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Effects of an integrated rice-crayfish farming system on soil organic carbon, enzyme activity, and microbial diversity in waterlogged paddy soil

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ABSTRACT

A 10-year (2005–2015) field experiment was conducted to study the effects of an integrated rice-crayfish (CR) model on soil organic carbon, enzyme activity, and microbial diversity at soil depths of 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm. Compared with a mid-season rice monoculture (MR) model, total organic carbon (TOC), particle organic carbon (POC), and water-soluble organic carbon (WSOC) were significantly higher in the 0–40 cm soil layers, and the content of microbial biomass carbon (MBC) was significantly higher in the 30–40 cm soil layer in the CR model. The ratios of WSOC to TOC and POC to TOC in the 0–40 cm soil layers in CR model exhibited an increasing trend, whereas the ratio of MBC to TOC in the 0–30 cm layers exhibited a decreasing trend with respect to that of the MR model, however, these differences were not statistically significant. The activity of soil invertase, acid phosphatase, and urease in the 0–40 cm soil layers in the CR model exhibited a decreasing trend with respect to that of the MR model, and the activity of urease in the 10–20 cm soil layer in the CR model was significantly lower than that in the MR model. Compared with the MR model, the CR model significantly enhanced the carbon utilization capacity of soil microbes, and the richness index, dominance index, and diversity index of the soil microbial community in the 20–30 cm layer, whereas it significantly decreased the number of dominant soil microorganism species and the carbon utilization capacity of soil microbes in the 0–10 cm layer. Soil organic carbon and its active components had a significant direct correlation with the microbial diversity index, and significantly positive correlations with invertase, urease, and acid phosphatase. With respect to the soil microbial diversity index, soil organic carbon and its active components had a closer relationship with soil enzyme activity.

1. Introduction

In the south of China, waterlogged paddies are primarily distributed in polders in plains along rivers, the lakeshore region, the coastal region, and the Pearl River Delta [1]. According to statistics, Jiangsu, Zhejiang, Fujian, Jiangxi, and Shanghai have a total of over 4,000,000 hm^2 of waterlogged paddies, accounting for over 30% of all arable land [2]. In the areas in the middle reaches of the Yangtze River, only the Jianghan Plain has waterlogged paddies with an area of approximately 767,000 hm^2, which accounts for 39.4% of the arable land of that area [3]. Because the groundwater level is high in waterlogged paddies, drainage is poor, the soil is viscous, air permeability is poor, soil reduction is strong, and organic matter decomposition is slow, thus disrupting the balance of water, fertilizer, air, and thermal conditions, seriously hindering the growth and development of crops [4].

The integrated rice-crayfish farming system is a highly effective artificial wetland ecosystem in which rice is mainly cultivated in the waterlogged paddy fields along with crayfish culture. This model fully uses the shallow water environment and the winter idle period of rice paddies, and organically combines the agriculture and aquaculture industries to raise the utilization and productivity rates of rice paddies to the maximum extent, and has become a primary cultivation model in the waterlogged areas of the middle and lower reaches of the Yangtze River. Currently, this model has been popular in an area of approximately 140,000 hm^2 in Hubei Province, on the basis of the results of an investigation showing that the average productivity of this model is 45,000 RMB/hm^2 higher than that of either the traditional "rice-rape rotation" model or the "rice-wheat rotation" model, showing that it has good economic and social benefits.

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The crayfish (*Procambarus clarkii*), classified under class Crustacea, order Decapoda, and family Cambaridae, has great economic value. Research has shown that *P. clarkii* has strong burrowing activity and lives in burrowed holes during winter and summer. The depth of most holes burrowed by adult *P. clarkii* is approximately 50–80 cm, with a few reaching depths of 80–150 cm [5]. In the integrated crayfish-rice (CR) model, *P. clarkii* penetrates the surface and base layers of the rice paddy soil, improving soil permeability, increasing water migration channels, and allowing the nutrients and dissolved oxygen in water to reach the base layer, thus perturbing the soil redox interface. These events affect the transformation of organic carbon in the soil, soil enzyme activity, and microbial diversity. Currently, research on the CR model is primarily focused on production technologies and economic benefits of the model [6–8], whereas research on paddy soil organic carbon, microbial diversity, and soil enzyme activity is lacking. By investigating the effect of the CR model on the organic carbon, soil enzyme activity, and microbial diversity of waterlogged paddy soil in the middle and lower reaches of the Yangtze River, this study aims to obtain data supporting further research on the processes of environmental change in soil, as well as provide a theoretical basis for promoting the CR model.

2. Materials and methods

2.1. General characteristics of the experimental plot

The experimental plot was located in the Guanshan subplot of the Bailuhu Farm in Qianjiang City, Hubei Province (30°11′36.07″N, 112°43′22.68″E), and is classified as a low lake of the Jianghan Plain. The winter static groundwater level is 40–60 cm, with a northern humid subtropical monsoon climate. The average annual temperature is 16.1 °C, with a frost-free period of 246 d. The annual rainfall is 1100 mm. The soil type is fluvo-aquic paddy soil developed from lake sediments.

