of AMF inoculation on the microbial community and carbon releases in paddy fields, and this information is vital to evaluate the associated environmental benefit.

The objectives of this study were (1) to investigate how AMF inoculation affects the microbial community structure by changing soil properties in a paddy field and (2) to determine the effects of AMF and changes in the microbial community on CH_4 and CO_2 releases. Phospholipid fatty acids (PLFAs) were used as biomarkers to identify community composition.

MATERIAL AND METHODS

Experimental site. The field experiment was conducted in a rice field in Yixing City, Jiangsu Province, China (119°52'06.17'N, 31°30'07.31'E) in 2012. The climate at this location is warm and wet year-round, with a mean annual rainfall of 1 177 mm and a mean annual temperature of 15.7 °C. The total organic carbon of the soil was 16.2 g/kg. Total N and P contents of the soil were 1.4 g/kg and 0.25 g/kg, respectively. The soil pH was 6.78.

Experimental design and field management. The experiment was set up in a randomised block design with three replicates of each of the three treatments: (*i*) traditional mineral fertilisation without inoculation as the control (C); (*ii*) inoculation with AMF without mineral fertilisation (M), and (*iii*) traditional mineral fertilisation with inoculation (FM).

The experiment was conducted *in situ*, subject to local natural conditions. To prevent contamination from neighbouring plots, each 5 × 5 m^2 plot was separated by waterproof sheets extending 0.5 m above and below ground. Each plot contained 289 plants planted in 17 rows and columns. Irrigation and fertilisation were managed separately.

Seeds were sown in a seedling bed on May 30, 2012. During the seedling stage, each trial bed was inoculated with stock cultures of *Rhizophagus irregularis* (RI), which contained spores, mycelia, and infected root fragments, and each control bed received an equal gram of sterilised soil and inoculum. The concentration of spores was 33–35 per gram of inoculum. Infected (10.9% colonisation rate) and uninfected seedlings were transplanted to the M, FM and control plots 20 days later, at three plants per hill density. The fields were managed according to the standard local practice (Table 1).

Sampling and measurements. Root biomass was determined after washing and drying at 75 °C until constant weight. The soil moisture sensor measured

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Table 1. Inoculation and fertilisation practice

Date	Fertiliser type	Quantity (kg per 5 × 5 m^2 plot)		
		C	M	FM
30 May	inoculum	0	0.5	0.5
20 Jun	N:P:K (16%:7%:13%)	2.0	0	2.0
30 Jun	urea	1.0	0	1.0
31 July	urea and KCl	0.6 and 0.3	0	0.6 and 0.3
10 August	urea	0.2	0	0.2

C – control; M – inoculation with arbuscular mycorrhizal fungi without mineral fertilisation; FM – traditional mineral fertilisation with inoculation

the topsoil volumetric water content (AQUA-TEL-TDR, METER, Pullman, USA). C and N concentrations (mg/g) in soil samples were determined using an elemental analyser (Vario EL cube; Elementar, Hanau, Germany). The carbon-to-nitrogen ratio (C/N) was calculated from the C concentration divided by the N concentration. Plant and topsoil (0~15 cm depth) samples collected on 22 July (midseason drainage), 27 August (flooding stage) and 23 September (end-of-season drainage) were chosen for analysis. The percentage of AMF colonisation was estimated using the method described by Biermann and Linderman (1981).

PLFA extraction and measurement methods were provided by the Cold Spring Biotech Corporation. The soil samples were freeze-dried before extraction. Each soil sample (2.0 g) was saponified, methylated, extracted and washed, producing fatty acid methyl esters (FAMEs). FAMEs were analysed using a 6890 series gas chromatograph (Agilent, Santa Clara, USA). PLFAs were identified using the MIDI Sherlock Microbial Identification System (Microbial ID, Newark, USA). Various types of PLFAs as biomarkers for different categories of microorganisms are described in Table 2, and the remaining unlisted PLFAs are non-specific. The Shannon diversity index was calculated for PLFA biomarkers.

Gas samples were collected using the static chamber technique over two hours, from 09:00~11:00, once a week. The details followed by Zhang et al. (2015a). Gas samples were analysed with a modified gas chromatograph (4890D, Agilent, Santa Clara, USA) equipped with an electron capture detector (ECD). Fluxes were calculated based on linear regressions of gas concentrations in the chambers *versus* time for 30 min. Average CO_2 and CH_4 fluxes and standard errors were calculated based on all plot replicates (three per treatment).

Statistical analysis. Differences between the treatments were examined using one-way ANOVA. Significant differences were determined using Duncan's new multiple range test ($P < 0.05$) in SPSS 19.0 statistical software (IBM, Armonk, USA). The relationships between the environmental variables, the soil microbial community, and the soil microbial community and carbon emissions were analysed *via* detrended canonical correspondence analysis (DCCA) using CANOCO 5.0 (Microcomputer, Ithaca, USA). Graphs were generated using Origin 8.0 (OriginLab, Northampton, USA).

