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# Soil organic carbon (SOC) accumulation in rice paddies under long-term agro-ecosystem experiments in southern China – VI. Changes in microbial community structure and respiratory activity

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Received: 8 January 2011 – Accepted: 28 January 2011 – Published: 21 February 2011

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Published by Copernicus Publications on behalf of the European Geosciences Union.

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## Abstract

Biological stabilization within accumulated soil organic carbon (SOC) has not been well understood, while its role in physical and chemical protection as well as of chemical recalcitrance had been addressed in Chinese rice paddies. In this study, topsoil samples were collected and respiratory activity measured in situ following rice harvest under different fertilization treatments of three long-term experimental sites across southern China in 2009. The SOC contents, microbial biomass carbon (SMBC) and nitrogen (SMBN) were analysed using chemical digestion and microbial community structure assessment via clony dilute plate counting methods. While SOC contents were consistently higher under compound chemical fertilization (Comp-Fert) or combined organic and inorganic fertilization (Comb-Fert) compared to N fertilization only (N-Fert), there was significantly higher fungal-bacterial ratio under Comb-Fert than under N-Fert and Comp-Fert. When subtracting the background effect under no fertilization treatment (Non-Fert), the increase both in SMBC and SMBN under fertilization treatment was found very significantly correlated to the increase in SOC over controls across the sites. Also, the ratio of culturable fungal to bacterial population numbers (F/B ratio) was well correlated with soil organic carbon contents in all samples across the sites studied. SOC accumulation favoured a build-up the microbial community with increasing fungal dominance in the rice paddies under fertilization treatments. While soil respiration rates were high under Comb-Fert as a result of enhanced microbial community build-up, the specific soil respiratory activity based on microbial biomass carbon was found in a significantly negatively correlation with the SOC contents for overall samples. Thus, a fungal-dominated microbial community seemed to slow SOC turnover, thereby favouring SOC accumulation under Comp-Fert or under Comb-Fert in the rice paddies. Therefore, the biological stabilization process is of importance in SOC sequestration in the rice paddies, operating with physical and chemical protection and chemical recalcitrance. However, sufficient understanding and prediction of SOM dynamics needs further quantitative characterization of the simultaneous operation of several mechanisms.

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## 1 Introduction

Soil carbon sequestration had been well-addressed to have double beneficial effects on both crop production and climate change mitigation (Lal, 2004a). The great potential of carbon sequestration has been recognized for world agriculture through enhancing soil C stock under good cropland management (Smith et al., 2008). Recently, the World Bank announced the *Roadmap for Action: Agriculture, Food Security and Climate Change* and called for action to make the agricultural sector part of the solution to climate change by sequestering more carbon into the soil and biomass (World Bank, 2010).

Studies have shown that Chinese croplands may have a great potential for C sequestration, while soil organic carbon enhancement may help increase crop productivity and sustainability (Lal, 2004b; Pan, 2009; Huang et al., 2010). As more productive croplands, rice paddies generally have higher SOC storage (Pan et al., 2004a, b) and sequestration capacity under fertilization (Wang et al., 2010), conservation tillage (Wang et al., 2009), as well as under conventional conditions (Pan et al., 2010) when compared to drier croplands. Using soil samples from long-term agro-ecosystem experiments across southern China, the enhanced SOC sequestration has been characterized by higher organic matter inputs (Zhou et al., 2009c), physical protection in coarse soil micro-aggregate fractions (Li et al., 2007; Zhou et al., 2006, 2009a), chemical binding to oxyhydrates (Zhou et al., 2009b), and chemical recalcitrance of molecular stabilization (Zhou et al., 2010; Spaccini et al., 2009).

There have been increasing concerns regarding the role of soil microbial community in biological stabilization of SOC in agricultural soils (Kögel-Knabner et al., 2006). Butler et al. (2003) reported a change in the soil microbial community within rhizospheres with changes in organic matter input. Kandeler et al. (2008) observed a significant fungal predominance over bacterial under free CO<sub>2</sub> enrichment experiment. These studies indicate that soil organic matter turnover is regulated by soil micro-organisms, which is subject to changes with soil conditions including soil organic matter accumulation

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itself. In previous studies, changes in the genetic diversity of soil bacteria (Zhang et al., 2004) and methanotrophs (Zheng et al., 2008) was seen under differential fertilization in a rice paddy from the Tai Lake region of China. However, the role of such changes in soil organic carbon sequestration and its stabilization has not yet been well understood.

We hypothesize that the microbial community and its functioning may have changed as soil organic matter accumulated under different long-term fertilization treatments. There would be increasing fungal predominance and lower soil respiratory activity as a biological stabilizing mechanism when SOC sequestration is facilitated. Using both topsoil samples from long-term field experiments across southern China and a plate incubation technique of in situ soil respiration measurements after rice harvest, this study: (1) depicts the changes in microbial population numbers and fungal predominance; (2) infers a biological stabilizing mechanism behind the C sequestration which includes physical-chemical stabilization as the major controlling process.

