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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:

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