RESULTS

Biomass and soil environmental properties. Representative samples from each treatment and

Table 2. Phospholipid fatty acid (PLFA) biomarkers representing various microbial taxa

Microbial taxa	PLFA biomarkers	Source
Gram-positive bacteria	i 12:0, i 13:0, i 14:0, a 14:0, i 15:0, a 15:0, i 16:0, i 17:0, i 22:0	Lu et al. (2004), Balasooriya et al. (2016)
Gram-negative bacteria	10:0 3OH, 13:1ω5c, 17:1ω6c, 17:1ω7c, 17:1ω8c, 18:1ω6c, 18:1ω7c, 18:1ω8c, 19:1ω6c, 19:1ω7c	Kao-Kniffin and Zhu (2013), Long et al. (2015)
Fungi	18:3ω6c (6,9,12)	Zelles (1999)
AMF	16:1ω5c, 18:1ω9c	Zelles (1999), Madan et al. (2002)
Eukaryote	15:4ω3c, 18:3ω6c, 19:3ω3c, 19:3ω6c	Zelles (1999)

AMF – arbuscular mycorrhizal fungi

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Table 3. Soil environmental properties

		Midseason drainage	Flooding stage	End-of-season drainage
C to N ratio	C	9.80 ± 0.01^{cC}	10.51 ± 0.03^{cB}	11.56 ± 0.00^{bA}
	M	11.28 ± 0.00^{aC}	11.69 ± 0.02^{aB}	11.81 ± 0.00^{aA}
	FM	9.93 ± 0.04^{bC}	11.61 ± 0.04^{bB}	11.81 ± 0.03^{aA}
Soil moisture (*v/v*)	C	0.45 ± 0.02^{aA}	0.46 ± 0.02^{aA}	0.35 ± 0.01^{aB}
	M	0.40 ± 0.03^{bAB}	0.45 ± 0.02^{aA}	0.37 ± 0.03^{aB}
	FM	0.45 ± 0.02^{aA}	0.45 ± 0.03^{aA}	0.35 ± 0.03^{aB}

Data shown are means (n = 3) ± standard error. Lower-case letters within columns compare different treatments within each developmental stage; upper-case letters within columns compare developmental stages within each treatment. Values followed by the same letter did not differ significantly based on Duncan's test ($P \leq 0.05$). C – control; M – inoculation with arbuscular mycorrhizal fungi without mineral fertilisation; FM – traditional mineral fertilisation with inoculation

growth stage were analysed to determine C/N, soil moisture (Table 3) and biomass of rice plants (Figure 1). The above-ground biomass, root biomass of rice plants and soil C/N ratio of each treatment increased seasonally. In all treatments, the soil moisture was significantly lower in the end-of-season drainage period than in the initial mid-season drainage and flooding stages.

Compared with the control, AMF inoculation significantly increased the soil C/N ratio during the three stages, especially in the M treatment. From the midseason to end-of-season drainage periods, the soil C/N ratio was increased by 15.2, 11.2 and 2.1% through inoculation with AMF alone and was increased by 1.4, 10.5 and 2.1% in the FM treatment. A higher C/N ratio was measured in the M compared with the FM treatment in the midseason drainage and flooding stages, but there was no significant difference in the end-of-season drainage period.

Soil moisture varied with water management. Numerically, soil moisture decreased as follows: flooding stage > midseason drainage period > end-of-season drainage period. However, there were no significant differences between the first two stages, while soil moisture in the end-of-season drainage period decreased significantly in all treatments. Overall, there were no significant differences among the three treatments at each stage.

Compared with the control, AMF inoculation significantly increased the root biomass during the three stages. From the midseason to end-of-season drainage periods, root biomass was increased by 20.0, 17.1 and 10.6% in the M treatment and was increased by 11.5, 16.7 and 10.1% in the FM treatment. However, the above-ground biomass was significantly increased by 7.2% and 11.0% in the M and FM treatment only at the end of the season, while the grain yield was significantly increased by 23.3%

