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Original article

Long-term fertilization of P coupled with N greatly improved microbial activities in a paddy soil ecosystem derived from infertile land

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ABSTRACT

Microcalorimetry was used to study the effects of long-term (20 years) fertilization regimes on microbial activities in a paddy soil in southern China derived from infertile land. Managements of phosphorus fertilizer coupled with nitrogen fertilizer significantly promoted the contents of total and available P, mineral N and microbial biomass C (MBC) ($P < 0.05$). Both principal component analysis (PCA) of calorimetric indices and metabolic quotient of heat (Q_T/MBC) showed that fertilization of P coupled with N, P-deficient fertilization and non-fertilized control significantly separated from each other. Redundancy analysis plot showed that rate of heat output (Q_T/t), peak power (P_{max}) and constant of growth rate (k) were significantly correlated with soil total and available P, total and mineral N, which were greatly increased by the P fertilizer coupled with N fertilizer. In contrast, Q_T/MBC and peak time (t_{max}) were greatly increased by the P-deficient treatments. In addition, Q_T/t as a new introduced parameter was negatively correlated well with Q_T/MBC ($R^2 = 0.93$, $P < 0.01$). Accordingly, integrating microcalorimetric result analyzed by PCA as well as sensitive indicators of Q_T/MBC, Q_T/t and t_{max} are useful to assess soil microbial activity. The higher Q_T/t, lower Q_T/MBC and t_{max} indicate higher microbial activity and soil quality. In conclusion, long-term fertilization of P coupled with N, especially combined organic fertilizer greatly improved soil fertility and microbial activity; in contrast, deficiency of soil P had lower microbial activity in the paddy soil derived from infertile land.

1. Introduction

Paddy soils cover approximately 155 million hectares (ha) of the Earth's surface and support more than half of the world's population, in which China accounts for 30% of total world production [1]. In subtropical China, it is a traditional approach to convert the infertile wasteland into paddy field for improving soil fertility and increasing land productivity [2,3]. Those heavily weathering and leaching soils are characterized by low pH and deficiencies of available nutrients, particularly N and P [4]. Therefore, many studies have focused on the effects of long-term fertilization such as using organic and inorganic fertilizers on improving nutrient availability in the soil and increasing crop yields in this region [2,4–6].

Phosphorus fertilizer, especially when combined with organic manure application, was effective in improving fertility of soils and rice yields, and increasing microbial biomass and community functional diversity in infertile land [2,6,7]. Mono-organic manure fertilization increased soil C and N sequestration [5] and promoted the population size of bacterial ammonia oxidizers rather than ammonia-oxidizing archaea, mainly because of the existence of mineral ammonia in soil [8]. Under mineral fertilization, continuous P application increased the soil C and N pools [4,7], microbial biomass, functional diversity of community and cultivable microorganisms [4], as well as significantly mitigated the emissions of N_2O and CH_4 when P fertilizer was more than 60 kg ha^{-1} [7]. Nitrogen application inhibited methane oxidation activity indicated by functional genes under higher concentration of ammonium fertilizers [9]. Soil microbial growth and activity are important properties and functions of soil, which are sensitive indicators of

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alterations in soil [10,11]. As compared with nutrient pools and microbial community, little is known about alterations of microbial activities in response to long-term fertilization in paddy soil ecosystems derived from infertile land.

Among the methods for measuring soil microbial activity, approaches based on microbial growth such as respiration and substrate utilization were the most advantageous and allowed simultaneously quantitative estimation of microbes in soil [12]. One of the calorimetric approaches, Isothermal microcalorimetry (IMC) meets those demands because it can continuously monitor heat dissipating process of microbial growth and give growth curve in live, providing qualitative and quantitative data to indicate soil microbial activity [13–15]. Moreover, IMC is a highly sensitive method to assess the overall activity of soil microorganisms, and the measurements can be made without any interference to soil system over long periods of time [13,14]. The validity of IMC also has been corroborated by traditional techniques such as fumigation, e.g. the output of heat was well correlated with the amount of CO_2 respired in glucose amended or non-amended soils [16,17]. Recently, several experimental parameters including peak power, peak time and output of heat per unit of biomass were developed based on new micocalorimetric equipment [13,14]. Consequently, IMC was successfully used to assess the effects of nutritional status, fertilizers, landscape, vegetation and pollutants on soil quality [14,15,18–23]. Micocalorimetric parameters encompassing peak time and peak power [15,19], heat output per unit of biomass [13,23] were recommended as indicators of soil microbial activity and soil quality. However, the ecological significance of some parameters such as total output of heat and rate of heat output has not been elucidated yet. In addition, as far as our information goes, the thermal effect method has never been used to assess microbial growth in paddy soils.

The aim of this study was to use micocalorimetry to investigate soil microbial activity in a paddy soil derived from infertile land after long-term (twenty years) fertilizer management, and to introduce new indicators of micocalorimetry to evaluate soil quality through comparing the ecological significance of different calorimetric parameters in paddy soil ecosystem.

2. Materials and methods

2.1. Description of the long-term experiment

This long-term experiment was conducted at the Ecological Experimental Station of Red Soil, Chinese Academy of Sciences, located in Yingtan, Jiangxi Province, south China (28°15′30″ N, 116°55′30″ E) in 1990. This region had a typical subtropical monsoon climate with mean annual temperature of 17.6 °C and mean annual rainfall of 1795 mm. The field was derived from Quaternary red clay covered by red pine with soil pH of 4.5 and clay (<1 μm) content of 38%. The initial soil organic C, total N, total P, and total K contents of the plough layer (0–15 cm depth) were 3.3, 0.43, 0.28, and 11.1 g kg^{-1}, respectively. Available N, P, and K contents were 90.2, 5.6, and 105.9 mg kg^{-1}, respectively.

