

Effects of crop type on soil microbial properties in the cropland of the Jiangnan plain of China

JUN LI¹, LIXIN LIU¹, CHUNLEI ZHANG¹, CHANG CHEN¹, GUANGYUAN LU¹, JUNLAN XIONG¹, HONGJUN YANG^{2,*}

¹Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, Wuhan, P.R. China

²College of Resources and Environment, Southwest University, Chongqing, P.R. China

*Corresponding author: meilirensheg-@swu.edu.cn

ABSTRACT

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Soil microbial properties are varied by growing different crops, ultimately reflecting the growth and reproduction of crops. In this study, two types of oilseed rape (*Brassica napus* L. ZS11 and ZY821) and wheat (*Triticum aestivum* L. ZM9023) were planted in the Jiangnan plain of China. Rhizosphere soil samples were collected three months after sowing. Soil physicochemical properties, enzyme activities and microbial diversity were determined. The results showed that soil available phosphorus significantly increased from 25.57 mg/kg (ZM9023) to 33.20 mg/kg (ZS11) and 35.72 mg/kg (ZY821), respectively. Invertase activity of ZS821 (0.86 mg glucose/g) was significantly lower than in ZS11 (1.04 mg glucose/g). Acid phosphatase activity under planting rapes was significantly higher than that under wheat. Urease activities significantly increased from 40.88 mg NH₄⁺-N/g soil/24 h (NFP) to 49.04 mg NH₄⁺-N/g soil/24 h (FNP) and 51.28 mg NH₄⁺-N/g soil/24 h (ZM9023), 51.60 mg NH₄⁺-N/g soil/24 h (ZY821) and 52.28 mg NH₄⁺-N/g soil/24 h (ZS11), respectively. The ACE (abundance based coverage estimator) and Chao1 indexes of bacteria of ZS11 were lower than ZY821, which were similar to ZM9023. Fertilization increased soil bacterial ACE and Chao1 indexes. However, ACE and Chao1, Shannon and Simpson indexes of soil fungi for ZS11 were significantly higher than in ZY821, which were similar to ZM9023 (except for the Shannon index).

Keywords: soil microorganism; microbiota; nutrients; fungal community; microbial ecology

The interaction between crop and soil microorganism maintains or even dominates the farmland ecological functions. The photosynthetic products produced by plants and released into soil provide carbon for soil microbes; soil microbes turn organic substances into inorganic nutrients that crops can effectively absorb and utilize (Davids et al. 2017).

The soil enzyme activities and physico-chemical properties were affected by crop types and soil fertility (Badiane et al. 2001, Acosta-Martínez et al. 2007). Previously, the variation of soil microbial diversity and composition of soil microbial

community occurred due to various factors such as vegetation, soil type, fertilization and climatic conditions (Nielsen et al. 2010, Ochoa-Hueso et al. 2016). Under natural conditions, the variability of plants and their influencing factors led to a response that rhizosphere microbial community formed (Breidenbach et al. 2017). Different crops have varied effects on the numbers and compositions of soil microbes that changed the soil biochemical environment, which ultimately reflected in the growth and reproduction of crops (Wiehe and Höflich 1995). However, little is known about

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the impact of different crop types on soil microbial diversity and their roles in soil biological processes.

This study had three objectives: (1) to understand the effect of planting different crops on soil enzyme activities in a single soil type; (2) to investigate differences of soil microbial diversity caused by growing oilseed rapes of low and high erucic acid and glucosinolate and wheat, and (3) to determine any correlations among soil physicochemical properties, enzyme activities and soil microbial diversity under different crops.

MATERIAL AND METHODS

Site description. The study area was located in the Yangluo Research Station, Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan, China (114°54'E, 30°37'N). The soil (Dystric Cambisol) had the following properties: pH (1:4, soil/water) 6.69; electrical conductivity 33.50 $\mu\text{S}/\text{m}$; soil organic carbon (SOC) 11.66 g C/kg; total nitrogen (TN) 1.31 g/kg; total phosphorus (TP) 0.87 g/kg; total potassium (TK) 19.67 g/kg; available nitrogen (AN) 88.44 mg/kg; available phosphorus (AP) 16.73 mg/kg; available potassium (AK) 103.53 mg/kg.