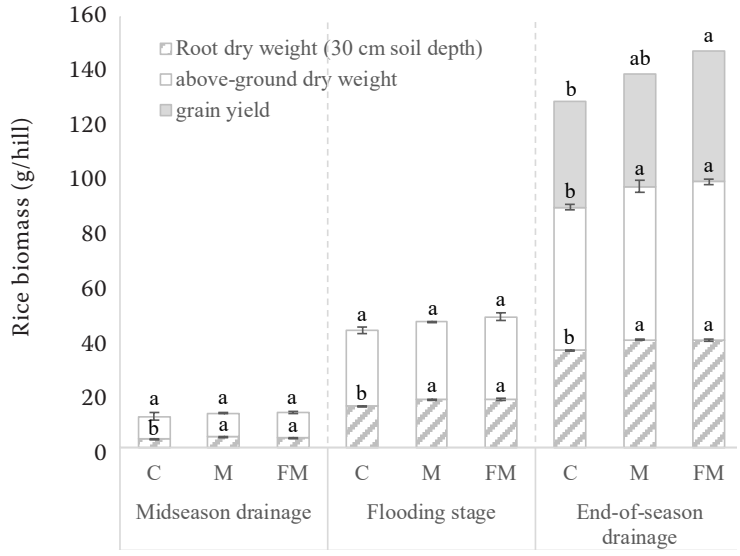

Figure 1. Biomass of rice plants at different stage, as affected by arbuscular mycorrhizal fungi (AMF) inoculation. Histograms represent means (n = 3) ± standard error. Lower-case letters compare different treatments within each developmental stage. Values followed by the same letter did not differ significantly based on Duncan's test ($P \leq 0.05$). C – control; M – inoculation with AMF without mineral fertilisation; FM – traditional mineral fertilisation with inoculation

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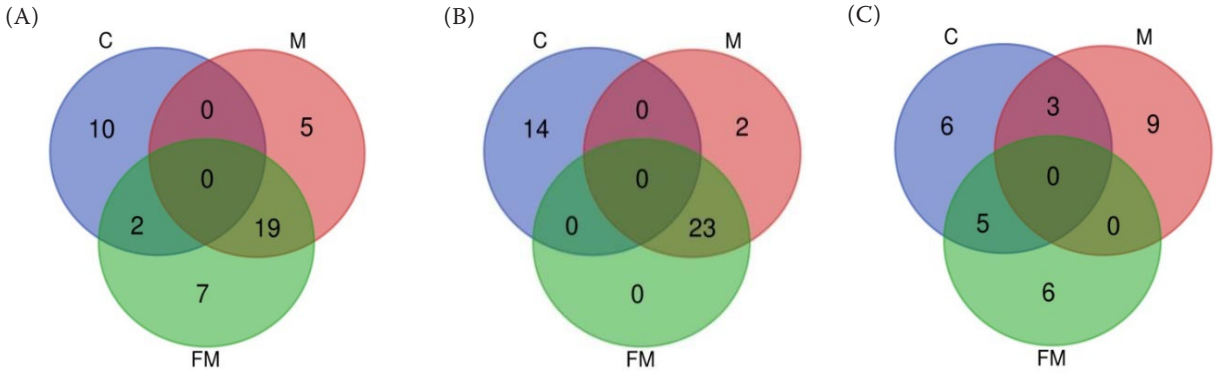

Figure 2. Venn diagrams of phospholipid fatty acid (PLFA) biomarkers in different treatments. (A) Midseason drainage; (B) flooding stage, and (C) end-of-season drainage. C – control; M – inoculation with arbuscular mycorrhizal fungi without mineral fertilisation; FM – traditional mineral fertilisation with inoculation

only in the FM treatment. There were no significant differences in root biomass, above-ground biomass and grain yield between M and FM.

Phospholipid fatty acids profiles. In the control plots subjected to traditional fertilisation, 12~14 PLFAs existed stably throughout the growing season (Figure 2). Inoculation with AMF alone or in combination with mineral fertiliser increased the number of soil PLFAs in the midseason period and flooding stage, and the number was approximately twice that measured in control. However, the PLFAs in the inoculated plots (M and FM) exhibited a dramatic decline in the end-of-season drainage period, roughly equal to that observed in the control. The FM plots shared many more common PLFAs with the M plots than the control in the midseason drainage period; this tendency increased during the flooding stage. This indicates that AMF inoculation had a greater influence on the microbial community structure than mineral fertiliser application. However, there was a convergence among the community structures of the different treatments with the decrease in AMF at the end of the rice-growing season.

General bacteria, eukaryotes and gram-negative bacteria were the dominant microbial taxa in the control treatment throughout the entire growing season. The group with the highest relative abundance shifted from general bacteria in the midseason drainage period to G^- bacteria during the remaining periods (Figure 3). Fungi did not appear in the control plots at any stage.

Next to general bacteria, gram-positive bacteria and fungi were the dominant microbes in the M treatment during the midseason drainage and flood-