The field was flooded for transplanting double rice (*Oryza sativa* L.) crops from early April to the end of October and was in fallow for the rest of year. Early rice was sown in April. Both the grain and straw were harvested in late July. Late rice was sown in July. The grain and straw of late rice were harvested in early November. Treatments were arranged in a randomized complete block design with three replicates. Each plot size was 30.0 m^2. The treatments included CK (control, without fertilization), N (mineral N fertilizer only), NK (mineral N and K fertilizer), NP (mineral N and P fertilizer), NPK (mineral N, P, K fertilizer), NPKO (mineral N, P, K fertilizer and organic manure) and NPKS (mineral N, P, K fertilizer and half amount of rice straw). Nitrogen, P and K were applied at annual rates of 115, 29.7 and 34.9 kg ha^{-1}, respectively, as urea, calcium magnesium phosphate and potassium chloride, respectively. Phosphorus and K were applied as basal fertilizers, while N was split into 55% and 45% as basal application and topdressing for rice, respectively. In treatments with organic manure, application involved full return of rice straw and addition of 833 kg ha^{-1} pig manure. Rice straw had a mean nutrient content of 387C, 9.9 total N, 1.1 total P, and 31.0 g kg^{-1} total K. Pig manure had a mean nutrient content of 267C, 21.2 total N, 27.6 total P, and 18.1 g kg^{-1} total K. Basal fertilizers were applied before rice transplanting and mixed with soil by plough, and topdressing was applied to the soil surface before the stage of tillerring. After 20 years of different fertilization, changes of nutrient properties in six treatments and control were significant. For instance, SOC were about 10.96–12.04 g kg^{-1}, total N about 0.85–1.18 g kg^{-1}, total P about 0.27–0.69 g kg^{-1}, total K about 12.17–13.55 g kg^{-1}, available N about 85.75–110.25 mg kg^{-1}, available P 2.99–22.63 mg kg^{-1} and available K about 68.33–157.50 mg kg^{-1}. Fertilization decreased soil pH slightly.

2.2. Soil sampling and chemical analyses

In December 2010 after late rice harvest, composite soil samples were taken from the plough layer at a depth of 0–15 cm using a 30-mm-diameter gouge auger. For each plot, 9 cores were randomly sampled and mixed. After removing visible plant debris including roots, the moist soils were sieved (2.0 mm) and kept at 4 °C. Soil water content was detected before biochemical and calorimetric analyses within 2 weeks. A quarter of soil samples were air-dried and stored at room temperature for chemical analyses.

Soil organic C and total N were determined by dichromate oxidation [24] and Kjeldahl digestion [25], respectively. Total P and K in soil were digested by HF–$HClO_4$ and determined by molybdenum-blue colorimetry and flame atomic absorption spectrometry, respectively [26]. Microbial biomass C was determined with the chloroform fumigation extraction method as reported by Jenkinson and Ladd [27].

2.3. Microcalorimetric measurements

Metabolic activities of soil microorganisms were evaluated with a third generation thermal activity monitor (TAM III, Järfälla, Sweden) [28]. All 4-ml steel ampoules were cleaned with ethanol and sterilized in an oven twice at 100 °C for 30 min before use. All soil samples were first placed at 28 °C for 6 h and then submitted to microcalorimetric measurement. Each soil sample of 1.0 g (dry weight) was put into a sterilized steel ampoule and then solution containing 5.0 mg of glucose and 5.0 mg of ammonium sulphate was added immediately, as well as the final moisture of each soil sample was kept as 29% [29]. The temperature of the calorimeter system and the isothermal box was controlled at 28 °C. The power–time curve of microbial growth was continuously monitored and recorded with a computer. The thermodynamic parameters including constant of growth rate (k), peak power (P_{max}), peak time (t_{max}), total heat output (Q_T) were obtained by integrating the power–time curves [15,29]. In detail, the Q_T showed the output of total heat during the metabolic process [29], and the rate of heat output (Q_T/t) was ratio of Q_T to the total time of metabolic process. The P_{max} and t_{max} were the power and time to reach the maximum of the peak, respectively [15,29]. The k provides important quantitative index of microbial growth rate and obeys the following thermal kinetic equation:

$$\ln P_t = \ln P_0 + kt$$

This index was calculated from the slope of semi-logarithm of the exponential phase, where t was the time, P was the power output at time t, P_0 was the power at time $t = 0$ and k was the constant of growth rate. At the same time, all of the correlation coefficients obtained were noted to be greater than 0.999, meaning a strong positive correlation.

2.4. Statistical analyses

All results presented in this study were based on a soil oven-dry weight (105 °C, 24 h). Three replicates of soil samples were prepared for each treatment. One-way analysis of variance (ANOVA) and the Tukey's test ($P < 0.05$) were performed to compare the mean values for different samples by using SPSS 17.0. Significant differences were analyzed with Duncan's method at $P = 0.05$. Redundancy analysis (RDA), a multivariate direct gradient analysis method, was calculated with Canoco version 4.5 to elucidate the relationships between microcalorimetric parameters, chemical properties and fertilizer management. Principal component analysis (PCA) was conducted to analyze the five microcalorimetric indices including t_{max}, P_{max}, k, Q_T and Q_T/t in each soil sample after Kaiser-Meyer-Olkin measure of sampling adequacy and Bartlett's test by using SPSS 17.0.

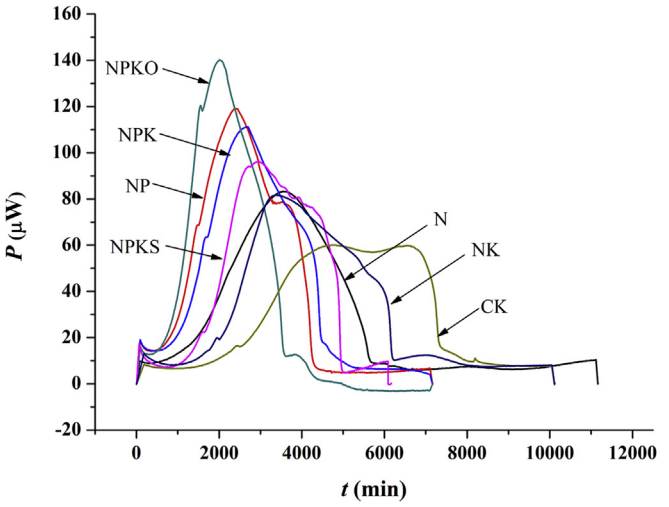

Fig. 1. Power-time curves of paddy soils under long-term (20-year) fertilization recorded microcalorimetrically. CK: control; N: mineral N fertilizer only; NK: mineral N and K fertilizer; NP: mineral N and P fertilizer; NPK: mineral N, P, K fertilizer; NPKS: mineral N, P, K fertilizer plus half amount of rice straw; NPKO: mineral N, P, K fertilizer and organic manure.