## 2 Materials and methods

### 2.1 Site descriptions and experimental design

As about 90 percent of China's paddy soils are located in the region south to the Huai River (Pan et al., 2004), including the red earth terraces and the lower reaches of Yangtze River valley of China with rice cultivation for hundreds of years. In this study, three separate long-term agro-ecosystem experiments were used with different fertilization treatments which were conducted separately on these soils since the late 1980s. These experiments are described as follows.

Soil JX is from an experiment with fertilization treatments located at the Experimental Farm of Jiangxi Institute of Red Soils (28°15' N and 116°20' E), Jinxian County, Jiangxi Province. Derived from quaternary red clay, the soil is classified as a typical Hapludult (Soil Taxonomy, USDA, 1999). The local climate is governed by a subtropical monsoon, with the mean annual temperature of 17.7 °C and annual precipitation of 1400 mm with

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70% falling in late April to early July for the last two decades. The treatments since 1981 included: (1) a control treatment as CK without fertilizers (Non-Fert); (2) nitrogen fertilizer only (N-Fert); (3) compound fertilizer of inorganic N, P, K (NPK) (Comp-Fert); compound fertilizer of inorganic N, P, K plus organic manure (NPKM) (Comb-Fert<sub>m</sub>); (4) constant cultivation with rice-rice rotation. The experimental design was described in detail by Li et al. (2006).

Soil WCH was from an experiment with different fertilization treatments, located in Wangcheng Municipality (28°37' N and 112°80' E), Changsha City, Hunan Province. The soil was similar in pedogenesis to Soil JX. The climate is also governed by a subtropical monsoon, with a mean annual temperature and precipitation of 17.0°C and 1385mm, respectively during the past two decades. The experiment with the fertilization treatments initiated in 1981 included: (1) no fertilizer application as CK (Non-Fert); (2) compound chemical fertilization with N and K (NK) (Comp-Fert<sub>nk</sub>); (3) compound chemical fertilization with N, P and K (NPK) (Comp-Fert); (4) compound chemical fertilizers of N and K combined with pig manure (NKM) (Comb-Fert<sub>nk</sub>); (5) compound chemical fertilization of N, P, and K combined with straw (NPKS) (Comb-Fert<sub>s</sub>). The experiment has been under continuous rice-rice rotation since 1981. The experimental design was described in detail by Liao et al. (2009).

Soil WJ was also from an experiment with different fertilization treatments, located in Wujiang Municipality (31°05' N and 120°46' E), Suzhou City, Jiangsu Province. The soil is derived from riverine-lucustrine sediments and classified as an entic Halpudept. A subtropical monsoon climate governs the area with mean annual temperature and precipitation of 18.3°C and 1,100 mm for the last two decades, respectively. The fertilizer treatments initiated in 1987 with continuous rice-rape rotation have consistently been as follows: (1) no fertilizer application (NF) as CK without fertilizers (Non-Fert); (2) compound chemical fertilization with N, P and K (NPK) (Comp-Fert); (3) compound chemical fertilization plus pig manure (NPKM) (Comb-Fert<sub>m</sub>); (4) compound chemical fertilization combined with rice straw (NPKS) (Comb-Fert<sub>s</sub>). The experimental design was described in detail by Pan et al. (2009b).

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All of the fertilization treatments were performed in triplicate with randomly allocated blocks. The basic conditions of the three experimental sites were summarized in Table 1.

## 2.2 Topsoil sampling

5 Three undisturbed topsoil (0–20 cm) samples were randomly collected, using an Eijkkamp soil core sampler, in each plot of the experimental treatments during the rice harvest in the autumn of 2009. Each collected sample was separated into two portions and placed in stainless steel cans. The portion for microbial study was immediately stored in a refrigerator at 4 °C after shipment to the laboratory. Another portion for soil  
10 analysis was air-dried, ground, and sieved through a 0.16 mm sieve.

## 2.3 In situ measurement of soil respiratory activity

To infer the biological control on SOC stabilization in association with the changes in microbial community structure, soil respiratory activity was measured in situ before soil samples were collected. This was done with a multi-channel soil respiration meter (Li-8100 auto-calculate system, LI-COR Company, USA, 2009). A polyethylene  
15 ring of 20 cm diameter was set on top of the soil one day before measuring to steady the soil environment within the ring. While measuring, the multi-channel soil respirator with both a soil temperature and soil moisture detector was placed tightly on top of the polyethylene ring, which was inserted in triplicate in the experimental plot prior  
20 to measurement. Soil respiration ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), soil temperature (°C), air temperature (°C) and soil moisture ( $\text{m}^3 \text{ H}_2\text{O m}^{-3} \text{ soil}$ ) were measured synchronously and data was managed with a Personal Digital Assistant (PDA) supplied with the instrument. Soil respiration rates ( $\text{g CO}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) were estimated using the recorded data in  
 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ .

