Variation in microbial biomass and community composition based on long-term fertilization regimes in paddy soil profiles

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Abstract

Fertilization is a common approach to increase or sustain soil fertility, but its impact on microbial biomass and community structure remains controversial, particularly in paddy soils. In this study, we investigated the effect of different long-term fertilization strategies, beginning in 1986, namely no fertilization, mineral fertilization, mineral fertilization combined with rice straw or chicken manure, on microbial biomass and community composition at four soil depths (0–10, 10–20, 20–30, and 30–40 cm). The extracted soil phospholipid fatty acids (PLFAs) were pooled into gram-positive (G+) bacteria, gram-negative (G-) bacteria, fungi, and actinomycetes groups. Results showed that irrespective of the fertilization type, the abundance of PLFAs decreased with soil depth in the following order due to nutrient decrease along soil profiles: fungi > G- bacteria > G+ bacteria > actinomycetes. Mineral fertilization induced G+ bacteria more than G- bacteria and actinomycetes, which suggested that the inorganic nutrients in mineral fertilizers are utilized more by G+ bacteria than by other microbial groups. Partial replacement of mineral fertilizer with manure further stimulates G+ bacteria at all depths. Redundancy analysis showed obvious microbial separation at the 0-20 and 20-40 cm soil depths due to the rhizodeposition effect and also revealed that the microbial communities were significantly correlated with nutrient content (soil organic carbon and available N) and pH. Overall, our findings highlight microbial community shifts due to different fertilizer types, which provides basic information for understanding how substrate availability controls the structure of soil microbial communities in paddy soil systems.

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pH. Overall, our findings highlight microbial community shifts due to different fertilizer types, which provides basic information for understanding how substrate availability controls the structure of soil microbial communities in paddy soil systems.

Key words : mineral fertilizer –rhizodeposition effect –straw addition – soil depth – organic manure – paddy soil.

INTRODUCTION

Paddy soil is estimated to cover a total area of approximately 161 million ha worldwide, with 18.9% of this area in China (FAOSTAT, 2016). Mineral or organic fertilizers, or their combinations, are usually applied to paddy fields to maintain soil fertility and increase rice crop yields. Organic fertilizers commonly consist of crop residues or animal manure. Crop residue and manure addition can increase soil organic carbon (SOC) content (Yan et al., 2007; Liu et al., 2014; Zhang et al., 2016), but they can also be a source of CH_4emissions in paddy soil (Shen et al., 2007; Tang et al., 2016). Application of manure or rice straw combined with mineral fertilizer in paddy soils was demonstrated to be effective in increasing soil fertility (mainly due to increased microbial biomass and SOC) (Xu et al., 2018). Similarly, a mixture of 70% manure in combination with 30% chemical fertilizer improved rice yields and bacterial diversity, and also alleviated soil acidification (Chen et al., 2017).

Soil microorganisms play a key role in nutrient cycling, and maintaining or increasing microbial diversity is crucial for soil health (Keeler et al., 2009; Whiteside et al., 2012; Fierer, 2017). Microbial diversity is affected by several factors, such as soil physical and chemical properties (Lynn et al., 2017; van Leeuwen et al., 2017), fertilization (Huang et al., 2018), and irrigation (Azziz et al., 2016; Das et al., 2016). Most previous studies on microbial communities focused on topsoil (based on tillage or direct fertilization)because the composition is more variable in the surface horizons (Eilers et al., 2012), which generally refers to depths of 0-20 cm for paddy soils. However, a previous study suggested that nutrients can translocate from the topsoil to the deeper layers in maize and wheat fields (Kramer et al., 2013). Another previous study determined that up to 30% of microbial biomass could still be found in the C horizon (55-65 cm in grassland and cropland) (van Leeuwen et al., 2017). Hence, soil depth should be considered as an important environmental gradient that structures soil-microbial communities (Eilers et al., 2012), especially the subsoil close to the plough layers. Mineral fertilizer plus manure addition has been demonstrated to increase different taxa of bacteria and archaea for topsoil (0–20 cm) and subsoil (below 20 cm) compared to mineral fertilizer alone in long-term paddy fields (Gu et al., 2017).