3. Results

3.1. Main chemical properties of soil

A summary of the chemical properties of soil after long-term (20 years) fertilizer management was presented in Table 1 ($P < 0.05$). Soil organic C (SOC) and total N were relatively higher in P-enriched fertilizer treatments of NPKS and NPKO. Total P in P fertilizer treatments was higher than that in P-deficient fertilizer treatments and control. There was no difference in total K. Microbial biomass C (MBC) increased in all fertilizer treatments, among which the highest MBC occurred in balanced fertilization of NPK, NPKS and NPKO. The shifting pattern of available P was the same as total P in fertilizer treatments. Mineral N increased in some N fertilizer treatments of NP, NPK and NPKO, but available K decreased in treatments of NP and NPK (data not shown).

3.2. Soil microbial activity indicated by calorimetric profiles

All recorded power–time curves presented a typical process of microbial growth as shown in Fig. 1. The thermal flow increased exponentially after the lag phase, which was followed by the stationary phase and then the decline phase. The lag phase of NPKO, NPK and NP were shorter than that of other treatments, whereas CK (control) had the longest stationary phase. The total heat released per gram of soil by microbial growth reactions, Q_T (J g^{-1}), the constant of microbial growth rate, k (min^{-1}), the power (P_{max}) and the time (t_{max}) at the maximum of the peak, and rate of heat output (Q_T/t), were obtained in three replicates of soil samples through the integration of each curve shown in Table 2. As a whole, the k, P_{max} and Q_T/t significantly increased whereas t_{max} significantly decreased in treatments of P coupled with N fertilizer (NP, NPK, NPKS and NPKO) in comparing with CK ($P < 0.05$). In contrast, the difference of those four calorimetric parameters (k, P_{max}, Q_T/t and t_{max}) between P-deficient treatments (N and NK) and CK was smaller than those between P coupled with N treatments (NP, NPK, NPKS and NPKO) and CK. The Q_T had no significant difference among all treatments and CK. In contrast, the output of heat per biomass unit (Fig. 2), i.e., metabolic quotient of heat (Q_T/MBC), ranging from 70 J mg^{-1} to 377 J mg^{-1} biomass C, significantly decreased in all fertilizer treatments ($P < 0.05$), in which the lowest values occurred in P coupled with N fertilizer treatments (NP, NPK, NPKS and NPKO).

The rate of heat output (Q_T/t) as a novel index was conveniently calculated from thermal curve (Table 2). Q_T/t was negatively correlated with Q_T/MBC ($R^2 = 0.93$, $P < 0.01$) and t_{max} ($R^2 = 0.79$, $P < 0.01$), positively correlated with P_{max} ($R^2 = 0.63$, $P < 0.05$), k ($R^2 = 0.65$, $P < 0.05$) and MBC ($R^2 = 0.83$, $P < 0.01$) (Figs. 3–4). Therefore, the lower Q_T/MBC and t_{max}, the higher MBC, Q_T/t, P_{max} and k indicated higher microbial activity. In addition, microbial biomass (MBC) intimately linked with microbial activity due to

Table 1
Chemical properties of a paddy soil under long-term (20-year) fertilizer management in Southern China.

Sample	SOC (g kg^{-1})	Total N (g kg^{-1}) [30]	Total P (g kg^{-1})	Total K (g kg^{-1})	MBC (mg kg^{-1})
CK	11.39 ± 0.21 ab	0.89 ± 0.06 ab	0.28 ± 0.01 a	13.19 ± 0.53 ab	42.32 ± 4.95 a
N	11.36 ± 0.17 ab	0.85 ± 0.15 a	0.27 ± 0.02 a	12.17 ± 0.87 a	93.07 ± 1.30 b
NK	11.47 ± 0.62 ab	0.97 ± 0.08 abcd	0.30 ± 0.02 a	13.31 ± 0.41 ab	135.86 ± 13.24 c
NP	10.96 ± 0.91 a	1.11 ± 0.06 bcd	0.57 ± 0.02 c	12.77 ± 0.90 ab	196.08 ± 27.95 d
NPK	11.25 ± 0.50 ab	0.93 ± 0.24 abc	0.57 ± 0.05 bc	13.20 ± 0.67 ab	229.69 ± 20.51 e
NPKS	11.62 ± 0.13 ab	1.14 ± 0.06 cd	0.51 ± 0.04 b	13.55 ± 0.24 b	205.22 ± 2.26 de
NPKO	12.04 ± 0.85 b	1.18 ± 0.07 d	0.69 ± 0.05 e	12.58 ± 0.63 ab	214.97 ± 8.99 de

The values in the table were means ± standard deviations of three replicates. Values with the same letter within each column indicated that there was no significant difference ($P < 0.05$).
CK: control; N: mineral N fertilizer only; NK: mineral N and K fertilizer; NP: mineral N and P fertilizer; NPK: mineral N, P, K fertilizer; NPKS: mineral N, P, K fertilizer plus half amount of rice straw; NPKO: mineral N, P, K fertilizer and organic manure.

Table 2
Thermal results of power–time curves measured with microcalorimetric method.