Experiment design. Seeds were winter oilseed rapes (*Brassica napus* L., ZS11 – low erucic acid (0) and glucosinolate content (18.84 $\mu\text{mol}/\text{g}$) in rapeseed meal; ZY821 – high erucic acid (48%) and glucosinolate content (123.5 $\mu\text{mol}/\text{g}$) in rapeseed meal) and winter wheat (*Triticum aestivum* L., ZM9023), which are widely cultivated in the Yangtze River basin China. All seeds of winter oilseed rapes were sown on 15 October 2016, in 2 m \times 10 m sized plots, in rows about 30–35 cm apart (three rows per meter) and plant density of 45 plants/ m^2 . The seeds of wheat were sown on the same day, at the rate of 200 seeds/ m^2 . Each plot of winter oilseed rapes and wheat was fertilized with a compound fertilizer (N:P:K, 25:10:15) at the rate of 750 kg/ha at the beginning of the experiment. The treatments of plots fertilized but without planting crops (FNP) and plots without fertilizing and planting crops (NFP) were used as control. The experimental design was randomized complete blocks with three replicates.

Soil sampling. On 1 March 2017 (winter oilseed rapes at the budding stage, wheat at the jointing stage), ten plants were removed from field by using S-shaped sampling pattern in each plot. The soil

still adhering to the roots was collected as rhizosphere soil after loose clumps of soil falling off when shaking the root system. These ten rhizosphere soil samples then were mixed thoroughly (Barillot et al. 2013). The fresh soil was sieved through a 2-mm mesh and divided into three subsamples. One subsample was stored at 4°C for analyses of soil enzyme activities and soil moisture content. Another subsample was air dried, and then sieved through a 0.25 mm mesh before chemical analyses. The last subsample was stored at –80°C for DNA extraction.

Soil physicochemical and enzyme activities measurements. Soil moisture (M), total nitrogen, total phosphorus, total potassium, soil organic matter, available P, available K and available nitrogen were measured using procedures as described by Xun et al. (2016). The soil invertase, acid phosphatase, dehydrogenase and urease activities were determined by Borowik et al. (2017).

Extraction of soil DNA and analysis of sequence data. Total soil DNA was extracted with a power soil DNA isolation kit (MoBio Laboratories, Carlsbad, USA) and the 16s and ITS (internal transcribed spacer) were investigated by Illumina MiSeq sequencing. The Purified PCR amplicons were sequenced using the Luoning Bio-pharm Technology Co., Ltd. (Chengdu, China).

Analyses of microbial community structure and diversity. Raw data were processed with the pipeline coupling of the Mothur software packages (Michigan, USA). The sequences were analysed according to the Usearch and QIIME4 pipeline. The diversity was measured based on a subset of randomly selected sequences from each sample.

Statistics. Differences in soil physicochemical properties and enzyme activities between samples were tested with separate ANOVAs for crops type, and *LSD* (least significant difference) tests to identify contrasts. Redundancy analysis (RDA) was used to test correlations of potential drivers of the microbial community structure with first and second principal component scores. Statistical analysis was carried out using SPSS 18.0 (Stanford, USA) and R (Auckland, New Zealand).

RESULTS AND DISCUSSION

Soil physicochemical properties. Soil moisture contents of cropping soil were significantly higher