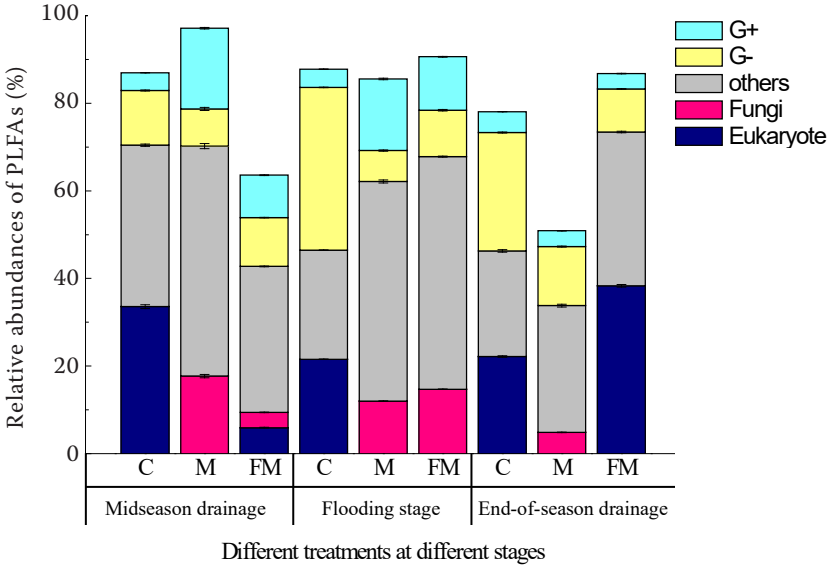

Figure 3. Relative abundances of the microbial taxa in the paddy soil. Phospholipid fatty acids (PLFAs) with < 1% relative abundance were not included in the analysis. C – control; M – inoculation with arbuscular mycorrhizal fungi without mineral fertilisation; FM – traditional mineral fertilisation with inoculation; G^- – gram-negative bacteria; G^+ – gram-positive bacteria

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Table 4. The gram-positive bacteria (G^+)/ gram-negative bacteria (G^-) and fungal/bacterial ratios in the paddy plots

		Midseason drainage	Flooding stage	End-of-season drainage
G^+ to G^- ratio	C	0.32 ± 0.00^{cA}	0.11 ± 0.00^{cC}	0.18 ± 0.00^{cB}
	M	2.18 ± 0.10^{aB}	2.31 ± 0.03^{aA}	0.27 ± 0.01^{bC}
	FM	0.87 ± 0.01^{bB}	1.15 ± 0.^{02}bA	0.36 ± 0.00^{aC}
Fungi to bacteria ratio	C	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bA}
	M	0.22 ± 0.01^{aA}	0.16 ± 0.00^{bB}	0.11 ± 0.00^{aC}
	FM	0.06 ± 0.00^{bB}	0.19 ± 0.00^{aA}	0.00 ± 0.00^{bC}

Data shown are means (n = 3) ± standard error. Lower-case letters within columns compare different treatments within each developmental stage; upper-case letters within columns compare developmental stages within each treatment. Values followed by the same letter did not differ significantly based on Duncan's test ($P \leq 0.05$). C – control; M – inoculation with arbuscular mycorrhizal fungi without mineral fertilisation; FM – traditional mineral fertilisation with inoculation

ing periods. At the same time, there were no obvious dominant groups in the end-of-season drainage period. Nevertheless, the G^+ to G^- bacterial ratio (G^+/G^-) and the fungi to bacteria ratio (F/B) of the M treatment were significantly higher than those in the control during the whole growing season (Table 4). From the midseason to the end-of-season drainage period, G^+/G^- of the paddy soil increased 5.7, 19.7 and 0.5 fold in response to the M treatment and 1.7, 9.3 and 1.0 fold in response to the FM treatment. The community composition of the FM plots showed characteristics similar to those of the control and M plots in the midseason drainage period. Besides general bacteria, G^+ and G^- bacteria held a dominant position in the FM plots. In addition, the FM treatment's G^+/G^- and F/B values lay between those of the control and M treatment. At the flooding stage, the microbial composition and abundance of the FM plots showed higher similarity with those of the M plots; moreover, G^+/G^- and F/B were significantly higher than in the control. However, the microbes from the FM plots shared more similarities with the control in terms of composition and abundance as the number of fungi decreased.

Methane and carbon dioxide emissions. During the growing period, CH_4 emissions fluctuated dramatically until 121 days. Emissions were in the range of –0.51~39.35 mg $CH_4/m^2/h$ in the control plots, –0.31~15.96 mg $CH_4/m^2/h$ in the M plots, and –1.10~24.34 mg $CH_4/m^2/h$ in the FM plots (Figure 4). There were four CH_4 emission peaks. The first peak appeared in the mid-season drainage period, and the second appeared in the end-of-season drainage period. The CH_4 emission peaks in M and FM were significantly lower than those in the control in these two periods, i.e., respective decreases of 59.4% and 76.0% in the M plots and 52.5% and 29.4% in the FM plots were recorded. Another two lower peaks were observed in the flooding stage. Compared with the