Sample	Q_T (J g^{-1})	t_{max} (min)	P_{max} (μW)	K (min^{-1}) × 10^{-3}	Q_T/t (J g^{-1} h^{-1})
CK	15.57 ± 1.62 a	4324 ± 423 d	64.5 ± 4.4 a	1.11 ± 0.19 a	0.09 ± 0.01 a
N	13.88 ± 4.45 a	3352 ± 174 c	57.7 ± 1.8 a	1.03 ± 0.10 a	0.11 ± 0.02 ab
NK	17.62 ± 1.63 a	3807 ± 415 c	78.9 ± 8.3 b	1.08 ± 0.03 a	0.12 ± 0.02 ab
NP	15.78 ± 4.43 a	2522 ± 182 b	133.7 ± 12.8 d	1.88 ± 0.21 b	0.14 ± 0.04 bcd
NPK	16.13 ± 2.56 a	2680 ± 112 b	110.9 ± 2.1 c	1.65 ± 0.07 b	0.17 ± 0.02 cd
NPKS	14.73 ± 1.76 a	2740 ± 301 b	85.9 ± 10.2 b	1.91 ± 0.02 b	0.14 ± 0.01 bc
NPKO	14.87 ± 2.73 a	1990 ± 22 a	135.2 ± 4.4 d	2.65 ± 0.30 c	0.18 ± 0.02 d

The values in the table were means ± standard deviations of three replicates. Values with the same letter within each column were not significantly different ($P < 0.05$).
CK: control; N: mineral N fertilizer only; NK: mineral N and K fertilizer; NP: mineral N and P fertilizer; NPK: mineral N, P, K fertilizer; NPKS: mineral N, P, K fertilizer plus half amount of rice straw; NPKO: mineral N, P, K fertilizer and organic manure.
Q_T: total heat output, t_{max}: peak time, P_{max}: peak power, k: growth rate constant; Q_T/t: rate of heat output.

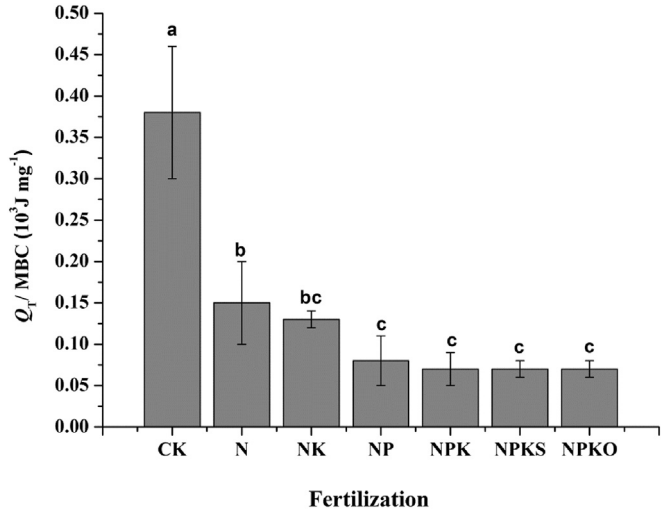

Fig. 2. The output of heat per microbial biomass C (Q_T/MBC) or metabolic quotient of heat in a paddy soil under different fertilizer management. Vertical T bars indicated standard deviations. Bars not topped by the same letter indicated a significant difference of values ($P < 0.05$). Q_T: values of total heat evolution recorded from soil samples; CK: control; N: mineral N fertilizer only; NK: mineral N and K fertilizer; NP: mineral N and P fertilizer; NPK: mineral N, P, K fertilizer; NPKS: mineral N, P, K fertilizer plus half amount of rice straw; NPKO: mineral N, P, K fertilizer and organic manure.

MBC was well correlated with calorimetric indices of Q_T/t ($R^2 = 0.83$, $P < 0.01$), t_{max} ($R^2 = 0.75$, $P < 0.01$), P_{max} ($R^2 = 0.57$, $P < 0.05$) and k ($R^2 = 0.51$, $P < 0.05$) (Fig. 4).

3.3. Principal component analyses (PCA)

In order to describe integrated changes of microbial activity shown by calorimetric parameters (t_{max}, P_{max}, k, Q_T and Q_T/t) in response to fertilization regimes, the PCA of calorimetric profiles was analyzed as shown in Fig. 5. The first two PCs of the PCA plot accounted for 66% and 17% of the overall variance, respectively. The control without fertilizers (CK) clearly separated from other two groups of fertilization treatments based on the variations. The group of four P coupled with N fertilizer treatments (NP, NPK, NPKS and NPKO) significantly separated from another group of P-deficient fertilization treatments (N and NK). In addition, P coupled with N fertilizer treatments were divided into two subgroups of NPKO and the others.

3.4. Redundancy analyses (RDA)

RDA clearly demonstrated that relationships between calorimetric parameters, soil chemical properties and six fertilization treatments and control (Fig. 6). Constant of microbial growth (k), peak power (P_{max}), and rate of heat output (Q_T/t) were positively increased by the application of N and P fertilization (NPKO, NPK, NPKS and NP) but decreased by the P-deficient fertilization (N and NK). Moreover, k and Q_T/t and P_{max} were significant positively correlated with soil total P, available P and mineral N. In contrast, metabolic quotient of heat (Q_T/MBC) and peak time (t_{max}) were increased by the P-deficient fertilization but decreased by the application of N and P fertilization, as well as t_{max} was significant negatively correlated with soil total P and available P.

4. Discussion

Since the growing and active microorganisms are sensitively in response to alterations in soil and provide an integrated and relevant vision of soil functioning, various parameters of microbial activity encompassing soil respiration CO_2, calorimetric parameters, soil microbial biomass and soil enzyme were proposed to be used as indicators of assessing soil quality [10,13,19,31–35]. In this case, the microcalorimetry was successfully used to measure microbial activity in paddy soil. To our knowledge, no previous investigation has performed such work. The CK (without fertilizers) had the longest lag phase and the lowest P_{max} and k, followed by the P-deficient treatments (NK and N), indicating that the treatment with fertilizers harboring more population of microorganisms or higher MBC (Table 1) and thus fertilizer-deficient treatments needed a long time to adapt and grow (Figs. 1 and 6 and Table 2). In contrast, P coupled with N fertilizer managements grew faster and had higher activity than other managements. Accordingly, microbial activity shown by calorimetric dynamics was very sensitively in response to alterations of nutrients in soil.