Table 1. Soil physicochemical characteristics under different treatments

Treatment	SOM	TN	TP	TK	AN	AP	AK	M (%)
	(g/kg)				(mg/kg)			
ZS11	22.97	1.68	0.99	21.65	146.38	33.20	162.93	21.06
	± 0.31 ^a	± 0.03 ^a	± 0.03 ^a	± 0.46 ^a	± 2.19 ^a	± 3.79 ^a	± 14.35 ^a	± 0.38 ^a
ZY821	23.57	1.58	0.93	20.51	148.50	35.72	149.67	20.71
	± 0.31 ^a	± 0.03 ^a	± 0.03 ^a	± 1.14 ^a	± 1.90 ^a	± 0.44 ^a	± 13.24 ^a	± 0.89 ^a
ZM9023	21.19	1.58	0.91	21.58	144.77	25.57	147.20	20.75
	± 0.80 ^{ab}	± 0.04 ^a	± 0.01 ^a	± 0.29 ^a	± 1.53 ^a	± 3.36 ^b	± 24.99 ^a	± 1.09 ^a
FNP	23.24	1.57	0.91	21.52	152.60	30.88	170.79	15.31
	± 0.28 ^a	± 0.04 ^a	± 0.04 ^a	± 0.75 ^a	± 2.89 ^a	± 4.87 ^{ab}	± 19.46 ^a	± 1.90 ^b
NFP	22.23	1.59	0.95	21.98	127.09	21.97	162.06	6.58
	± 0.59 ^a	± 0.05 ^a	± 0.02 ^a	± 0.41 ^a	± 5.52 ^b	± 0.60 ^b	± 24.69 ^a	± 6.58 ^c

Mean ± standard error with ANOVA results ($n = 3$). Within each column, different letters indicate significant differences at the 0.05 probability level. SOM – soil organic matter; TN – total nitrogen; TP – total phosphorus; TK – total potassium; AN – available nitrogen; AP – available phosphorus; AK – available potassium; M – soil moisture. ZS11 – *Brassica napus* L. of low erucic acid and glucosinolate content; ZY821 – *B. napus* L. of high erucic acid and glucosinolate content; ZM9023 – *Triticum aestivum* L.; FNP – plot fertilized without planting crops; NFP – plot without fertilizing and planting crops treatment

than soil without crops planted (Table 1). The results showed that soil AP significantly increased from 25.57 mg/kg (ZM9023) to 33.20 mg/kg (ZS11) and 35.72 mg/kg (ZY821), respectively. Application of fertilizer significantly increased AN compared to treatment without fertilizer applied. Gong et

al. (2015) showed nitrogen (N) addition significantly enhanced the activities of urease (34.5%) and increased available N (19.8%) in a meadow steppe. Despite the addition of fertilization, soil with different crops in this study did not show any differences of soil available nitrogen during