Previous studies have shown that different fertilization strategies could alter different microbial-community structures. Mineral N fertilizer was reported to increase the gram-positive to gram-negative bacteria (G+ to G–) ratio in paddy soil (Zhang et al., 2007) and temperate grassland soil (Denef et al., 2009), while no ratio difference was observed in other studies (Zhang et al., 2012; Dong et al., 2014). It has been suggested that G–bacteria preferentially use labile plant-derived C and G+ bacteria that are able to utilize recalcitrant compounds from soil organic matter (SOM) (Kramer & Gleixner, 2008; Fanin et al., 2019). Complex litter addition was found to greatly promote fungi than bacteria and also increased the G– to G+ bacteria ratio in subtropical forest soil (Wang et al., 2014); the straw residue was demonstrated to promote more G– bacteria than G+ bacteria (Tang et al., 2018). Manure was reported to promote G–bacteria in rotation cropping (Peacock et al., 2001) and paddy soils (Zhang et al., 2012). However, there is still limited information regarding the microbial-community change along the soil profiles of paddy soil supplied with equal amounts but different types of fertilizer based on long-term observation. It has been demonstrated that, unlike inorganic fertilizer, straw and manure addition increase the relative abundance of*Gemmatimonadetes* and *Planctomycetia* (Tang et al., 2019).

In the present study, we investigated the microbial biomass and responses of specific microbial groups (the fungi to bacteria, G+ to G–, G+ to actinomycetes, and fungi to G– bacteria ratios) to different fertilizer types (mineral and organic combination with equal amounts of mineral fertilization [NPK]) at 0–10, 10–20, 20–30, and 30–40 cm soil depths in paddy soil systems. The microbial biomass and community composition were

measured using chloroform fumigation extraction and phospholipid fatty acid(PLFA) analyses, respectively. We hypothesized that 1) the microbial abundance and biomass would decrease with soil depth; 2) fertilization would increase microbial abundance and biomass; and 3) mineral fertilizer would promote G+ bacteria and thereby increase the G+ to G– bacteria ratio, while more complex organic fertilizer (straw and manure) would stimulate more G– bacteria.

MATERIALS AND METHODS

2.1 Site description and fertilization strategies

Soil samples were collected in April 2015 (before early rice transplanting) from long-term fertilization experiment stations at Ningxiang in the subtropical region of the Hunan Province, China (111°54'-112deg18'E, 28deg07'-28deg37'N). The mean annual temperature and rainfall of the site are 17degCand1681 mm, respectively. The soil was derived from river alluvium, Quaternary red clay, and plate shale, and the cropping system used was milk vetch–rice–rice. The main physical and chemical properties of the soil at the start of the long-term field fertilization trials were as follows: pH 5.8, SOC17.1 g kg^{-1}, total N 1.76 g kg^{-1}, available N 144mg kg^{-1}, and Olsen-P 12.8 mg kg^{-1} (Yuan et al., 2012).

The experiment started in 1986 with the following four treatments: no fertilizer (CK), mineral fertilizer (NPK) (urea, superphosphate, and potassium chloride), rice straw combined with mineral fertilizer (NPK + ST), and 70% NPK +30% chicken manure (NPK +OM). The rice straw produced from the straw plots was returned to the corresponding plots in July and November after the early and late rice harvests at rates of 2775 kg ha^{-1} a^{-1} and 3600 kg ha^{-1} a^{-1}, respectively. The experiment design ensured that all fertilizer treatments received the same amount of N, phosphorus pentoxide (P_2O_5), and potassium oxide (K_2O). The total amount of N, P_2O_5, and K_2O was the sum of the chemical fertilizer plus that from the rice straw residue or manure, corresponding to143 kg N ha^{-1}, 23.2 kg Pha^{-1}, and 52.3 kg K ha^{-1}; and 158.0 kg N ha^{-1}, 18.6 kg P ha^{-1}, and 67.2 kg K ha^{-1}per fertilized treatment for the early and late rice seasons every year, respectively. The total amounts of fertilizer applied in each fertilized treatment during the milk vetch season were identical to those for the late rice season.

2.2 Soil sampling and chemical analysis

Soil samples were collected from three replicate plots for each treatment. In each 33 m^2 plot, five soil cores were taken from the four layers at 0-10, 10–20, 20–30, and 30–40 cm depths and mixed thoroughly. The composite soil samples were immediately placed in a gas-permeable plastic bag and stored at 4degCuntil analysis. Subset soil samples were air-dried and sieved through a 2-mm mesh to remove plant debris and stones. The physical and chemical properties of the soil were then analysed, as presented in Table 2.