In generally, lower peak time (t_{max}) was coupled with higher peak power (P_{max}) and constant of growth rate (k), indicated the high microbial activity [15]. Peak time (t_{max}) is a good indicator to assess soil microbial activity because it was negatively correlated with soil enzymes, microbial biomass C (MBC), and the number of microorganisms [19]. In this case, t_{max} was well correlated with MBC ($R^2 = 0.75$) (Fig. 4). However, calorimetric results is still limited to elucidate some microbial process. It is often necessary to obtain support by specific analytical techniques and combine other techniques [14]. In this study, metabolic quotient of heat (Q_T/MBC), rate of heat output (Q_T/t) and PCA profile to elucidate the integrated activity were developed to indicate soil microbial activity and soil quality. Due to the output of total heat (Q_T) is not a good indicator of soil microbial properties, output of heat per biomass unit was proposed as metabolic quotient as function as qCO2 to indicate soil quality [13,33]. In this case, Q_T/MBC can clearly distinguish the differentiation of microbial activity even with the thin difference

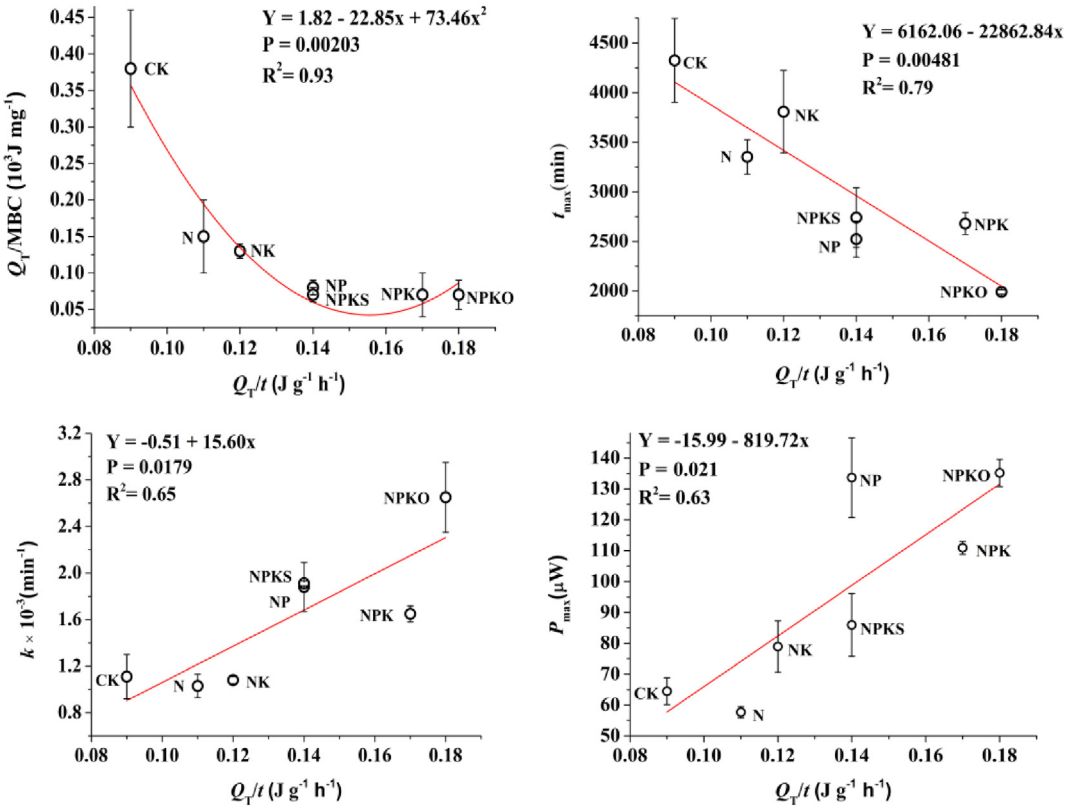

Fig. 3. The correlations between rate of heat output and calorimetric parameters including metabolic quotient of heat (Q_T/MBC), peak time (t_{max}), peak power (P_{max}) and growth rate constant (k).

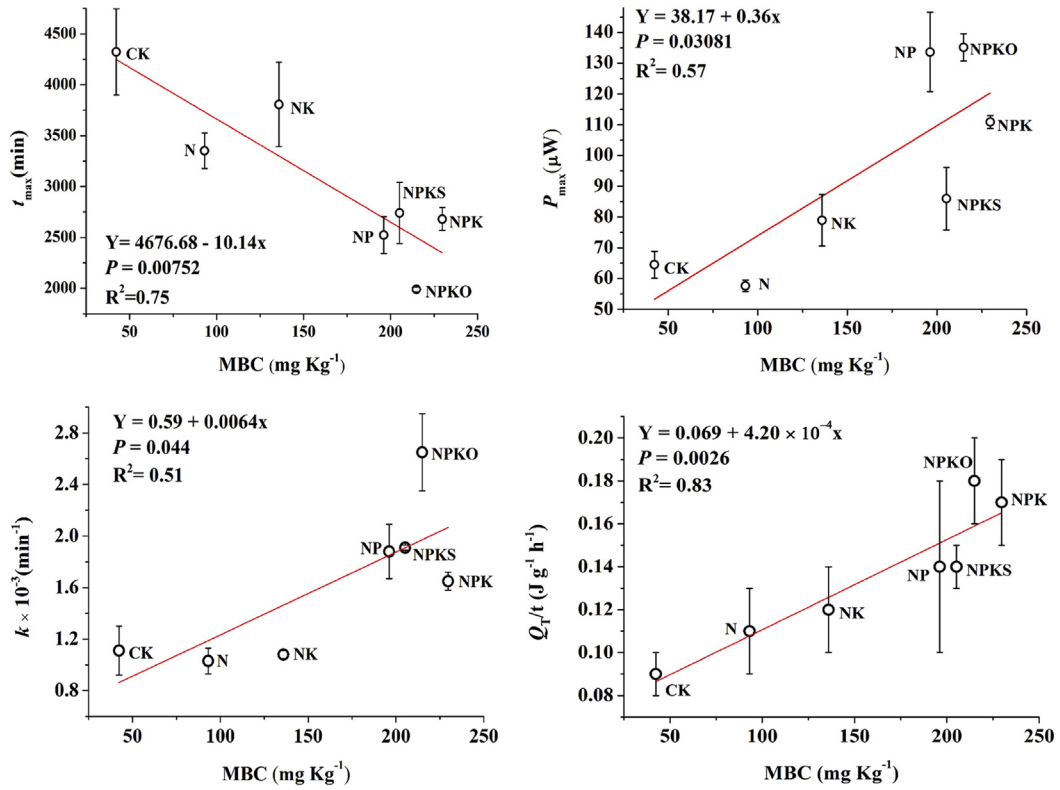

Fig. 4. The correlations between MBC and calorimetric parameters of peak time (t_{max}), peak power (P_{max}), constant of growth rate (k) and rate of heat output (Q_T/t).