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## 2.4 Culturable microbial population counting

The plate counting of culturable microorganisms was performed via the following procedure: Each sample (5 g) was homogenized with distilled water to form a suspension of 45 mL, and then diluted to form a series of suspensions at concentrations of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  respectively. An aliquot of 50 or 100  $\mu\text{L}$  of the diluted suspension of  $10^{-4}$  or  $10^{-5}$  was spread on a beef-protein medium plate and an aliquot of 100  $\mu\text{L}$  at  $10^{-2}$  or  $10^{-3}$  dilution spread on a Martin's medium plate to count bacteria and fungi respectively. The number of colonies on each medium plate was counted after inoculation at 28 °C with Petri dishes inverted. Plates that carried 20 to 200 colonies of fungi were counted on day 2 and that carried 30 to 300 colonies of bacteria were counted on day 4. The Colon Forming Units (CFUs) per gram of dry soil was calculated. The procedure of individual plate incubation and counting was done in triplicate.

## 2.5 Soil analysis

Determination of the basic soil properties was done following the conventional protocol described by Lu (2000). Briefly, soil pH was measured with a glass electrode using a 1:2.5 soil-to-water ratio. Organic C was determined by wet digestion. Total nitrogen was determined with the Kjeldahl method. The basic soil properties are given in Table 2.

The fumigation-extraction method was used to determine soil microbial biomass-C and biomass-N. The content of  $\text{K}_2\text{SO}_4$ -extracted C from the  $\text{CHCl}_3$ -treated and untreated soils was determined by an automated TOC Analyzer (Shimazu, TOC-500, Japan) and a  $K_{\text{EC}}$  of 0.45 was used to convert the measured flushed C to biomass C. The total N in soil extracts was measured by the Kjeldahl digestion-distillation procedure.

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## 2.6 Data processing and statistics

The data presentation and treatment was processed with Excel 2003. The results of the aforementioned analysis were expressed in means plus standard deviation. The stepwise regression analysis was performed using SPSS 16.0 statistical package for Windows (SPSS Inc., 2004). The significance of the difference between polluted and control plots was tested using LSD test at a probability of 0.05.

## 3 Results

### 3.1 Soil organic carbon, microbial biomass C and N

Data for SOC and total N content, of SMBC and SMBN content under the treatments of the sites are shown in Table 2 and Fig. 1 respectively. While all the long-term agro-ecosystem experiments lasted more than 20 yr, there existed differences in both SOC and total N contents among the different fertilization treatments at a single site. Both topsoil SOC and total N storage tended to be higher under Comb-Fert treatments when compared to chemical fertilization either of N-Fert or Comp-Fert at a single site though more significant differences for N content occurred between these treatments. In addition, the high level of SOC contents under Comb-Fert treatments ranged from 18–19 g kg<sup>-1</sup> in WJ soil in a slightly acidic reaction to 20–26 g kg<sup>-1</sup> in JX and WCH soils in acidic reaction. The late two soils are rich in free iron oxyhydrates (Table 1).

SMBC and SMBN exerted much wider differences between the fertilization treatments in a single site and between the sites (Fig. 1). While soil microbial C was generally higher under Comb-Fert treatments (except in WJ site), there existed much wider differences in SMBN between the chemical fertilization only and combined fertilization at a single site. This indicated a prompt response of microbial biomass N to fertilization practices. The calculated microbial quotient (SMBC/SOC) was 23.22 ± 3.3 mg g<sup>-1</sup> in WJ site, 50.6 ± 6.6 mg g<sup>-1</sup> in JX site and 30.6 ± 2.12 mg g<sup>-1</sup> in WCH site, not

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significantly different between the treatments at a single site. As shown in Table 2, soil C/N ratio was more or less consistent between the treatments and across the sites, being close to 10 for all the analyzed samples. Nevertheless, there was greater difference in the calculated microbial C to N ratio between the treatments at a single site and between the sites, being lower under pure N or chemical fertilization compared to the other fertilizer treatments at two sites of WJ and WCH except in site JX where the treatment of Comb-Fert<sub>m</sub> received green manure as an organic amendment with a biologically-fixed N input (Li et al., 2006).

### 3.2 Culturable microbial population numbers

Data of culturable microbial population numbers analysed using plant counting of the studied samples is listed in Table 3. Ranging from 10 MCFU g<sup>-1</sup> to 70 MCFU g<sup>-1</sup>, the number of bacteria exerted slight differences between the treatments in a single site though the populations were greater in Soil WJ with higher soil pH than in sites of JX and WCH with lower pH. However, in a wide range of 47–133 thousand CFU g<sup>-1</sup>, there exist greater differences between the N-Fert, Comp-Fert and Comb-Fert treatments at a single site. Moreover, the population ratio of culturable fungi to bacteria ranged from 1.5 × 10<sup>-3</sup> to 12.5 × 10<sup>-3</sup> and is much higher under Comb-Fert than under chemical fertilization at a single site.