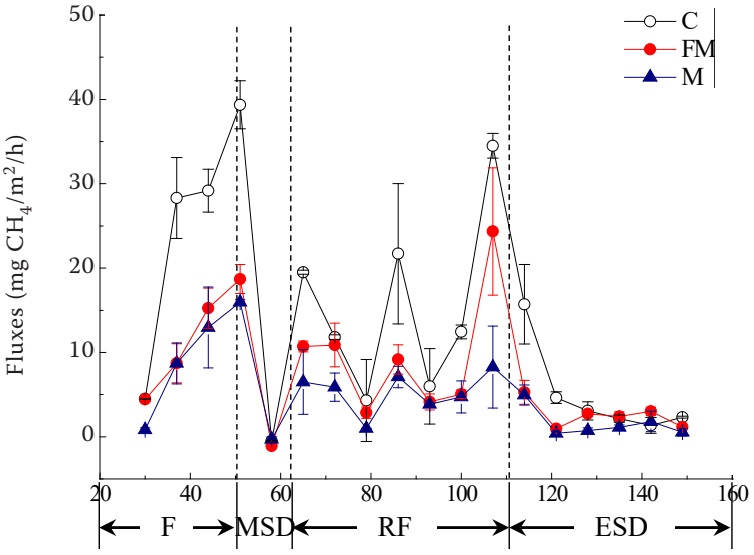

Figure 4. Methane (CH_4) emission fluxes from rice paddy plots under different treatments. F – flooding stage; MSD – midseason drainage; RF – reflooding stage; ESD – end-of-season drainage. C – control; M – inoculation with arbuscular mycorrhizal fungi without mineral fertilisation; FM – traditional mineral fertilisation with inoculation

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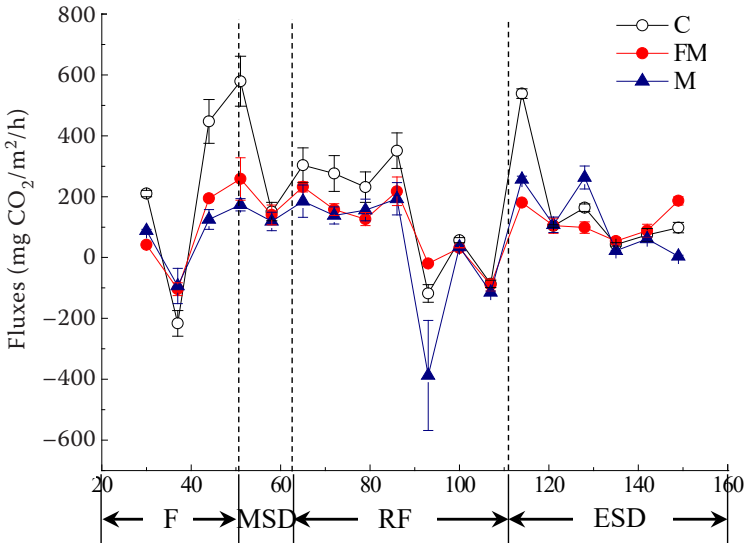

Figure 5. CO_2 emission fluxes from rice paddy plots under different treatments. F – flooding stage; MSD – midseason drainage; RF – reflooding stage; ESD – end-of-season drainage; C – control; M – inoculation with arbuscular mycorrhizal fungi without mineral fertilisation; FM – traditional mineral fertilisation with inoculation

control, the CH_4 emission peaks were significantly reduced by 66.7% and 67.3% in the M plots and by 45.1% and 57.8% in the FM plots.

During the growing period, CO_2 emissions fluctuated in the range –216.19~579.03 mg $CO_2/m^2/h$ in the control plots, –387.58~262.76 mg $CO_2/m^2/h$ in the M plots, and –104.04~258.37 mg $CO_2/m^2/h$ in the FM plots (Figure 5). Two positive emission peaks appeared in the midseason and end-of-season drainage periods. The peaks observed in the control were significantly higher than those in the M and FM treatments. Compared with the control, the peak emission was reduced by 70.1% and 52.3% in the M plots and by 55.4% and 66.4% in the FM plots during the two stages. There were also two negative emission peaks, which appeared at the flooding stage before the respective midseason and end-of-season drainage periods. The first peak in the control group was significantly lower than those in the M and FM treatments, while the second peak in the M treatment group was significantly lower than those in the control and FM treatments. The CO_2 fluxes depend on the balance between foliage photosynthetic assimilation and autotrophic and heterotrophic respiration, which was particularly sensitive to environmental changes. The negative peaks indicated photosynthesis might dominate the process, while respiration may be restricted with the variations of water and heat conditions (Neogi et al. 2021).

DISCUSSION

Soil environmental factors and microbial community structure. DCCA was performed to investigate relationships between the structure of the microbial community from the paddy soil and environmental factors (Figure 6). The first two axes explained 81.3% of the total variance of the microbial community attributed to the soil environmental factors. The results suggested that the microbial community was significantly affected by the five selected factors ($P < 0.01$). Of these, the applications of mineral fertiliser and *Rhizophagus irregularis* were dominant in shaping the composition of the microbial community. In addition to these variables, the C/N ratio was the most influential environmental factor, based on a comparison with root biomass and soil moisture. As nutrients and cell components, C and N are key factors for microbial growth in paddy soils and thus had significant effects in shaping the microbial community structure. Li et al. (2018) also reported that the C content and C/N ratio of bulk soil from a wheat-maize double cropping system were significantly ($P < 0.05$) and positively correlated with microbial communities along the first axis, accounting for 75.3% of the total variation in PLFAs. Although the root biomass of rice is closely correlated with the organic C return of roots (Luo et al. 2018), a relatively weak effect on the microbial community was observed in this study, possibly because root biomass could not provide the microbes with labile organic C in this short-term trial.