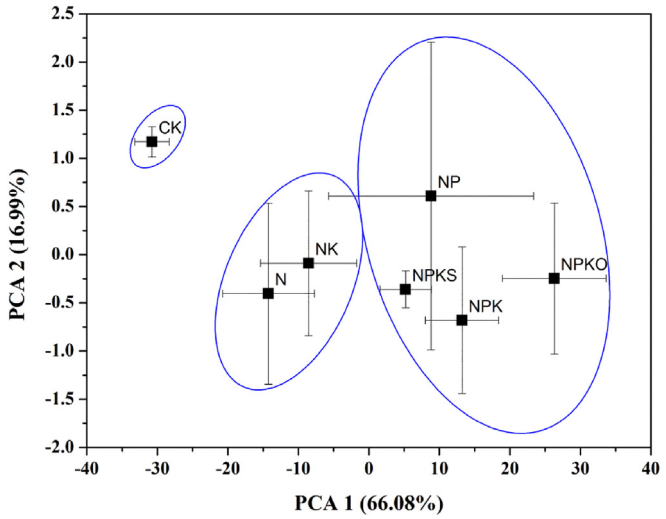

Fig. 5. Principal component analyses (PCA) of calorimetric parameters (t_{max}, P_{max}, Q_T, k and Q_T/t) in a paddy soil under long-term (20-year) fertilization. CK: control; N: mineral N fertilizer only; NK: mineral N and K fertilizer; NP: mineral N and P fertilizer; NPK: mineral N, P, K fertilizer; NPKS: mineral N, P, K fertilizer plus half amount of rice straw; NPKO: mineral N, P, K fertilizer and organic manure.

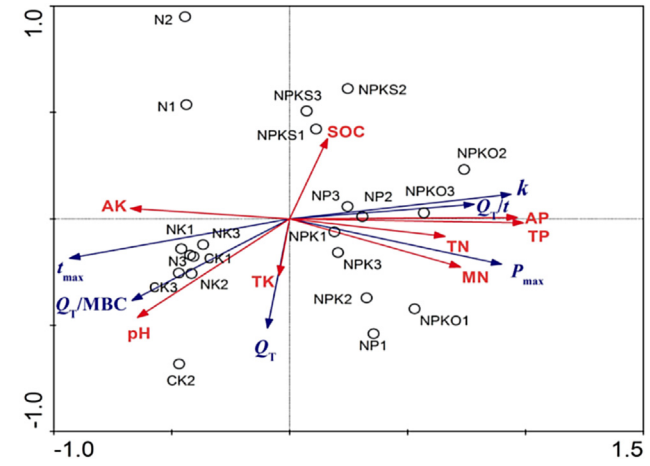

Fig. 6. Redundancy analysis of the relationships between calorimetric parameters, MBC and chemical properties of soil. CK: control; N: mineral N fertilizer only; NK: mineral N and K fertilizer; NP: mineral N and P fertilizer; NPK: mineral N, P, K fertilizer; NPKS: mineral N, P, K fertilizer plus half amount of rice straw; NPKO: mineral N, P, K fertilizer and organic manure. Q_T: total heat output; t_{max}: peak time; P_{max}: peak power; k: growth rate constant; Q_T/MBC: metabolic quotient of heat; Q_T/t: rate of heat output.

between NK and N treatments (Fig. 2). The highest Q_T/MBC and the lowest soil fertility appeared in control, whereas the lowest Q_T/MBC under P coupled with N fertilizer treatments showed the best soil fertility (Figs. 2 and 6) and the highest rice yields in the same field [2]. It suggested that the P coupled with N fertilizer treatments increased the capacity of the soil biomass (Table 1) to keep SOC and the external carbon (glucose in this case) while the P-deficient fertilizers increased energy spilling mediated by a futile cycle of protons or other ions because of stress [36], as well as P-deficient fertilizers could stimulated C losses as CO_2 through respiration [16,37]. The Q_T/t had a high level of correlations with Q_T/MBC, t_{max} and MBC (Figs. 3–4), suggesting Q_T/t is a good indicator to assess soil microbial activity. Finally, considering too many thermal parameters, principal component analysis (PCA) was served as integrated analyses of thermal dynamics to indicate microbial activity for the first time (Fig. 5). The PCA profile successfully elucidated the shifting pattern of microbial activity under different fertilization, which was consistent with the result of metabolic quotient (Q_T/MBC) (Fig. 2) and other results such as rice yields in the same experiment as in this case [2]. In particular, PCA profile clearly differed NPKO (NPK mineral fertilizer plus organic manure) from other N–P- treatments (NP, NPK and NPKS), indicating the importance to apply organic manure in agricultural system. Taken together, integrating microcalorimetric result analyzed by PCA as well as sensitive indicators of Q_T/MBC, Q_T/t and t_{max} are useful to assess soil microbial activity in future studies. The higher Q_T/t, lower Q_T/MBC and t_{max} indicate higher microbial activity and soil quality.