### 3.3 Soil respiratory activity

As rice-rape crop rotation has been in operation at site WJ where rice is harvested in late October while at sites JX and WCH with double rice crops, late rice is harvested in mid November. Accordingly, soil temperature was 1–2 degrees higher in WJ than in JX and WCH when sampling. As soil microbial metabolic activity is related to temperature, field soil respiration activity observed in situ was generally higher at the WJ site than at the JX and WCH site. While there was no detectable difference in soil temperature between the treatments at the WJ site, soil temperature tended to be lower under

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Comb-Fert treatments compared to under non Comb-Fert treatments (Fig. 3). Ranging from  $6.91 \text{ gCO}_2 \text{ m}^{-2} \text{ d}^{-1}$  to  $16.84 \text{ gCO}_2 \text{ m}^{-2} \text{ d}^{-1}$  the total  $\text{CO}_2$  respired in field condition varied with treatments and soil temperature. The normalized soil respiration intensity ( $\text{CO}_2$  respired on base of total SOC) and specific metabolic respiratory activity ( $\text{CO}_2$  respired on base of SMBC) is shown in Table 3. Both soil respiration intensity and metabolic respiratory activity was generally higher under N-Fert treatment than under Comp-Fert treatments at sites JX and WCH, but not at the WJ site. When compared to the controls, there were no significant differences in both soil respiration intensity and metabolic respiratory activity between the Comp-Fert and Comb-Fert treatments across the sites.

## 4 Discussions

### 4.1 SOC accumulation and microbial biomass and community structure changes

It is generally accepted that microbial biomass carbon is positively correlated with soil organic carbon content (Jenkinson et al., 1981; Woods et al., 1986; Chen et al., 2008). In this study, SMBC content generally increased under Comb-Fert treatments at all sites. Again in this study, both SMBC and SMBN in  $\text{mg kg}^{-1}$  was found significantly correlated with SOC in  $\text{g kg}^{-1}$  ( $\text{SMBC} = 55.78 \times e^{0.12 \times \text{SOC}}$ ,  $p < 0.01$ ;  $\text{SMBN} = 10.094 \times \text{SOC} - 151.74$ ,  $p < 0.01$ ). Furthermore, the F/B ratio of population number was correlated well with soil organic carbon content for all the sites studied (Fig. 3). However, when taking into account the background effect under Non-Fert treatment, the increase both in SMBC and SMBN was found significantly correlated with the increase in SOC under fertilization treatments over controls across the sites (Fig. 4a and b), contributing to 80% of the total variation. These results indicate that SOC accumulation favours a build-up of the soil microbial community with increasing fungal

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dominance in rice paddies under the Comp-Fert and Comb-Fert treatments. Butler et al. (2003) reported a fungal-dominated microbial community build-up under high C inputs in rhizosphere soils compared to bulk soils. In a study of microbial community changes during the rice growing season in a paddy from Yixing, China, Hussain et al. (2010) also observed increases in fungal gene copies with a higher fungal-bacterial ratio in rhizospheres compared to bulk soil and also during rice growing stages when compared to soils before rice transplantation. Synthesizing a series of investigations from a long-term field experiment addressing the turnover of organic matter in soil, Kirchmann et al. (2004) was able to find that the quality and amount of organic matter input had no significant effect on the community structure of soil bacteria, but fungal activity increased significantly mainly when coarse-sized fractions increased under organic matter amendments. Recently, Kandeler et al. (2008) also observed increased microbial biomass carbon and fungal dominance under FACE treatment and attributed it to high C inputs from rhizodeposition which favoured the microbial C substrate supply. They claimed that fungal abundance was greatly affected by the increased C substrate at a high C:N ratio under transient elevated CO<sub>2</sub> in a semi-arid grassland. Bossuyt et al. (2001) argued, in a study using wide range of C:N for the C substrate, that fungal growth could be favoured by low quality (high C:N ratio) organic inputs, but bacterial growth by high quality (low C: N ratio) inputs. Here the rice soils under treatment were much richer in total N and the C;N ratios seemed not significantly different between the treatments; the increased microbial biomass and fungal dominance could not be accounted for by C:N variations. In contrast, fungi are well-known to live in coarse soil aggregates (Kirchmann et al., 2004), which were generally increased with new carbon inputs in rice soils under combined organic/inorganic fertilization (Li et al., 2007). The observed increase in microbial biomass carbon and fungal dominance may be related to the increase in coarse soil micro-aggregates which physically protected new carbon inputs under combined fertilization with higher crop yields (Pan et al., 2009b; Zhou et al., 2009a). However, this requires further study by soil aggregation and microbial molecular ecology approaches.