The application of *Rhizophagus irregularis* resulted in separating the different sample groups along the two axes, especially the first axis (Figure 6A). The M samples were distributed in the forward direction of RI, the C/N ratio, and the reverse direction of CF,

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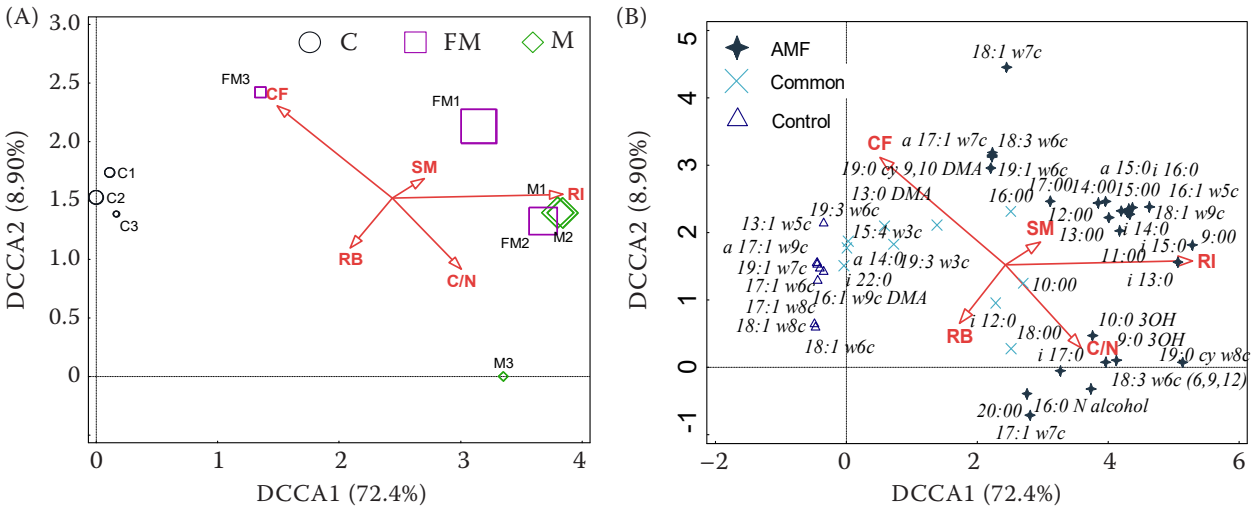

Figure 6. Relationships among microbial community structure and environmental characteristics revealed by detrended canonical correspondence analysis (DCCA). (A) Samples and environmental variables biplot. Symbol size represents the sample Shannon's diversity index (H) value and (B) species and environmental variables biplot. Selected formal variables, including the application of mineral fertiliser (CF) and *Rhizophagus irregularis* (RI), and selected soil variables, including soil moisture (SM), carbon to nitrogen ratio (C/N), and root biomass (RB) for DCCA of phospholipid fatty acid (PLFA) data

while the control samples exhibited opposite patterns. This indicates that inoculation with AMF changed the microbial community structure of paddy soil to which traditional mineral fertiliser was applied. The microbial community composition of FM gradually approached that of M from the midseason to the flooding stage but was similar to that of the control in the end-of-season drainage period. Inoculation with AMF increased the microbial species diversity in the paddy plots, as indicated by Shannon's diversity index (H); however, AMF inoculation resulted in a dramatic decrease in microbial species diversity in all treatments at the end of the season.

All PLFAs in the paddy soil were divided into three groups: (1) inhabiting only the control soil; (2) inhabiting both the control and inoculated soil, and (3) inhabiting only the inoculated soil (Figure 6B). Among the representative PLFAs, 10 biomarkers were present in all treatments, 8 ones were present in the control treatment, and 27 ones were present in the M and FM treatments. Based on the loading scores on the first axis, the PLFAs in the control clustered along the forward direction of CF, suggesting N-reliant or autotrophic microbes. Except for a17:1ω7c, 18:1ω7c, 18:3ω6c and 19:1ω6c from the FM samples, the PLFAs in the inoculated soil mainly clustered along the forward direction of RI and the C/N ratio, suggesting C-driven microbes. With respect to the common PLFAs distributed between the RI and CF axes, 10:00, i12:0 and 18:00 clustered along the forward direction of RI, whereas the remainder clustered along that of CF.