It is a key requirement for microbial activity that the availability of complementary nutrients at optimal proportions in the soil [4,5,7,23,34]. In this case, CK (without fertilizers) had the lowest microbial activity (Figs. 2 and 5). Comparing the importance of main mineral fertilizers N, P and K, the lower microbial activity occurred in P-deficient treatment (NK and N), while the higher microbial activity occurred in P coupled with N fertilizer treatments (NPKO, NPK, NPKS and NP). P-deficient fertilization have not improved the nutrition status (Table 1). These results validated the essential role of P for microbial activity in soil. The microorganisms were under stress to cope with the deficiency of necessary nutrients such as soil P for their proper metabolic activity and thus led to longer lag phase, lower P_{max} and k. Soil P, especially available P was a limiting factor in those infertile paddy soils, and thus continuous P fertilization improved the microbial biomass, diversity of microbial community, soil fertility and rice yields [2,4,6,7,38]. Continuous mono-N fertilization (N) significantly improved the microbial activity and MBC in comparison with CK but did not increase SOC, total N, total P and total K (Table 1, Figs. 2 and 5), emphasizing the limitation of mono-N and the importance of application of N coupled with P fertilizer. This result also confirmed that alterations of microbial activity measured by microcalorimetry were more sensitive than shift of soil nutrients. In contrast, the negative effect of K-deficiency on microbial activity was not strong as P-deficiency in soil, e.g., the similar microbial activity was observed between treatments of N and NK, and between NP and NPK (Figs. 2 and 5). Notably, N and P application in particular combined with organic manure (NPKO) had the best nutritional status and highest microbial activity (Table 1 and Fig. 5) and thus the highest rice yields for last two decades in the same experiment as this case [2], emphasizing the importance of balanced fertilization and organic manure in promoting microbial activity and rice yields in soils derived from infertile land. The result was consistent with reports in other upland soils for soil fertility, microbial diversity and activity, and crops yields [19,21,23,31,39]. Taken together, long-term fertilization of P coupled with N, especially combined organic fertilizer greatly improved soil fertility, rice yields and microbial activity in the paddy soil derived from infertile land. In contrast, P-deficient treatments have not improved soil nutritional status and thus resulted in lower microbial activity, emphasizing the essential role of P in improving soil fertility and microbial activity.

The content of SOC and total N was considered as the most important index of paddy soil fertility by traditional concepts [6]. After long-term fertilization in infertile paddy soils, the content of SOC and total N was still less than those in highly productive paddy soils in this region [2,6,7,38]. However, the rice yields of P couple with N fertilization derived from infertile land have reached an average level as those in highly productive paddy fields [2,6,7,38]. In this case, total P, available P and mineral N significantly affected indices of microbial activity encompassing MBC, Q_T/t, P_{max}, k, Q_T/MBC and t_{max} (Figs. 4 and 6). However, SOC had lower correlations

with those indices of microbial activity due to minor difference between various fertilizer managements (Table 1 and Fig. 6). These results were consistent with report that the rice yields were better correlated with indices related to microbial activity such as MBC, soil available P and mineral N than SOC [2].

5. Conclusions

Long-term (20 years) application of P coupled with N fertilizers, especially combined with organic manure, significantly improved soil fertility, rice yields and microbial activity in a paddy soil derived from infertile land. P-deficient fertilization have not improved soil nutritional status and thus led to the lower microbial activity, emphasizing the essential role of P in improving soil fertility and microbial activity. In contrast, K-deficient fertilization had less negative effect than P-deficient fertilization on microbial activity. Metabolic quotient of heat (Q_T/MBC), peak time (t_{max}) and new introduced rate of heat output (Q_T/t) are all good indicators to assess soil microbial activity and soil quality. PCA profile of thermal dynamics can be served as an excellent method to analyze integrated microbial activity and indicate soil quality in future studies.