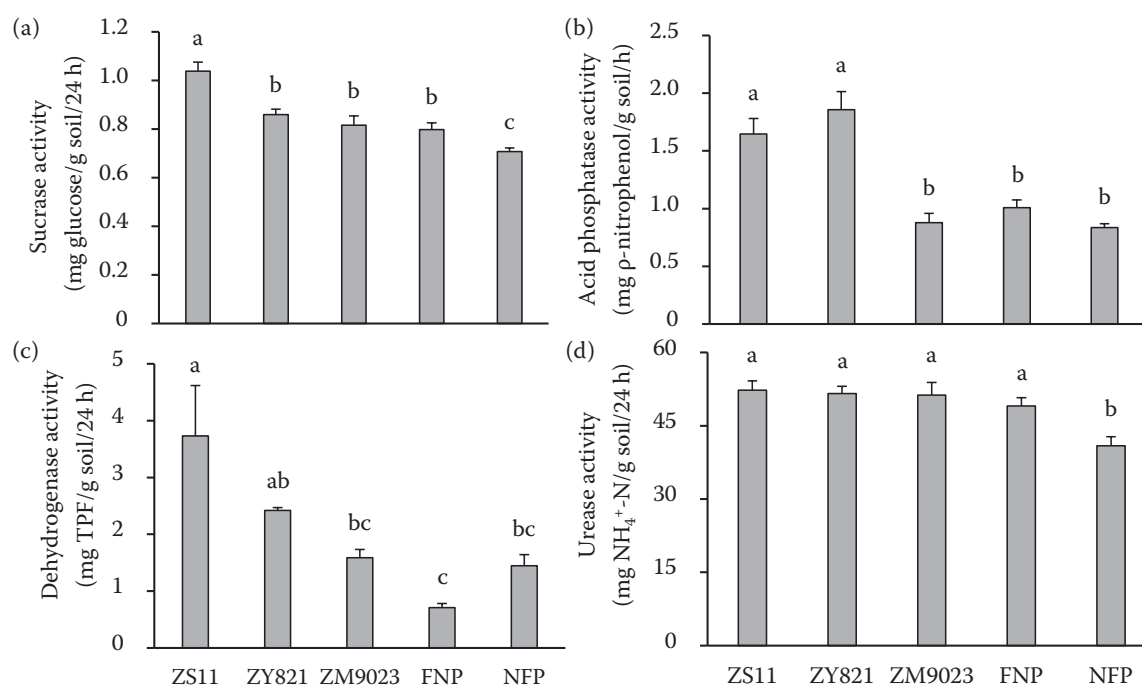


Figure 1. Soil enzyme activities. Columns with different letters indicate statistical differences at $P < 0.05$. ZS11 – *Brassica napus* L. of low erucic acid and glucosinolate content; ZY821 – *B. napus* L. of high erucic acid and glucosinolate content; ZM9023 – *Triticum aestivum* L.; FNP – plot fertilized without planting crops; NFP – plot without fertilizing and planting crops treatment; TPF – triphenyl formazan

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early growth stage after applying nitrogen fertilizer, which demonstrated that there was enough available nitrogen in the soil for plant uptake and utilization during this period.

Soil enzyme activities. Invertase activity in cropping soils increased significantly compared with NFP (Figure 1a). Invertase not only participates in the direct metabolism of soil organic matter, but also plays an important role in the enhancement of soil soluble nutrients (Yuan et al. 2017). Moreover, invertase activity of ZS11 was significantly higher than that of ZY821. It is presumed that it may be caused by high glucosinolate content from root exudates of ZY821 inhibiting invertase activity. Living roots of rape can release glucosinolates into rhizosphere (Schreiner and Koide 1993), which are hydrolysed by myrosinase into glucose, sulphate and biocidal products such as isothiocyanates, which are considered to be the most toxic and this level of the toxicity could decrease enzyme activities (Ntalli and Caboni 2017).

A significant effect of planting rapes on acid phosphatase activity was recorded (Figure 1b). Acid phosphatase activities significantly increased from 1.007 mg p-nitrophenol/g soil/h (FNP) to 1.648 mg p-nitrophenol/g soil/h (ZS11) and 1.856 mg p-nitrophenol/g soil/h (ZY821), respectively. However, planting wheat did not improve acid phosphatase activity. During growing season, the root system of rape initiates active metabolism and continuously secretes organic substances to rhizosphere soil, which are important nutrients and energy sources for rhizosphere microbes. Significant

increases of phosphatase activity in rhizosphere soil of rape assisted and improved conversion and supply of soil available phosphorus and promoted root growth and nutrient uptake for rapes.

The results demonstrated that higher activity of dehydrogenase in cropping soil was observed than in soil without crops (Figure 1c). The highest dehydrogenase activity of ZS11 increased 5.25 and 2.57 fold compared to FNP and NFP, respectively. Soil dehydrogenase activity of ZY821 and ZM9023 increased by 240.90% and 123.5% compared to FNP, respectively. Dehydrogenase was present in all viable organisms (Dick 1994). Therefore, it was considered to be the soil microbial oxidative capacity and was supposed to be correlated with the total number of viable microorganisms (Chaperon and Sauvé 2007).

Urease activity significantly increased in soil after application of mineral fertilizers (Figure 1d). Urease activities significantly increased from 40.88 mg NH_4^+ -N/g soil/24 h (NFP) to 49.04 mg NH_4^+ -N/g soil/24 h (FNP) and 51.28 mg NH_4^+ -N/g soil/24 h (ZM9023), 51.60 mg NH_4^+ -N/g soil/24 h (ZY821) and 52.28 mg NH_4^+ -N/g soil/24 h (ZS11), respectively. However, urease activities did not significantly vary in different cropping soils. Fertilization increased soil enzyme activity via promoting soil enzymatic reactions, which contributed to improved soil fertility. Variation of soil urease activity was closely related to the level of fertilization, and it was an important indicator for soil nitrogen transformation (Demoling et al. 2008).