16:1ω5c, an indicator of AMF, and 18:1ω9c, frequently present in the spores of *Glomus* (Zhao et al. 2019), were positively correlated with inoculation. In the current study, they were detected only in the inoculated soil in the midseason period and flooding stage, when non-inoculated roots were sparsely colonised by AMF, with 5.4~6.3% colonisation rates in the control plots; by contrast, the colonisation rates of the M and FM treatments were 22.6~42.4% and 15.9~21.5%, respectively (Zhang et al. 2015a). Additionally, 16:1ω5c and 18:1ω9c were positively correlated with the C/N ratio. Our observation of the increased relative abundance of 18:1ω9c with higher carbon inputs agrees with the reports of Bossio and Scow (1998) and Balasooriya et al. (2016). In this study, the soil C/N ratio of the M treatment was much higher than that of the control, but the difference gradually reduced from the mid-season to the end-of-season drainage period. As the control plots indicate, the soil C/N ratio during the growing stage would increase along with carbon accumulation and nitrogen uptake by rice. Thus, the greater increase of the C/N ratio in the early stages indicates that AMF inoculation accelerated the C and N turnover

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within the topsoil. AMF obtain all their carbohydrates from the photosynthates of the host plant (Zhu and Miller 2003). Thus, mycorrhizal colonisation can increase hyphal biomass by promoting the amount and allocation of photosynthates belowground, which predominantly contribute to soil organic matter (Langley and Hungate 2003, Parihar et al. 2020). However, AM fungi were sensitive to N fertilisation. In a comprehensive global-scale meta-analysis of data from 94 publications and 101 sites, Han et al. (2020) found that adding N significantly reduced AM fungal abundance. Other studies also indicated the negative effects of N application on root length colonisation, AM fungal richness, and the degree of plant-AMF association (Lu et al. 2022, Peng et al. 2023). Additionally, a significant increase in the CO_2 flux under N fertilisation in a paddy field was observed (Chen et al. 2022). Therefore, N application may dampen the effect of AMF on soil carbon sequestration and stabilisation. Promoting the C/N ratio in the FM plots showed hysteresis, reaching a much higher level than control until the flooding stage.

Photosynthates in the form of exudates and AM hyphae may rapidly drive soil microbial activity. Lu et al. (2004) reported that 14:00, 15:00, 18:00, 20:00, i15:0, and a15:0 from a wetland rice soil positively responded to photosynthate input, with high ^{13}C incorporation. Similarly, Balasooriya et al. (2016) reported that several gram-positive PLFAs (i14:0, i15:0, i16:0) were active in rhizosphere-derived C uptake and were highly ^{13}C enriched after $^{13}CO_2$ pulse labelling of rice. In addition to the PLFAs reported above, G^+ i13:0 and i17:0, fungi 18:3ω6c (6,9,12), AMF markers 16:1ω5c and 18:1ω9c, and general PLFAs 9:00, 9:0 3OH, 11:00, 16:0N alcohol, and 19:0 cy ω8c positively responded to AM inoculation and the C/N ratio in this study. By contrast, G^- bacteria from the control plot were strongly and positively correlated with mineral fertilisation. Our observation agrees with Ai et al. (2012), who reported that chemical fertilisers could increase the abundance of G^- bacteria. Few G^- bacteria were in the inoculated plots, including M and FM, indicating an inhibition of G^- bacteria by AMF.

Microbial community structure and carbon release. The DCCA results provided further information about the relationships between the microbial community and carbon released in the form of CO_2 and CH_4 (Figure 7). The PLFAs of soil microorganisms were clearly distributed along the first axis, which was highly correlated with carbon release (canonical correlation coefficient = 0.8209; $P < 0.01$). All the PLFAs from the control and the most common PLFAs were positively correlated with CO_2 and CH_4 emissions. By contrast, most PLFAs except for 18:1ω7c in the inoculated soil were negatively correlated with CO_2 and CH_4 emissions.

CO_2 and CH_4 are derived from the decomposition of organic matter in paddy soils by microorganisms and contribute to global carbon emissions. Carbon uptake mechanisms maintain the relative equilibrium of carbon concerning input and output in natural systems. In this study, 18:1ω6c and