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References

[1] I. Kögel-Knabner, W. Amelung, Z. Cao, S. Fiedler, P. Frenzel, R. Jahn, K. Kalbitz, A. Kölbl, M. Schloter, Biogeochemistry of paddy soils, Geoderma 157 (2010) 1–14.
[2] M. Lv, Z.P. Li, Y.P. Che, F.X. Han, M. Liu, Soil organic C, nutrients, microbial biomass, and grain yield of rice (*Oryza sativa* L.) after 18 years of fertilizer application to an infertile paddy soil, Biol. Fertil. Soils 47 (2011) 777–783.
[3] Z.T. Gong, Q. Xu, Paddy soils, in: Q.K. Li, O. Sun (Eds.), Soils of China, Science Press, Beijing, 1990, pp. 233–260.
[4] W.H. Zhong, Z.C. Cai, Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay, Appl. Soil Ecol. 36 (2007) 84–91.
[5] W. Dong, X. Zhang, H. Wang, X. Dai, X. Sun, W. Qiu, F. Yang, Effect of different fertilizer application on the soil fertility of paddy soils in red soil region of southern China, PLoS ONE 9 (2012) e44504.
[6] Z. Li, M. Liu, X.C. Wu, F.X. Han, T.L. Zhang, Effects of long-term chemical fertilization and organic amendments on dynamics of soil organic C and total N in paddy soil derived from barren land in subtropical China, Soil Till. Res. 106 (2010) 268–274.
[7] X. Li, H. Wang, S. Gan, D. Jiang, G. Tian, Z. Zhang, Eco-stoichiometric alterations in paddy soil ecosystem driven by phosphorus application, PLoS ONE 5 (2013) e61141.
[8] Y. Wang, G.B. Zhu, L.Y. Song, S.Y. Wang, C.Q. Yin, Manure fertilization alters the population of ammonia-oxidizing bacteria rather than ammonia-oxidizing archaea in a paddy soil, J. Basic Microbiol. 54 (2014) 190–197.
[9] M.S. Alam, Z. Jia, Inhibition of methane oxidation by nitrogenous fertilizers in a paddy soil, Front. Microbiol. 3 (2012) 246–258.
[10] P.M. Mele, D.E. Crowley, Application of self-organizing maps for assessing soil biological quality, Agric. Ecosyst. Environ. 126 (2008) 139–152.
[11] R. Romaniuk, L. Giuffré, A. Costantini, P. Nannipieri, Assessment of soil microbial diversity measurements as indicators of soil functioning in organic and conventional horticulture systems, Ecol. Indic. 5 (2011) 1345–1353.
[12] E. Blagodatskaya, Y. Kuzyakov, Active microorganisms in soil: critical review of estimation criteria and approaches, Soil Biol. Biochem. 67 (2013) 192–211.
[13] N. Barros, J. Salgado, S. Feijoo, Calorimetry and soil, Thermochim Acta 458 (2007) 11–17.
[14] I. Wadsö, Characterization of microbial activity in soil by use of isothermal microcalorimetry, J. Therm. Anal. Calorim. 95 (2009) 843–850.
[15] L. Núñez-Regueira, J. Proupin-Castiñeiras, J.A. Rodriguez-Añón, M. Villanueva-López, O. Nunez-Fernandez, Design of an experimental procedure to assess soil health state, J. Therm. Anal. Calorim. 85 (2006) 271–277.
[16] G.P. Sparling, Heat output of the soil biomass, Soil Biol. Biochem. 13 (1981) 373–376.
[17] G.P. Sparling, Microcalorimetry and other methods to assess biomass and activity in soil, Soil Biol. Biochem. 13 (1981) 93–98.
[18] F. Wang, J. Yao, H. Chen, K. Chen, P. Trebse, G. Zaray, Comparative toxicity of chlorpyrifos and its oxon derivatives to soil microbial activity by combined methods, Chemosphere 78 (2010) 319–326.
[19] B. Ahamadou, Q. Huang, W. Chen, S. Wen, J. Zhang, I. Mohamed, P. Cai, W. Liang, Microcalorimetric assessment of microbial activity in long-term fertilization experimental soils of Southern China, FEMS Microb. Ecol. 70 (2009) 186–195.
[20] B. Ye, H. Feng, J. Zhao, J. Fang, W. Shen, Microcalorimetry study on the microbial activity of permafrost on the Tibetan plateau of China, J. Therm. Anal. Calorim. 111 (2013) 1731–1736.
[21] Q. Zhang, X. Liu, X. Ma, J. Fang, T. Fan, F. Wu, L. An, H. Feng, Microcalorimetric study of the effects of long-term fertilization on soil microbial activity in a wheat field on the Loess Plateau, Ecotoxicology 23 (2014) 2035–2040.
[22] S. Zheng, J. Yao, B. Zhao, Z. Yu, Influence of agricultural practices on soil microbial activity measured by microcalorimetry, Eur. J. Soil Biol. 43 (2007) 151–157.
[23] S. Zheng, J. Hu, K. Chen, J. Yao, Z. Yu, X. Lin, Soil microbial activity measured by microcalorimetry in response to long-term fertilization regimes and available phosphorous on heat evolution, Soil Biol. Biochem. 41 (2009) 2094–2099.
[24] L.J. Mebius, A rapid method for determination of organic carbon in soil, Anal. Chim. Acta 22 (1960) 120–124.
[25] J.M. Bremner, Total nitrogen, in: C.A. Black, D.D. Evans, L.E. Ensminger, J.L. White, F.E. Clark, R.C. Dinauer (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, Agronomy Series 9, American Society of Agronomy, Inc., Madison, WI, 1965, pp. 1149–1178.
[26] M.L. Jackson, Soil Chemical Analysis, Prentice-Hall, Inc., Englewood Cliffs, NJ, 1958, pp. 111–133.
[27] D.S. Jenkinson, J.N. Ladd, Microbial biomass in soil: measurement and turnover, in: E.A. Paul, J.N. Ladd (Eds.), Soil Biochemistry, vol. 5, Marcel Dekker Inc, New York, 1981, pp. 451–471.
[28] I. Wadsö, Isothermal microcalorimetry in applied biology, Thermochim. Acta 394 (2002) 305–311.
[29] N. Barros, I. Gomez-Orellana, S. Feijoo, R. Balsa, The effect of soil moisture on soil microbial activity studied by microcalorimetry, Thermochim. Acta 249 (1995) 161–168.
[30] X. Chen, Z. Li, M. Liu, C. Jiang, Effects of different fertilizations on organic carbon and nitrogen contents in water-stable aggregates and microbial biomass content in paddy soil of subtropical China, Sci. Agric. Sin. 5 (2013) 950–960.
[31] P. Mäder, A. Fließbach, D. Dubois, L. Gunst, P. Fried, U. Niggli, Soil fertility and biodiversity in organic farming, Science 296 (2002) 1694–1697.
[32] T.H. Anderson, O.H. Einemeyer, H.J. Weigel, Changes in the fungal-to-bacterial respiratory ratio and microbial biomass in agriculturally managed soils under free-air CO2 enrichment (FACE) – a six-year survey of a field study, Soil Biol. Biochem. 43 (2011) 895–904.
[33] N. Barros, S. Feijoo, S. Fernandez, Microcalorimetric determination of the cell specific heat rate in soils: relationship with the soil microbial population and biophysic significance, Thermochim. Acta 406 (2003) 161–170.
[34] D. Geisseler, K.M. Scow, Long-term effects of mineral fertilizers on soil microorganisms- A review, Soil Biol. Biochem. 75 (2014) 54–63.
[35] W. Hassan, W. Chen, P. Cai, Q. Huang, Estimation of enzymatic, microbial, and chemical properties in brown soil by microcalorimetry, J. Therm. Anal. Calorim. 116 (2014) 969–988.
[36] J.B. Russell, The energy spilling reactions of bacteria and other organisms, J. Mol. Microbiol. Biotechnol. 13 (2007) 1–11.
[37] J.A. Harris, K. Ritz, E. Coucheney, S.M. Grice, T.Z. Lerch, M. Pawlett, A.M. Herrmann, The thermodynamic efficiency of soil microbial communities subject to long-term stress is lower than those under conventional input regimes, Soil. Biol. Biochem. 47 (2012) 149–157.
[38] X. Chen, Z. Li, M. Liu, C. Jiang, Y. Che, Microbial community and functional diversity associated with different aggregate fractions of a paddy soil fertilized with organic manure and/or NPK fertilizer for 20 years, J. Soils Sedim. 15 (2015) 292–301.
[39] J. Hu, X.G. Lin, J.H. Wang, J. Dai, R.R. Chen, J.B. Zhang, M.H. Wong, Microbial functional diversity, metabolic quotient, and invertase activity of a sandy loam soil as affected by long-term application of organic amendment and mineral fertilizer, J. Soils Sedim. 11 (2011) 271–280.