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the fumigation-extraction method (Brookes et al., 1985; Wu et al., 1990) and analysed using a total organic carbon analyser (TOC-VWP; Shimadzu Corporation, Kyoto, Japan) and flow-injection auto-analyser (Tecator FIA Star 5000 Analyzer, Foss Tecator, Sweden), respectively. Soil pH was measured using a pH meter (Delta 320; Mettler Toledo, Columbus, OH, USA) with a soil/water ratio of 1:2.5. SOC content was determined using dry combustion in an elemental analyser (VarioMAX C/N; Elementar, Langenselbold, Germany). The soil alkali-hydrolysable nitrogen content (available N) was determined using alkali solution diffusion absorption. Olsen-P was extracted using 0.5mol$L^{-1}$$NaHCO_3$(Olsen et al., 1954).

2.3 PLFA extraction

For the PLFA extraction, 2 g of freeze-dried (2-mm sieved) soil was extracted and derivatized to fatty acid methyl esters following the previously reported method of Bligh and Dyer (1959) as adapted by White et al.(1979). Briefly, the soil was first extracted twice using a 22.8-mL single-phase solution of chloroform: methanol: citrate buffer (1:2:0.8 v/v/v,0.15 M, pH 4.0). The supernatant was transferred to test tubes and split into two phases by adding equal amounts of $CHCl_3$ and citrate buffer. Phospholipids were then separated from neutral lipids and glycolipids on a silica acid column (Supelco, Bellefonte, PA, USA). Methyl nonadecanoate fatty acid (19:0) was added prior to derivatization as an internal standard to quantify the

concentration of phospholipids. Following methylation of the phospholipids, the PLFA methyl esters were separated and identified using gas chromatography (GC; N6890; Agilent Technologies, Inc., Santa Clara, CA, USA) fitted with a MIDI Sherlock microbial identification system (version 4.5; MIDI, Inc., Newark, DE, USA). Individual PLFAs were quantified from the combined area of the peaks with mass-to-charge values (m/z 44, 45, and 46) relative to the internal standard added to each sample (Thornton et al., 2011). Iso- and anteiso-branched fatty acids (except for 10Me-branched PLFAs) were used as indicators for G+ bacteria, while monounsaturated and cyclopropyl fatty acids were used as indicators for G- bacteria (Wang et al., 2016; Ma et al., 2018). The 10Me-branched PLFAs were used as actinomycete biomarkers, while 18:2 ω6c and arbuscular mycorrhizal fungi 16:1 ω5c were used as fungal biomarkers. The sum of the G+ bacteria, G- bacteria, and actinomycetes was used for ratio calculation as bacteria PLFAs.

2.4 Soil respiration measurement

The subset of air-dried soil samples was then pre-incubated for 14 days at 25°C under flooded conditions. For each measurement of respiration rate, approximately 20 g (equivalent dry weight) of each soil sample was incubated in a 500-mL container at 25°C for 24 h. At the end of this period, CO_2 concentrations in the headspace were measured using an Agilent-7890a gas chromatograph equipped with a flame ionization detector (Agilent Technologies, Inc.). Soil respiration rates were calculated from the net accumulation of CO_2 over time. The soil metabolic quotient (qCO_2) was calculated by dividing the soil respiration rate by the MBC content (Plaza et al., 2004).

2.5Statistical analysis

Data were analysed using one-way analysis of variance for different groups of soil depth within each fertilization regime and different groups of fertilization regimes within each soil depth, and were compared using Duncan's test in SPSS 19.0 (SPSS Inc., Chicago, IL, USA), with significance defined as P <0.05. The relationship between the microbial structures and physicochemical properties was analysed using redundancy analysis (RDA) using Canoco 5.0 for Windows (Microcomputer Power, Ithaca, NY, USA).

RESULTS

3.1 Effect of fertilization strategies on microbial communities along the paddy soil profile

The absolute abundance of total and specific microbial groups (G-, G+ bacteria, actinomycetes, and fungi) in all treatments significantly decreased with soil depth (Table 1, P <0.05). The abundance of total and specific PLFAs were the lowest and highest in the control and NPK + OM treatments, respectively, at the 0-40 cm soil depth. Compared with that in the NPK treatments, NPK + ST showed significantly higher bacterial (G-, G+, and actinomycetes) and fungal abundance at the 10-20 cm soil depth (P <0.05). The fungi to bacteria, G+ bacteria to actinomycetes, and fungi to G- bacteria ratios gradually decreased with soil depth in all treatments between 0 and 30 cm. The G+ to G-bacteria ratio increased with soil depth for all treatments (Figure 1).