Alpha diversity index. Crops types and fertilizer application can affect soil microbial diversity (Zhao

Table 2. Indexes of diversity and richness for soil bacterial and fungal communities among different treatments

Treatment	Bacterial 16S				Fungal ITS			
	ACE	Chao1	Shannon	Simpson	ACE	Chao1	Shannon	Simpson
ZS11	1876.51 ± 6.56 ^c	1783.53 ± 6.56 ^c	6.38 ± 0.02 ^b	0.95 ± 0.01 ^a	487.42 ± 8.96 ^a	456.84 ± 8.51 ^a	3.04 ± 0.30 ^a	0.91 ± 0.01 ^a
ZY821	2602.52 ± 20.32 ^b	2420.06 ± 2.08 ^b	6.56 ± 0.03 ^{ab}	0.97 ± 0.01 ^a	286.85 ± 8.96 ^b	291.26 ± 33.96 ^b	1.71 ± 0.29 ^b	0.64 ± 0.01 ^b
ZM9023	2638.36 ± 50.19 ^b	2510.23 ± 58.57 ^b	6.61 ± 0.03 ^{ab}	0.97 ± 0.02 ^a	296.46 ± 13.97 ^b	308.37 ± 26.42 ^b	2.73 ± 0.32 ^a	0.80 ± 0.01 ^{ab}
FNP	2695.66 ± 6.70 ^a	2720.46 ± 74.65 ^a	6.68 ± 0.03 ^a	0.97 ± 0.01 ^a	317.09 ± 10.15 ^b	324.28 ± 11.32 ^b	2.77 ± 0.27 ^a	0.83 ± 0.08 ^{ab}
NFP	2518.10 ± 42.38 ^b	2367.64 ± 27.60 ^b	6.40 ± 0.17 ^b	0.94 ± 0.02 ^a	295.25 ± 30.66 ^b	307.77 ± 43.00 ^b	2.63 ± 0.05 ^a	0.70 ± 0.06 ^b

Mean ± standard error with ANOVA results ($n = 3$). Different letters in a single column indicate significant differences between the treatments at $P < 0.05$. ZS11 – *Brassica napus* L. of low erucic acid and glucosinolate content; ZY821 – *B. napus* L. of high erucic acid and glucosinolate content; ZM9023 – *Triticum aestivum* L.; FNP – plot fertilized without planting crops; NFP – plot without fertilizing and planting crops treatment; ACE – index abundance based coverage estimator

