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Monitoring Soil Microbial Activities in Different Cropping Systems Using Combined Methods

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ABSTRACT

Cropping activities may affect soil microbial activities and biomass, which would affect C and N cycling in soil and thus the crop yields and quality. In the present study, a combination of microcalorimetric, enzyme activity (sucrase, urease, catalase, and fluorescein diacetate hydrolysis), and real-time polymerase chain reaction (RT-PCR) analyses was used to investigate microbial status of farmland soils, collected from 5 different sites in Huazhong Agriculture University, China. Our results showed that among the 5 sites, both positive and negative impacts of cropping activities on soil microbial activity were observed. Enzyme activity analysis showed that cropping activities reduced soil sucrase and urease activities, which would influence the C and N cycles in soil. Much more attentions should be given to microbial status affected by cropping activities in future. According to the correlation analysis, fluorescein diacetate hydrolysis showed a significantly ($P < 0.05$) negative correlation with the time to reach the maximum power output ($R = -0.898$), but a significantly ($P < 0.05$) positive correlation with bacterial gene copy number ($R = 0.817$). Soil catalase activity also showed a significantly ($P < 0.05$) positive correlation with bacterial gene copy number ($R = 0.965$). Using combined methods would provide virtual information of soil microbial status.

Key Words: cropping activity, enzyme activity, fluorescein diacetate hydrolysis, microbial biomass, microcalorimetric analysis, real-time polymerase chain reaction

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Cropping activities are closely related with human behavior. Different crops are planted on the land to meet human needs. Cropping activity benefits people by providing high-quality vegetables to improve health while generating employment opportunities and high incomes (Weinberger and Lumpkin, 2007). Crop cultivation has also affected soil microbial community composition and activity (Carney *et al.*, 2004; Zhang *et al.*, 2007). For instance, soil microbial diversity and dehydrogenase activity decreased significantly in the greenhouse vegetable land (Lin *et al.*, 2004; Shen *et al.*, 2008). Soil microorganisms play an essential role in cycling of mineral compounds and decomposing organic materials. It is important to analyze the response of microorganisms to different cultural practices, because soil microorganisms respond quickly to environmental changes. As a result, they are expected to be efficient bioindicators of soil conditions.

Microcalorimetry as a technique has been widely used in environmental sciences because of its high sensitivity, high accuracy, and automaticity (Guo *et al.*, 2012). This technique has a great advantage over studies of complex living systems, and provides a particularly useful tool for the characterization of the microbial growth processes. Soil microbial metabolic rates measured by calorimetry correlate well with soil microbial activities detected by other methods. Microcalorimetry has already been performed to measure soil microbial growth rate and metabolic heat production under different contamination conditions and agricultural practices (Chen *et al.*, 2010; Wang *et al.*, 2010; Ge *et al.*, 2011).

Since soil enzyme activities reflect the structure and functions of microbial communities, they are considered as integrative bioindicators. Hence, soil enzyme activities are often used for monitoring the impacts of soil management, agricultural practices or environmental contamination on soil health (Deng and

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Tabatabai, 1996; Kandeler *et al.*, 1996; Gianfreda *et al.*, 2005; Mamy *et al.*, 2011; Lebrun *et al.*, 2012). In the present study, we focused on sucrase, catalase, urease, and fluorescein diacetate (FDA) hydrolysis. Sucrase, a common soil enzyme, is important in the degradation of sucrose and is involved in the direct metabolism of soil organic matter, playing an important role in the enhancement of soil soluble nutrients (Ge *et al.*, 2011). Catalase is a common enzyme found in nearly all living organisms exposed to oxygen and is highly related with soil microbial activity. Urease is closely linked to N cycle and availability as it converts urea into ammonia, a form available to plants (Guo *et al.*, 2012). Activity of FDA hydrolysis has been suggested as a possible indicator for measuring overall microbial activity, because the ubiquitous lipase, protease, and esterase are involved in the hydrolysis of FDA (Green *et al.*, 2006; Achuba and Peretiemo-Clarke, 2008).

Real-time polymerase chain reaction (RT-PCR) is a technique of molecular biology based on the polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule. This method has been applied to estimate target-gene copy as a proxy for population size in soil systems, for example bacterial quantification using fragments of the 16S gene (Stubner, 2002), or quantifying genes to target functional groups such as the ammonium monooxygenase A subunit to estimate nitrifier population size (Okano *et al.*, 2004). In this study, a combination of microcalorimetric, enzyme activity and RT-PCR analyses was used to investigate the effect of cropping activities on soil microbial status. Correlation analysis was also conducted to compare the results of these analysis methods.

MATERIALS AND METHODS

Study area and sample collection

Soil samples were collected from Huazhong Agriculture University, China, which is situated in the middle of Hubei Province and is the confluence of the middle reaches of the Yangtze River and Hanshui River. The climate belongs to humid subtropical with abundant rainfall and four distinctive seasons. All the samples were collected in December (3 weeks after harvest) when there were no crops in the field.

In this study, soil samples were collected from 5 different sites: greenhouse 1 of tomato (GT1), greenhouse 2 of tomato (GT2), greenhouse of Hongshan *Brassica* (GH3), open field of Hongshan *Brassica* (OH4), and open field without crops (OF5). At each sampling site, 8 random soil cores were taken from the top 10 cm of the profile and then mixed together. The soil texture was clay-loam. All samples were air-dried, sieved through a < 2-mm mesh to remove plant residuals, soil macro-fauna and stones, and then stored in polyethylene bags at 4 °C for further analysis. The main chemical properties of soil samples are shown in Table I.

Microcalorimetric measurements

The microcalorimetric measurements were conducted on a TAM-III multi-channel thermal activity microcalorimeter (Thermometric, Sweden). Soil samples were stored at room temperature for 24 h prior to the measurements. Immediately before the measurements, one gram of soil was weighted into a 4.5-mL steel ampoule that had been cleaned and sterilized in an oven at 105 °C for 30 min and 0.2 mL of a solution containing 5.0 mg glucose and 5.0 mg ammonium sulfate was added (Chen *et al.*, 2010; Wang *et al.*, 2010). Glucose and ammonium sulfate were provided as C and N sources to stimulate soil microbial activity. The measurements were carried out at 28 °C. Heat flow was recorded over time until the signal returned to baseline, indicating no further measurable microbial metabolic activity (Braissant *et al.*, 2010).

Determination of soil microbial activities

For sucrase activity determination, 5 g of air-dried soil was incubated for 24 h at 37 °C with 15 mL of 8%

TABLE I

Main chemical properties of soil samples collected from 5 different sites: greenhouse 1 of tomato (GT1), greenhouse 2 of tomato (GT2), greenhouse of Hongshan *Brassica* (GH3), open field of Hongshan *Brassica* (OH4), and open field without crops (OF5)

Site	pH (H_2O)	Total N	Organic matter	Available P	Available K
		g kg^{-1}		mg kg^{-1}	
GT1	7.97	3.62	20.79	7.79	147.08
GT2	7.30	0.77	14.13	37.89	128.38
GH3	4.71	2.19	8.34	50.00	88.50
OH4	4.99	1.21	8.66	43.57	135.86
OF5	5.26	1.75	3.97	53.79	173.26