Both mineral and organic fertilizers significantly increased the G+ to G- bacteria and actinomycetes ratios compared with those in the control group at 0–30 cm soil depths (Figures 1b and c,P <0.05). NPK + ST showed the lowest fungi to bacteria and G- bacteria ratios at 0–10 cm compared with those in the other treatments (Figures 1a and d, P <0.05). At 10-40 cm, the fertilized treatments showed higher fungi to bacteria and G- bacteria ratios compared to those of the control.

There was no uniform pattern throughout the soil profile regarding the influence of organic fertilizers on microbial functional PLFA ratios. With equal amounts of fertilizer input, compared with NPK, NPK + OM further stimulated more G+ bacteria than G- bacteria and actinomycetes at all depths (Figures 1b and c). Soil respiration rates gradually decreased with soil depth and the lowest values for each soil depth were observed in the control (Figure 2a, P <0.05). NPK + ST significantly increased the soil respiration rate at 0-20 cm (P <0.05). NPK + OM showed a constant soil qCO_2 between the 0 and 40 cm soil depths, while both the NPK treatments and the control significantly increased the qCO_2 values with soil depth. NPK + ST treatment showed the lowest qCO_2 at 10–20 cm compared to that at other depths (Figure 2b, P <0.05).

3.2 Relationship between microbial communities and soil properties

SOC content and available N decreased with soil depth (Table 2,P <0.05). Compared to that of the control, NPK +ST and NPK +OM showed significantly higher SOC contents at soil each depth. NPK addition alone resulted in lower SOC content at 0–10 cm compared to that in the control. In contrast, soil pH exhibited the opposite pattern of SOC, which was significantly increased with soil depth, with the highest value observed in the control (Table 2,P <0.05).

MBC and MBN decreased with soil depth in all treatments (Table 2,P <0.05). Compared to those in the control, NPK and NPK+ OM significantly increased MBC and MBN at0–40 cm, except for 10–20 cm in NPK. In contrast, NPK + ST significantly increased MBC at0–30 cm compared to that in the control (P <0.05). Compared to those under NPK addition alone, the NPK + ST treatment increased MBC and MBN at 10–30 cm and 0–30 cm, respectively; NPK+ OM treatments showed significantly higher MBC and MBN at 0–40 cm (Table 2,P <0.05).

The RDA showed that SOC significantly contributed to the soil microbial communities and explained 87% of the variance in the first two axes (Figure 3a, P <0.001). Soil pH was also significantly correlated with the microbial communities and explained 3.7% of the total variation. Available N and SOC contributed significantly to the microbial communities at both the 0–20 and 20–40 cm soil depths and explained 79.3% and 4.5%, and 3.1% and 86.4% of the total variation for each depth, respectively (P <0.05). Soil pH only showed a significant correlation to the microbial communities at the 0–20 cm soil depths and explained 4.9% of total the variation (P <0.013) (Figures3b and c).

DISCUSSION

4.1 Microbial groups along soil depth

The decline in the abundance of microbial PLFAs with soil depth (Table 1, P <0.05) could arise from the reduction in energy (carbon) and nutrient availability along the profile. A reduction in the ratio of fungi to bacteria with soil depth (Figure 1a) is also an indication that the fungal population declined much faster than that of bacteria along soil profiles. Similarly, the decreasing trends of the fungi to G-bacteria and G+ bacteria to actinomycetes ratios, as well as the increasing G+ to G-bacteria ratio with soil depth could mean that microbial dependence on nutrients adhered to the following order: fungi > G- bacteria> G+ bacteria > actinomycetes. Fungal abundance and activity are considered low in paddy soil because of prolonged anaerobic conditions. However, in paddy soils, rice plants could release oxygen from the root through aerenchyma (Frenzel et al., 1992) and created an aerobic environment in the rhizosphere. Previous studies have demonstrated that fungi have the highest capacity for assimilating rhizodeposits in paddy soils (Ge et al., 2016). Such translocation to fungi can be rapidly detected in PLFAs due to the direct connection of fungal hyphae and mycorrhizal fungi with rice roots (Yuan et al., 2016). Therefore, oxygen limitation caused by root biomass reduction with soil depth could largely suppress fungal abundance. G- bacteria have been reported to preferentially use labile compounds such as rhizodeposits and exudates from plant biomass, and G+ bacteria are able to use recalcitrant compounds from SOM (Kramer and Gleixner, 2008; Fanin et al., 2019). Zhu et al. (2017)also found that G+ bacteria increased after rhizo- and micro-C addition. Labile substrates decrease with increasing soil depth, thereby causing G- bacteria to decline faster than G+ bacteria. As actinomycetes are well known for their key role in degrading complex compounds (Acosta-Martinez et al., 2008), they are relatively the least sensitive to labile substrate reduction with soil depth. Thus, the sensitivity trends of the PLFAs with soil depth were not altered by fertilization type.