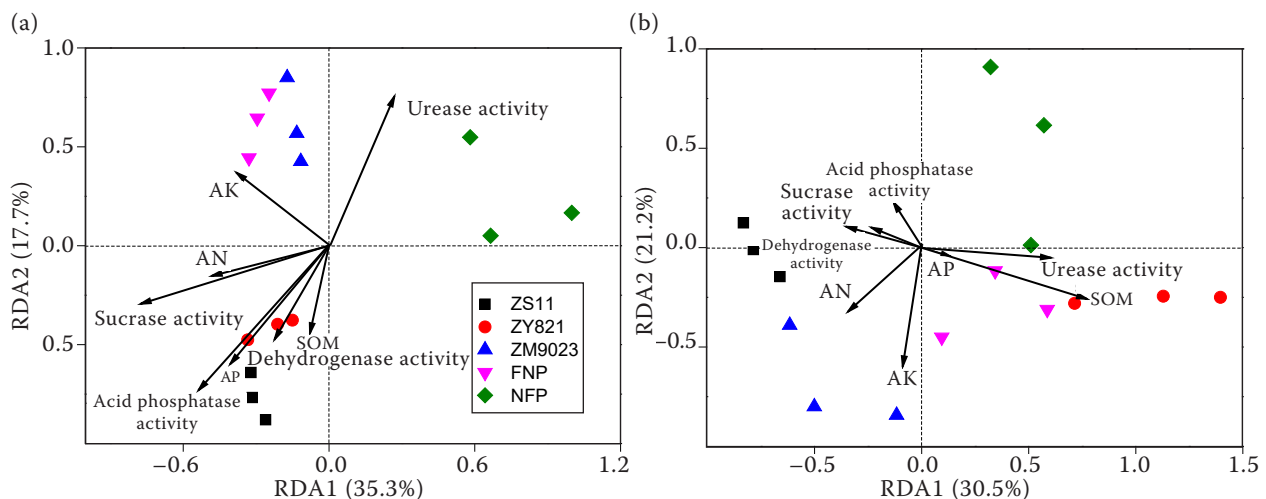


Figure 2. Redundancy analysis (RDA) of (a) 16S rRNA and (b) ITS (internal transcribed spacer) rRNA gene sequencing data at the OTU (operational taxonomic unit) level of soil properties and enzyme activities from samples collected in five treatments ($n = 3$). AK – available potassium; AN – available nitrogen; AP – available phosphorus; SOM – soil organic matter; ZS11 – *Brassica napus* L. of low erucic acid and glucosinolate content; ZY821 – *B. napus* L. of high erucic acid and glucosinolate content; ZM9023 – *Triticum aestivum* L.; FNP – plot fertilized without planting crops; NFP – plot without fertilizing and planting crops treatment

et al. 2016). Influence of plant species on microbial diversity was selective in the rhizosphere and diversity of microbes associated with different plants may have risen due to the variation in root exudates (Grayston et al. 1998). Different types of crops significantly influenced the listed alpha diversity indexes of the bacterial 16S rRNA and fungal ITS (Table 2). In our study, the ACE and Chao1 indexes of bacteria of ZS11 were lower than those of ZY821, which were similar to ZM9023. FNP treatment significantly increased soil bacteria ACE and Chao1 indexes compared to the rest of treatments (Table 2). Higher diversity of bacteria was found in soil amended with mineral fertilizer in an agricultural field (Zhao et al. 2016). The influence of fertilizer on soil microbial community diversity may be due to the changes of soil nutrient or indirect changes of plant properties. Previous studies indicated that microbial responses to fertilizer addition were plant species-specific, and might have influence the microbial community and diversity via altering the quality of plant-derived C, particularly root exudates (Weand et al. 2010). Plants provided nutrients and energy to soil microbes through its root exudates. Soil microbial growth had a selective stimulus, thereby affecting the microbial diversity (Prashar et al. 2014). In the present study, ACE and Chao1 indexes of fungus in ZS11 were higher than those of ZY821 (Table 2). These results demonstrate that high content of glu-

cosinolate from root rapeseed exudates of ZY821 and its major breakdown products inhibited the growth of fungi and oomycetes (Smith and Kirkegaard 2002), and reduced the fungal diversity of ZY821.

Redundancy analysis (RDA). RDA reflected the influence of soil properties and enzyme activities on bacterial community structure (Figure 2a). The first and second axes accounted for 35.3% and 17.1%, respectively, of total variation in bacterial community structure. Invertase activity, as a potential driver for the differentiation in bacterial community, showed the highest correlation with the first axis, followed by soil acid phosphatase activity. Soil urease activity had the highest correlation with the second axis, followed by soil acid phosphatase activities. Conversion of soil carbon and nitrogen was accelerated by soil invertase and urease, which enhanced soil microbial activities and further increased soil microbial diversity (Liu et al. 2017).

RDA reflected the influence of soil properties and enzyme activities on fungal community structure (Figure 2b). The first and second axes accounted for 30.5% and 21.2%, respectively, of total variation in fungal community structure. The fungal community was significantly correlated with SOM ($F = 2.617$, $P < 0.008$), followed by AN ($F = 2.081$, $P < 0.043$). SOM showed the highest correlation with the first axis, followed by soil urease activity. AK had the highest correlation with the second axis. Soil fungi

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activity improved by SOM, then, fungal diversity was also increased. Moreover, fungi could secrete various oxidases to promote decomposition of organic matter in soil (Lou et al. 2017).

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