## 4.2 SOC accumulation and biological stabilization of SOC

Soil respiratory activity is a measure of soil microbial utilization of soil organic matter (carbon) and reflects the SOC substrate availability, the stability of SOM during microbial attack (Franzuebbers et al., 2001; Liu et al., 2006). Soil microbiological stabilization of organic carbon can be inferred by the extent to which organic carbon is respired and released as CO<sub>2</sub> during a certain temperature regime or by warming (Fang, 2005; Zheng et al., 2006, 2007). In this study, the field soil respiration rates measured in situ varied with the sites and the treatments. The soil respiration rates were higher under Comb-Fert treatments compared to other treatments across the sites as a result of an enhanced microbial community build-up. This has been well-documented with soil conditions for microbial activities being improved by organic amendments (Singh et al., 2009; Iqbal et al., 2009). However, there are no detectable differences in soil respiration rates on the basis of soil microbial biomass carbon and soil organic carbon. Yet, the specific soil respiratory activity on the basis of microbial biomass carbon was found to correlate in a significantly negative way with the contents of SOC for all the samples (Fig. 5). This indicates that microbial utilization of the C substrate becomes weak when SOC accumulates as high as > 20 g kg<sup>-1</sup>. When compared to Non-Fert treatment, no increase or even a significant decrease in the specific respiratory activity was observed under Comp-Fert or Comb-Fert treatments across the sites (Fig. 6). Using lab incubation of topsoil sample from the WJ site, Zheng et al. (2007) could show the ready decomposition of SOC and higher release of CO<sub>2</sub> and CH<sub>4</sub> under chemical fertilization than under Comb-Fert fertilization. The fact that SOC decomposition as measured by soil respiratory activity was not increased with SOC accumulation can be supported by the positive correlation of culturable F/B ratio with SOC content of the overall samples (Fig. 4). This finding is in accordance with the reports of Bulter et al. (2003) and Kandeler et al. (2008) that a fungal-dominated microbial community slowed SOC turnover in grasslands with increased C input and/or under FACE conditions.

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In previous studies, the role of chemical stabilization by binding to oxyhydrates and by transformation into high recalcitrant components had been already addressed for SOC sequestration of rice soils from southern China (Zhou et al., 2009a and b). Here, using measurements of SOC and soil respiratory activity under long-term experiments for over 20 yr, it can be concluded that SOC accumulation as high as  $> 20\text{g kg}^{-1}$  in the rice soils also involves a mechanism of changes in the soil microbial community by increasing fungal dominance and, in turn, decreasing the SOC substrate utilization in the long run. This is likely in contrast to the argument by Krull et al. (2003) that chemical recalcitrance would be the only mechanism for SOC protection for long periods of time. As stressed in the comment by Kögel-Knaber et al. (2006) chemical recalcitrance as a hidden mechanism in SOC sequestration may be relatively important and biological inaccessibility may become significant for stabilization of SOC in long-term turnover. The physical protection, chemical and biological stabilization, may interactively operate in the soil system while SOC is accumulating.

For rice soils within a wide range of climatic conditions, crop cultivars, and crop management practices (Pan et al., 2008) the understanding and prediction of SOM dynamics needs further quantitative characterization of the simultaneous operation of several mechanisms.

*Acknowledgements.* This work was funded by Natural Science Foundation of China under grants number 40710019002 and 40830528. The authors are grateful for Huang Qinghai and Yu Xichu, and Ji Xionghui for their helpful advice and assistance in the field sampling and measurements at sites of JX and WCH respectively.

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**Table 1.** Site conditions and topsoil pedogenic features of the longterm fertilization trials studied from south China.

| Site | Location                | GPS position        | Annual Temperature (°C) | Annual Precipitation (mm) | Soil classification | Crop rotation | Clay content (g kg <sup>-1</sup> ) | Fe <sub>d</sub> (g kg <sup>-1</sup> ) |
|------|-------------------------|---------------------|-------------------------|---------------------------|---------------------|---------------|------------------------------------|---------------------------------------|
| WJ   | Wujiang County Jiangsu  | 31°05' N, 120°46' E | 16.1                    | 975.0                     | Entic Halpudept     | Rice-rape     | 302.9                              | 18.0                                  |
| JX   | Jinxian County, Jiangxi | 28°15' N, 116°20' E | 18.2                    | 1600.2                    | Kandic Paludult     | Double rice   | 259.0                              | 46.6                                  |
| WCH  | Wangcheng County, Hunan | 28°37' N, 112°80' E | 18.4                    | 1452.9                    | Typic Paludult      | Double rice   | 364.0                              | 53.8                                  |

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**Table 2.** Soil pH, organic carbon and nitrogen of topsoil under different treatments from the sites.

| Site | Treatment | pH (H <sub>2</sub> O) | SOC (g kg <sup>-1</sup> ) | TN (g kg <sup>-1</sup> ) | C/N        |
|------|-----------|-----------------------|---------------------------|--------------------------|------------|
| WJ   | CK        | 5.66                  | 16.48±0.42b               | 1.59±0.07b               | 10.38±0.25 |
|      | NPK       | 5.46                  | 17.96±0.57ab              | 1.59±0.07b               | 10.18±0.23 |
|      | NPKM      | 5.34                  | 18.04±0.67ab              | 1.78±0.08a               | 10.15±0.09 |
|      | NPKS      | 5.16                  | 19.29±1.59a               | 1.86±0.12a               | 10.35±0.35 |
| JX   | CK        | 4.86                  | 19.10±0.59b               | 1.95±0.03b               | 49.81±0.19 |
|      | N         | 4.54                  | 19.44±0.95b               | 1.99±0.074b              | 9.77±0.32  |
|      | NPK       | 4.64                  | 20.04±0.43b               | 2.04±0.07b               | 9.85±0.15  |
|      | NPKM      | 4.60                  | 25.68±0.74a               | 2.65±0.05a               | 9.68±0.12  |
| WCH  | CK        | 5.10                  | 20.73±0.33c               | 2.16±0.13c               | 9.63±0.49  |
|      | NK        | 4.90                  | 22.43±0.18ab              | 2.33±0.09ab              | 9.65±0.33  |
|      | NPK       | 4.90                  | 21.77±0.84bc              | 2.26±0.024bc             | 49.63±0.47 |
|      | NKPM      | 4.68                  | 23.11±0.99a               | 2.44±0.06a               | 9.46±0.23  |
|      | NPKS      | 4.66                  | 22.98±0.81ab              | 2.40±0.04ab              | 9.56±0.15  |