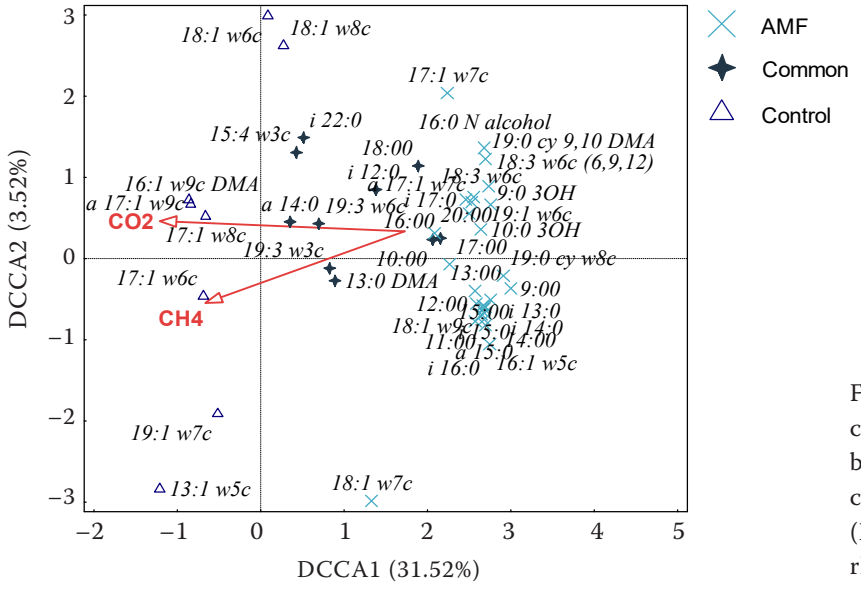

Figure 7. Relationships between microbial community structure and carbon release revealed by detrended canonical correspondence analysis (DCCA). AMF – arbuscular mycorrhizal fungi

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18:1ω8c were present in the control plots and are highly specific signatures for type II aerobic methanotrophs (Green and Scow 2000, Zigah et al. 2015), which can synthesise 50~70% of their biomass from formaldehyde through the serine pathway (Hanson and Hanson 1996). In this process, the CH_4 concentration is mainly determined by the enrichment of methanotrophic bacteria; thus, their PLFA markers clustered along the forward direction of CH_4. CH_4 oxidation in flooded systems is the main determinant of net CH_4 emissions; approximately 80% of the CH_4 that diffuses through the oxic sediment-water interface is consumed by methanotrophs (Hanson and Hanson 1996). The fatty acids i15:0, a15:0 and 17:0 from inoculated plots are diagnostic of sulfate-reducing bacteria, which can anaerobically oxidise 3% of methane *via* sulfate reduction (Zigah et al. 2015). Nevertheless, CH_4 oxidation will cause an increase in CO_2 emissions. Chemoautotrophs such as nitrifying, sulfur-oxidising and sulfate-reducing bacteria can buffer CO_2 release through various inorganic carbon fixation pathways (Long et al. 2015, Vasquez-Cardenas et al. 2016). The PLFAs 16:00 and 18:1ω7c, which are highly abundant in nitrifying bacteria, indicate a significant incorporation of ^{13}C-labeled dissolved inorganic carbon in investigated soils (Veuger et al. 2013, Vasquez-Cardenas et al. 2016). In the current study, 16:00 was the dominant fatty acid with high relative abundance in the inoculated plots in the midseason drainage and flooding stages, but a lower abundance of this fatty acid was measured in the control plots in the end-of-season drainage period. 18:1ω7c was a low-frequency PLFA that only appeared in FM in the midseason drainage period. Significant amounts of ^{13}C-labels were also recovered in i15:0 and a15:0 in the inoculated plots and 17:1ω8c in the control plots; these fatty acids have been suggested as specific biomarkers of sulfate-reducing bacteria. However, the abundance of sulfate-reducing bacteria is typically lower than that of aerobic chemoautotrophs (Boschker et al. 2014, Long et al. 2015, Vasquez-Cardenas et al. 2016).

Although specific G^- bacteria, as chemoautotrophs, show a certain capacity to fix C, G^+ bacteria play a more important role in mediating C retention in terrestrial ecosystems. This is because G^+ bacteria generally possess a higher proportion of peptidoglycan, which contains significant quantities of N-acetylglucosamine, a precursor of relatively decay-resistant soil organic matter (Simpson et al. 2004). This explains our observation that diverse G^+ bacteria indicated by branched PLFAs from the inoculated plots were negatively correlated with CH_4 and CO_2 emissions (Figure 6). Many reports suggest that a shift in the microbial community composition to more G^+ bacteria or a higher G^+/G^- bacterial ratio is favourable for soil organic C accumulation (Zhang et al. 2013, 2015b, Wang et al. 2017). Similarly, our observations show that in the inoculated plots (especially M, which had a higher G^+/G^- bacterial ratio), CH_4 and CO_2 emissions decreased significantly compared with the control and that the C/N ratio increased during the growing season.

Furthermore, the resistance of fungal products to decay and their interaction with soil aggregates also contributed to the stability of carbon in the paddy soil. Glomalin, which is associated with AMF, and chitin, i.e., the major constituent of AMF hyphal cell walls (up to 60%), are both comparatively resistant to decomposition, with a maximum residence time in soil of more than 40 years (Langley and Hungate 2003, Zhu and Miller 2003, Singh et al. 2013, Hawkins et al. 2023). Moreover, AMF hyphae can help entangle and enmesh soil particles to form stable macroaggregate structures, which protect soil carbon and microbial biomass (Oades and Waters 1991, Zhu and Miller 2003, Six et al. 2006). Compared with the control, the inoculated M and FM plots maintained a certain proportion of AMF, and the F/B ratio significantly increased during the midseason and flooding stages, contributing to the reduction in the amount of C released.

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