2.2. Experimental design

The experiment in the paddy began in 2005, with the plots set up as a crayfish-rice (CR) model and a mid-season rice monoculture (MR) model. Each plot was set up in triplicate, with each subplot having a surface area of 300 m^2. Each subplot was surrounded by a ridge measuring 60 cm in width and 40 cm in height and covered in film. In order to prevent flow of water or crayfish between plots, gutters 0.4 m in width and 1.0 m in depth were set up between plots. Crayfish gutters 3.0–4.0 m in width and 0.8–1.0 m in depth were dug on one side of each CR subplot, and nylon crayfish-catching nets were buried along all four sides at a depth of 1.0 m with 0.3 m exposed above ground, supported by small bamboo stakes. The CR model used the irrigation water after the harvest of mid-season rice for culture crayfish, and all rice straw was returned to the paddy. The MR model used winter fallow without crayfish culture, and all rice straw was returned to the paddy. Generally, 9000 kg/hm^2 of rice straw was directly returned to the paddy without tilling. The experimental rice used was mid-season Jianzhen No. 2 rice, and the experimental crayfish used was *P. clarkii*.

2.3. Plot maintenance

Generally, in mid- or late June each year, mechanical rotary tilling and artificial transplantation are used in rice paddies, with a wide row and narrow row spacing planting system. The row width and spacing in the CR model and the MR model were both 16.7 × 26.6 cm. Plants were harvested at the end of September. Generally, rice plants were fertilized with 120 kg/hm^2 N, 36.0 kg/hm^2 P_2O_5, and 60.0 kg/hm^2 K_2O per year. In the MR model plots, a standard number of 1.0–1.5 × 10^4 crayfish larvae were placed in each plot in October 2005, after which the crayfish self-propagated inside the rice paddies. At the same time in subsequent years, additional broodstock were added as appropriate for the conditions. From March to May of each year, an average of 120 kg of shrimp feed was added to each plot. The principal components of the feed were 46.6 g/kg total nitrogen, 11.0 g/kg total phosphorus, and 10.5 g/kg total potassium. Fishing of mature crayfish was completed in the beginning of June each year. Immature crayfish flowed into the gutters with the flow of water, and they continued growing in the rice paddy after replanting, drying, tilling, and rehydration. The second season of crayfish was harvested before harvest of mid-season rice, and the next season of crayfish culture commenced during irrigation in the beginning and mid of October. Under the CR model, an average burrow density of 0.4 burrows/m^2 could be observed.

2.4. Soil sample collection

Soil samples were collected in the middle of October 2015. The S-shaped 5-point collection method was used for sample collection. In each subplot, samples were collected from 0 to 10 cm, 10–20 cm, 20–30 cm, and 30–40 cm soil layers. During collection of soil samples, plant root remnants and rocks were removed and samples were mixed well and divided into two portions. One portion of fresh soil was passed through a 2 mm sieve and stored in a 4 °C refrigerator for use in determining biological attributes; the other portion was air dried and filtered for determining relevant indices.

2.5. Soil sample determination and methods

2.5.1. Methods for determination of soil organic carbon and its active components

The concentrated sulfuric acid potassium dichromate external heating method was used to assess total organic carbon (TOC) [9]. The chloroform fumigation potassium sulfate extraction method was used to assess soil microbial biomass carbon (MBC) [10]. Water-soluble soil organic carbon (WSOC) was filtered using a 0.45 μm filter and detected using a TOC analyzer. Particulate soil organic carbon (POC) was detected using the sodium hexametaphosphate dispersion-potassium dichromate external heating oxidation method [11].

2.5.2. Soil enzyme activity

The activities of invertase, urease, and acid phosphatase, which have a close relationship with the metabolism of soil carbon, nitrogen, and phosphorus, were selected to represent total soil enzyme activity. Determination of enzyme activity was performed as described by Guan et al. [12]. Specifically, 3,5-dinitrosalicylic acid colorimetry was used for invertase, and activity was expressed as mg glucose/g soil after 24 h. Phenol sodium hypochlorite colorimetry was used for urease, and activity was expressed as mg ammonium nitrogen/g soil after 24 h. Disodium phenyl phosphate colorimetry was used for acid phosphatase, and activity was expressed as mg phenol/100 g soil.

2.5.3. Analysis of soil microbial community functional diversity

The Biolog ECO MicroPlate was used to determine soil microbial community functional diversity [13]. Ten grams of fresh soil was passed through a 2 mm sieve, added to 100 mL 0.85% (*w*/*v*) sterile NaCl solution, and agitated for 15 min. In a sterile hood, the mixture was diluted to 10^{-3} with sterile 0.85% NaCl solution, and an 8-channel pipette was used to add 150 μL of the diluted suspension to each well of the Biolog ECO plate. Each soil sample was done in triplicate. Cultures were grown at a constant temperature of 25 °C. The absorbance at 590 nm was measured for each well after 1, 2, 3, 4, 5, 6, 7, 8, and 9 d.

2.6. Data processing

2.6.1. Average well-color development (AWCD)

$$\mathrm{AWCD} = \frac{\sum(A_i - A_{A1})}{31}$$