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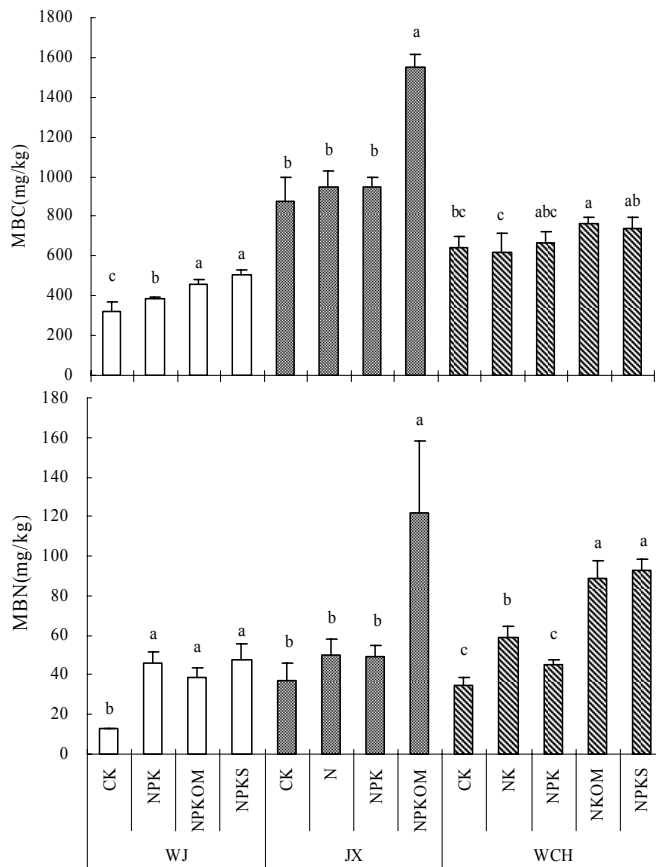
**Table 3.** Clony population number and fugal-bacterial ratio under different long-term experiments from the sites.

| Site | Treatments | Fungi (10 <sup>4</sup> CFUg <sup>-1</sup> ) | Bacteria (10 <sup>7</sup> CFUg <sup>-1</sup> ) | F/B (10 <sup>-3</sup> ) | Mineralization intensity (gCO <sub>2</sub> -C/gSOC m <sup>-2</sup> d <sup>-1</sup> ) | Metabolic quotient (mgCO <sub>2</sub> -C/mgSMBC m <sup>-2</sup> d <sup>-1</sup> ) |
|------|------------|---|--|-------------------------|--|---|
| WJ   | CK         | 11.3±0.11b                                  | 5.67±1.04a                                     | 2.04±0.38b              | 0.27±0.049a  | 14.27±3.90a   |
|      | NPK        | 11.5±1.70b                                  | 6.69±0.97a                                     | 1.72±0.05b              | 0.18±0.033a  | 8.51±0.96b  |
|      | NPKM       | 10.8±0.57b                                  | 6.90±0.91a                                     | 1.57±0.13b              | 0.24±0.025a  | 9.39±1.16b  |
|      | NPKS       | 17.0±0.72a                                  | 3.71±0.50b                                     | 4.67±0.86a              | 0.21±0.024a  | 7.92±1.05b  |
| JX   | CK         | 7.36±2.07a                                  | 4.23±2.69a                                     | 2.95±0.26b              | 0.13±0.015b  | 2.93±0.30a  |
|      | N          | 8.07±2.02a                                  | 5.36±1.05a                                     | 1.51±0.30c              | 0.16±0.006a  | 3.34±0.17a  |
|      | NPK        | 8.37±3.98a                                  | 3.01±1.42a                                     | 2.80±0.49b              | 0.09±0.003c  | 1.99±0.17b  |
|      | NPKM       | 27.84±6.77a                                 | 2.60±0.60a                                     | 11.47±5.40a             | 0.18±0.025a  | 2.97±0.47a  |
| WCH  | CK         | 4.66±2.15c                                  | 0.84±0.08c                                     | 5.58±2.57b              | 0.12±0.019ab   | 3.75±0.22b  |
|      | NK         | 8.31±0.73bc                                 | 0.93±0.15bc                                    | 9.14±2.02ab             | 0.14±0.010a  | 5.00±1.20a  |
|      | NPK        | 10.77±1.55ab                                | 1.64±0.14a                                     | 6.62±1.16b              | 0.10±0.005b  | 3.28±0.33b  |
|      | NKPM       | 12.31±3.65a                                 | 1.00±0.13bc                                    | 12.53±2.18a             | 0.10±0.013b  | 2.97±0.40b  |
|      | NPKS       | 13.33±3.65a                                 | 1.21±0.33b                                     | 11.3±2.26a              | 0.12±0.009ab   | 3.59±0.39b  |



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**Fig. 1.** Microbial biomass carbon (top) and nitrogen (bottom) of topsoil under different fertiliza-tions from the study sites.