The decrease in MBC and MBN with soil depth corresponded with the decrease in SOC and available N content along the soil profile (Table 2). This indicates that the growth of microorganisms is related to the availability of C and N nutrients (Loeppmann et al., 2016; van Leeuwen et al., 2017). SOC levels are fundamentally determined by the balance between organic matter inputs and their losses through decomposition (Six, Frey, Thiet, & Batten, 2006). In our current study, rhizodeposition and fertilization were the two main sources of microbial C nutrient availability. Zhu et al. (2017) found that up to 45% of rice rhizo-C was stabilized within SOC. In paddy soils, more than 50% of the total root biomass is allocated to the first 5

cm of surface soil; therefore, the upper soil layer can receive more C input than the soils below (Li et al., 2004; Li & Yagi, 2004). The decrease in the $\delta^{13}C$ isotopic signature of soil and PLFA from C_4 plants with soil depth after C_3-C_4 vegetation change in a previous study also demonstrated the decreasing influence of plants on deeper soil (Kramer & Gleixner, 2008). Higher MBC and MBN contents were observed at 0–20 cm in the control, indicating that soil layers with fresh C input can increase microbial biomass. RDA results also showed an obvious separation of the 0–20 and 20–40 cm soil layers, even in the control without fertilizer, confirming the rhizodeposition effect on the 0–20 cm soil layer (Figure 3a).

4.2 Effect of fertilization strategies on microbial community structure

All fertilized treatments increased microbial PLFAs (Table 1), showing a fertilizer-induced increase in microbial abundance. Large amounts of N from mineral and organic fertilizers could benefit microbes through a reduction in nutrient competition with rice plants (Jackson, Burger, & Cavagnaro, 2008; Zhu et al., 2018). Moreover, the increase of plant biomass caused by N fertilization would have a direct impact on rhizodeposition and the high availability of labile C to microbes (Christopher &Lal, 2007; Zhu, Vivanco, & Manter, 2016). G- bacteria are usually considered to benefit more from rhizodeposits (Fanin et al., 2019). However, the G+ to G-bacteria ratio was enriched (0.55˜0.83 after fertilizers vs. 0.45˜0.53 in control) at 0-30 cm soil depths (Figures 2b and c, P <0.05). Moreover, the abundance of G+ bacteria exceeded that of actinomycetes at 0-30 cm. This indicated that both mineral and organic fertilizers preferentially promote G+ bacteria. Pure manure has been demonstrated to induce G-bacteria and reduce G+ bacteria (Peacock et al., 2001). Wang et al. (2014) also found more C incorporation by G-bacteria, which had originally been dominant in the soils. In parallel, they found that litter addition would reduce the G+ to G- bacteria ratio, but its combination with mineral N could buffer this decrease. This indicated that complex substrates induced more G-bacteria and mineral N promoted G+ bacteria, thereby reducing the G+ to G-bacteria ratio. Relatively increased G+ bacteria levels due to mineral N fertilization have also been reported in grassland soils (Denef et al., 2009) and corn fields (Peacock et al., 2001). Consistent with these observations, all treatments with mineral N fertilizers in the present study induced G+ bacteria, even when the soil was predominated by G-bacteria. This indicates a positive response of soil G+ bacteria to mineral N fertilizers.