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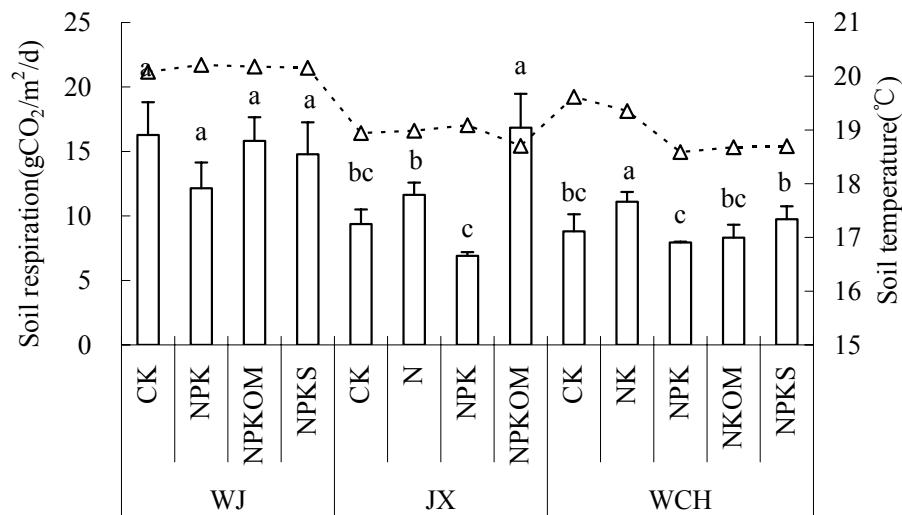
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**Fig. 2.** Soil temperature (dashed triangles) at a depth of 5 cm and soil respiration (blank blocks) measured at 08:00–11:00 a.m. during a clear day after rice harvest.

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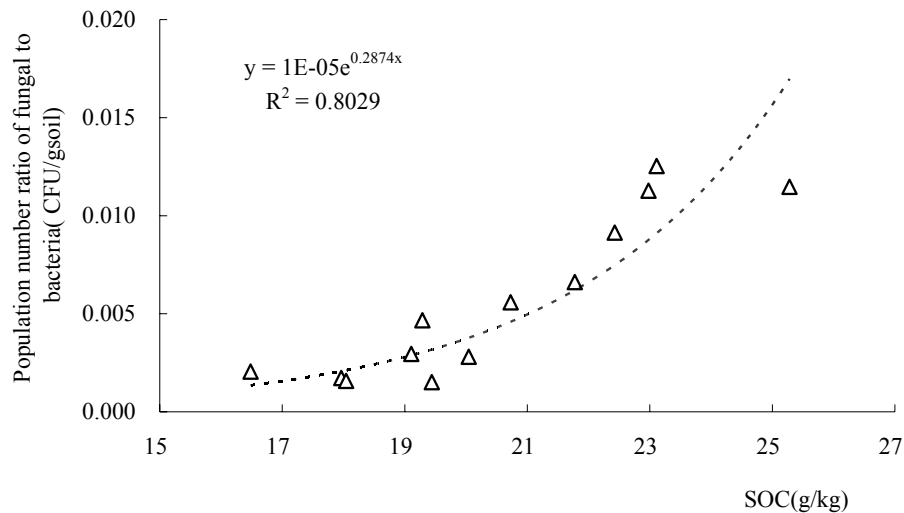
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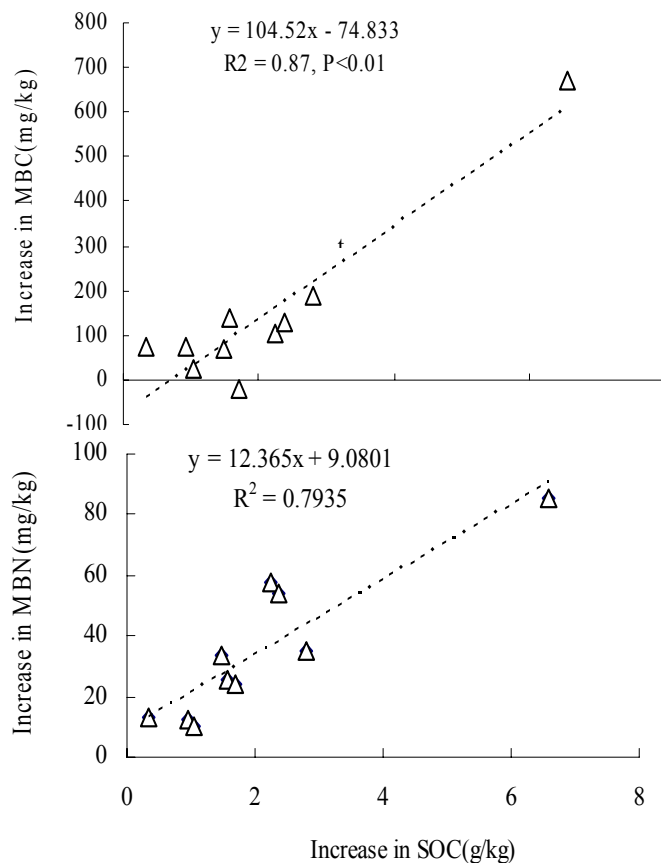
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**Fig. 3.** Correlation of the fungal-bacterial population number ratio with soil organic carbon content under different long-term fertilization treatments.