After partial replacement of inorganic fertilizer with straw, PLFA biomarkers increased at 10–20 cm compared with those under NPK addition alone (Table 2, P <0.05). The fungi to bacteria and G- bacteria ratios were also relatively increased at this soil depth (Figures 1a and d). This indicated that fungi were especially promoted at 10–20 cm, probably owing to more suitable anaerobic conditions for straw decomposition. Simultaneously, qCO_2 was observed to be the lowest at this soil depth. Some studies have suggested that higher qCO_2 means higher stress or disturbance for microorganisms (Wardle & Ghani, 1995). Under the same level of fertilizer input comparable to straw addition, MBC and PLFAs were largely induced by chicken manure throughout the soil profile (Tables 1 and 2, P < 0.05). Manure addition further increased the G+ to G- bacteria and G+ bacteria to actinomycetes ratios (Figures 1b and c), which may indicate that manure induced more G+ bacteria. In addition, the NPK + OM treatments showed a consistently low qCO_2 throughout the 0-40 cm soil profile (Figure 2). Plaza et al. (2004) observed that qCO_2 initially decreased after pig manure amendment to soil and then increased with further manure addition. Hence, qCO_2 was not negatively correlated with soil fertility or organic fertilizer input. The findings above may preferentially support the connection between low qCO_2 and favourable soil conditions for microorganisms.

In addition to its effect on microbial communities through nutrient availability, fertilization can indirectly alter microbial communities through its effect on pH at 0–20 cm soil depths (Figures 3a and b). More organic acids such as oxalic acid (Keiluweit et al., 2015) and acetate (Farrar, Hawes, Jones, & Lindow, 2003) released from rhizodeposits could partly cause a reduction in soil pH, especially in the rooted layers compared with the deeper layers (Table 2, Fig 3a-b,P <0.05). In addition, N fertilizer has been reported to cause soil acidification during N cycling (Bolan, Headley, & White, 1991; Geissler & Scow, 2014). However, in contrast to general assumptions, higher SOC contents were observed in the control compared with those in NPK at 0–10 cm. The soil respiration rate and qCO_2 were higher in NPK in this soil layer (Figures 2a and b, P <0.05), and carbon use efficiency was also found to be lower in NPK (Zhran et al., 2020). A greater amount of

respired than accumulated C in biomass can partially explain the decrease in SOC. In addition, Loeppmann et al. (2016) found that maize rhizodeposition can decrease the proportional activity of C- to N-cycling enzymes. Mineral NPK fertilizers can increase root biomass and then increase rhizodeposition, but it can also stimulate microorganisms that need both C and N. More C-cycling enzymes will be released by microbes to decompose SOC to maintain a certain microbial C:N ratio (Devi & Yadava, 2006) if microorganisms need more C than rhizodeposits can supply.

5. Conclusions

Long-term mineral and organic fertilization were demonstrated to have a positive effect on microbial biomass enhancement. Our data clearly showed that the soil microbial biomass and PLFAs decreased with soil depth owing to decreasing available nutrients along soil profiles. The microbial sensitivity of nutrients declined with soil depth in the following order: fungi > G- bacteria > G+ bacteria > actinomycetes; this order cannot be changed by fertilization type. Moreover, mineral fertilizers induced G+ bacteria more than G- bacteria and actinomycetes, which suggested a close link between G+ bacteria and mineral fertilizers. With equal fertilizer input, straw addition especially promoted fungi at 10-20cm soil depth, while chicken manure further stimulated G+ bacteria. In broader perspective, understanding the mechanisms of microbial group shift due to fertilization in paddy systems may help improving soil quality through better fertilization management. Further studies should include isotope analyses (^{13}C, ^{14}C,^{15}N) and microbial molecular analyses (16S rRNA sequencing, PLFA, SIP, etc) to fully reveal the fertilizer nutrient turnover *in situ* in paddy soil systems.

Conflict of interest statement: The authors declare no conflict of interest.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1 Total phospholipid fatty acids (PLFAs) (mg C kg^{-1}) in paddy soil under fertilization regimes at different soil depths (mean+- SE, n=3). Statistical significances based on one-way analysis of variance and Duncan's test ($P< 0.05$). Different lower-case letters represent significant differences in soil depth within a fertilization regime. Different uppercase letters represent significant differences in fertilization regime within soil depth. Fame: fatty acid methyl esters; CK: no fertilizer; NPK: chemical fertilizers; ST: rice straw combined with chemical fertilizers; OM: 70% NPK + 30% chicken manure. G-: Gram-negative bacteria; G+: Gram-positive bacteria.

Treatments	Soil depth (cm)	G-	G+	Actinomycetes	Fungi	General Fame	Total PLFA
CK	0–10	13.17±0.50Ca	5.92±0.47Ca	6.11±0.23Ca	1.96±0.10Ba	10.58±0.56Ca	39.36±1.84Ca
	10–20	5.81±0.15Db	2.54±0.26Db	3.33±0.10Db	0.62±0.01Cb	4.88±0.19Db	17.83±0.72Db
	20–30	1.50±0.08Cc	0.79±0.03Dc	1.11±0.01Cc	0.15±0.01Dc	1.62±0.06Cc	5.32±0.16Cc
	30–40	0.53±0.06Cd	0.41±0.07Cd	0.54±0.05Cd	0.05±0.01Cd	0.59±0.06Cd	2.20±0.25Cd
NPK	0–10	15.60±0.18Ba	9.16±0.12Ba	8.01±0.25Ba	2.35±0.03Ba	13.29±0.40Ba	50.14±0.87Ba
	10–20	9.63±0.19Cb	6.08±0.33Cb	5.66±0.20Cb	1.33±0.05Bb	8.43±0.30Cb	32.40±1.01Cb
	20–30	5.44±0.18Bc	3.57±0.10Cc	3.51±0.01Bc	0.67±0.01Bc	5.09±0.11Bc	19.03±0.47Bc
	30–40	1.12±0.03Bd	0.98±0.01Bd	1.07±0.01Bd	0.13±0.00Bd	1.27±0.04Bd	4.85±0.09Bd
NPK + ST	0–10	17.01±0.06Ba	9.36±0.25Ba	8.44±0.01Ba	2.31±0.02Ba	14.53±0.07Ba	52.78±1.46Ba
	10–20	15.32±0.41Bb	8.79±0.10Ba	8.05±0.13Ba	2.11±0.14Aa	13.88±0.12Ba	50.50±0.79Ba
	20–30	4.96±0.38Bc	4.08±0.27Bb	4.04±0.35Bb	0.57±0.04Cb	5.17±0.43Bb	19.45±1.41Bb
	30–40	0.93±0.12Bd	1.04±0.10Bc	1.00±0.12Bc	0.11±0.02Bc	1.04±0.10Bc	4.02±0.56Bc
NPK+ OM	0–10	19.29±0.90Aa	13.01±0.44Aa	10.21±0.56Aa	2.96±0.24Aa	17.13±1.11Aa	64.14±3.90Aa
	10–20	16.98±0.64Ab	11.12±0.37Aa	9.55±0.30Aa	2.26±0.03Ab	15.30±0.60Aa	57.47±1.90Aa
	20–30	6.89±0.22Ac	5.58±0.18Ab	5.14±0.20BAb	0.82±0.04Ac	6.71±0.27Ab	25.93±0.89Ab
	30–40	1.82±0.05Ad	1.63±0.10Ac	1.63±0.05Ac	0.27±0.01Ad	2.05±0.08Ac	7.65±0.30Ac

Table 2 Basic physicochemical properties of paddy soil under fertilization regimes at different soil depths (mean± SE, n=3). CK: no fertilizer; NPK: chemical fertilizers; ST: rice straw combined with chemical fertilizers; OM: 70% NPK + 30% chicken manure; SOC: soil organic content; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen. Statistical significances based on one-way analysis of variance and Duncan's test ($P < 0.05$). Different lower-case letters represent significant differences in soil property between soil depths within a fertilization regime. Different uppercase letters represent significant differences in a soil property between fertilization regimes within a soil depth.