**Fig. 4.** Increase in microbial carbon (top) and microbial N (bottom) correlated to increases in soil organic carbon under fertilization as compared to no fertilization.



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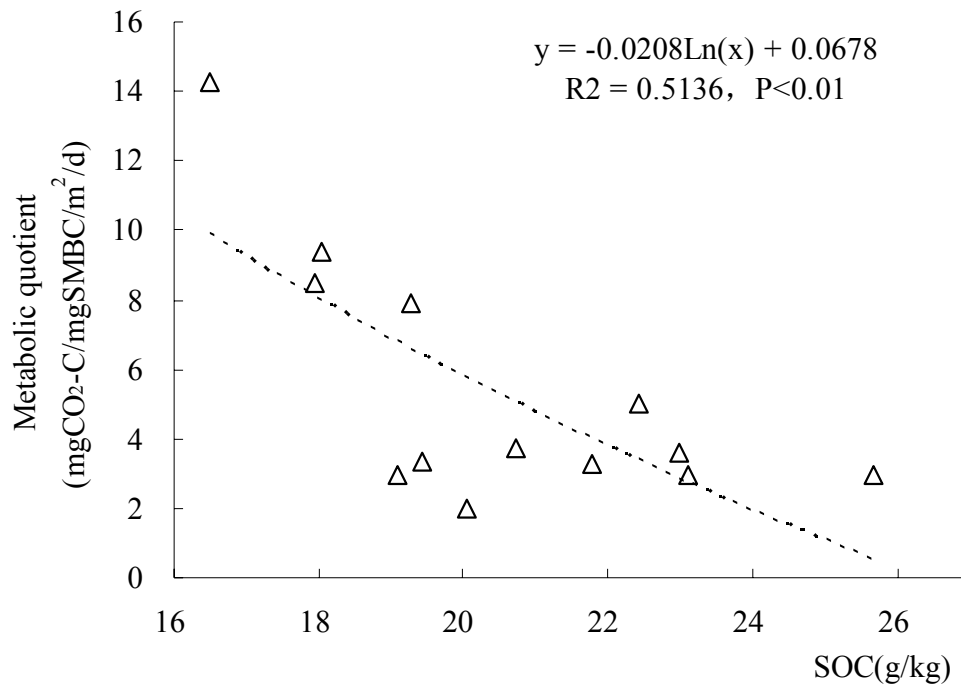
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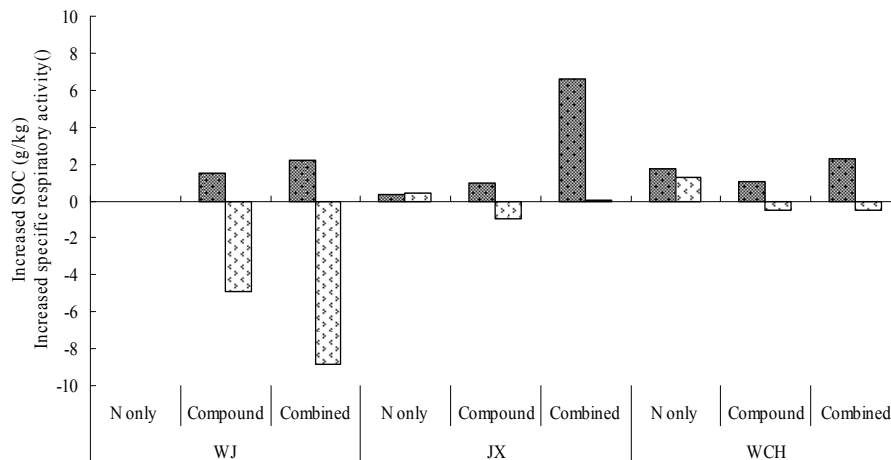
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**Fig. 5.** Correlation of microbial metabolic quotient with soil organic carbon content under the long-term experiments.

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**Fig. 6.** Increase in SOC ( $\text{g kg}^{-1}$ ) versus increased specific respiratory activity ( $\text{mgCO}_2\text{-C mgMBC m}^{-2} \text{d}^{-1}$ ) under fertilization treatments compared to no fertilization across the sites. N-only: fertilization with only chemical N fertilizer; Compound: compound fertilizers of chemical N, P and K; Combined: combined fertilization with organic/inorganic fertilizers.

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