Treatments	Treatments	pH	SOC (g kg^{-1})	MBC (mg kg^{-1})	MBN (mg kg^{-1})	Available N (mg kg^{-1})	Olsen-P (mg kg^{-1})
CK	0–10	7.15±0.01Ad	18.32±0.06Ca	740.40±32.52Ca	1.81±0.00Da	151.30±2.20Da	6.57±0.56Ca
	10–20	7.40±0.02Ac	15.78±0.21Cb	351.35±18.50Db	1.52±0.00Cb	108.39±1.18Cb	4.93±0.17Bb
	20–30	7.52±0.01Ab	6.48±0.07Dc	123.78±5.45Dc	0.79±0.01Dc	46.71±1.18Cc	5.56±0.37Cab
	30–40	7.61±0.02Aa	5.42±0.07Bd	59.37±1.71Cd	0.71±0.01Cd	44.92±0.77Bc	6.48±0.14Ca
NPK	0–10	6.48±0.01Dd	17.48±0.52Da	829.72±8.50Ba	1.87±0.03Ca	181.69±5.08Ca	11.37±0.64Ba
	10–20	6.82±0.01Cc	16.13±0.04Cb	527.14±4.60Cb	1.59±0.14Cb	129.40±0.80Bb	9.23±0.14Bb
	20–30	7.21±0.02Bb	9.40±0.13Cc	219.88±6.49Cc	1.04±0.02Cc	71.29±0.89Bc	6.80±0.75Cc
	30–40	7.25±0.02Ca	6.09±0.00Ad	81.66±7.02Bd	0.77±0.01Ad	43.13±0.49Bd	7.01±0.53BCc
NPK + ST	0–10	6.64±0.02Cd	22.04±0.10Ba	880.98±27.07Ba	2.21±0.02Ba	209.85±2.05Ba	10.38±0.49Bab
	10–20	6.84±0.01Cc	20.07±0.25Bb	807.86±29.72Bb	1.99±0.04Bb	185.27±0.45Ab	9.25±0.15Bbc
	20–30	7.12±0.01Db	11.90±0.10Bc	259.15±10.78Bc	1.25±0.02Bc	96.77±2.49Ac	11.04±0.88Ba
	30–40	7.19±0.02Da	6.08±0.06Ad	62.55±3.47BCd	0.77±0.01Ad	49.84±0.45Ad	8.00±0.07Bc
NPK + OM	0–10	6.74±0.02Bd	25.46±0.42Aa	1019.9±52.4Aa	2.60±0.08Aa	251.42±1.34Aa	111.25±6.47Aa
	10–20	6.93±0.01Bc	24.98±0.68Aa	869.2±22.1Ab	2.50±0.01Ab	193.32±6.49Ab	116.50±4.40Aa
	20–30	7.17±0.02Cb	12.12±0.03Ab	421.3±1.53Ac	1.32±0.01Ac	97.66±4.47Ac	56.99±2.16Ab
	30–40	7.34±0.02Ba	5.89±0.15Ac	165.134±2.1Ad	0.75±0.01Bd	42.24±2.05Bd	10.19±0.25Ac

Figure captions

FIGURE 1 Microbial community changes to the (a)ratio of fungal to bacterial phospholipid fatty acids (PLFAs); (b) ratio of gram-positive to gram-negative bacterial PLFAs; (c) ratio of gram-positive to actinomycetes PLFAs; and (d) ratio of fungal to gram-negative bacterial PLFAs in paddy soil under fertilization regimes at different soil depths. CK: no fertilizer; NPK: chemical fertilizers; NPK + ST: rice straw combined chemical fertilizers; NPK + OM: 70% NPK + 30% chicken manure. Statistical significance is based on a one-way analysis of variance and Duncan's test ($P< 0.05$). Different lower-case letters represent significant differences in soil depths within a fertilization regime. Different uppercase letters represent significant differences in fertilization regimes within a soil depth (n= 3)

FIGURE 2 (a) Soil respiration rate and (b) soil metabolic quotient (qCO_2) in paddy soil under fertilization regimes at different soil depths. CK: no fertilizer; NPK: chemical fertilizers; NPK + ST: rice straw combined with chemical fertilizers; NPK + OM: 70% NPK + 30% chicken manure. Statistical significance is based on a one-way analysis of variance and Duncan's test ($P< 0.05$). Different lower-case letters represent significant differences in soil depths within a fertilization regime. Different uppercase letters represent significant differences in fertilization regimes within a soil depth (n= 3)

FIGURE 3 Redundancy analysis of phospholipid fatty acid content from soil samples at different depths. (a) 0–40 cm; (b) 0–20 cm; (c) 20–40 cm. Shapes represent the type of fertilizer, while colours represent the different soil depths. CK: no fertilizer; NPK: chemical fertilizers; NPK + ST: rice straw combined with chemical fertilizers; NPK + OM: 70% NPK + 30% chicken manure. 0–10 cm (black), 10–20 cm (red), 20–30 cm (yellow), and 30–40cm (orange)