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Effects of straw management and nitrogen application rate on soil organic matter fractions and microbial properties in North China Plain

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Abstract

Purpose A 2-year field experiment was conducted on the North China Plain to assess the effects of two straw management practices and different nitrogen fertilizer addition levels on soil organic matter (SOM) fractions and microbial properties under a winter wheat-summer maize cropping system.

Materials and methods Eight treatments (two straw management practices and four nitrogen fertilizer input levels) were established using a randomized complete block design. The straw management practices were no straw return and straw return. Each straw management practice received nitrogen fertilizer rates at 0 (N0), 270 (N270), 360 (N360), or 450 (N450) kg N ha^{-1} $year^{-1}$. The soil properties measured included SOM fractionation, enzyme activities, community level physiology profile (CLPP), and microbial community abundance and composition, which were represented by phospholipid fatty acid (PLFA).

Results and discussion Compared to no straw return, the addition of straw significantly increased soil total organic carbon and nitrogen, particulate organic carbon and nitrogen, and occluded particulate organic carbon and hot water extractable organic carbon by 7–29%. Invertase, protease, urease, and dehydrogenase activities increased by 22–40%. The biomasses of Gram-positive bacteria and fungi also increased. The increase in SOM fractions may be attributed to the decomposition of the added straw by more active enzymes, which may result from the shift of microbial community composition. In addition, hot-water extractable organic carbon, NO_3-N, urease activity, and fungal biomass in the N270, N360, and N450 treatments significantly increased, independent of the nitrogen application rate, which indicated that N input level had a non-linear effect on soil properties in this area.

Conclusions Short-term straw return combined with a modest nitrogen fertilizer input (270 kg N ha^{-1} $year^{-1}$) can increase SOM contents and microbial activity, which may help improve soil fertility in this region.

Keywords Crop straw return · N fertilizer rate · SOM fractions · Enzyme activity · CLPP · PLFA

1 Introduction

The North China Plain covers 27% of the nation's farmland and produces 60–80 and 35–40% of its wheat and maize, respectively. Therefore, it fulfills an important food security role in China. Since the early 1980s, the wheat and maize yields in this region have increased dramatically, which has been attributed to the introduction of high-yielding varieties and large chemical fertilizer inputs (Li et al. 2009). Recently, concerns have been raised regarding the negative effects of oversupplying chemical fertilizers when there are no organic material inputs, such as nitrate leaching, soil organic matter (SOM) loss, and soil acidification (Ju et al. 2009; Huang et al. 2015). These adverse changes in soil properties could jeopardize the productivity and sustainability of the winter wheat-summer maize double-cropping system in this region. Therefore, highly productive and sustainable food production practices need to be developed if China is to meet its continually increasing food requirements.

Returning crop straw to the soil is considered a good management practice in agricultural production systems because it

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is a valuable recycling strategy (Cayuela et al. 2009). It is also well known that crop straw return can increase agroecosystem productivity and sustainability by improving soil structure, increasing SOM and nutrient availability, and by enhancing soil microbial activity and diversity (Zhu et al. 2015; Zhao et al. 2016a, b). However, crop straw return has previously produced slow and variable effects on the following crop yield and has led to an increase in disease incidences (Zhen et al. 2009; Liu et al. 2017). Therefore, farmers are often reluctant to use crop straw in intensive agriculture systems that operate under fluctuating environmental conditions. The negative effects on crop yield can result from the immobilization of soil and fertilizer N during the early stages of straw decomposition, which leads to N deficiency in the succeeding crop (Yadvinder-Singh et al. 2009). Singh and Sharma (2000) have suggested higher N applications in crop straw amended fields compared to the recommended N levels when straw is removed. However, although mineral N could be temporarily immobilized by microbes when crop straw is added as an available C source, straw-derived N could make an important contribution to the subsequent crop N uptake and soil N sequestration (Xu et al. 2010). Furthermore, temporary N immobilization can reduce N loss to the environment and delay N release until later in the season, thereby improving N synchrony between soil supply and plant demand (Gentile et al. 2008). The increases in N supply through straw decomposition and fertilizer N utilization efficiency suggest that chemical fertilizer input could potentially be reduced after crop straw return. Therefore, it is necessary to understand the response of soil properties to the combined application of crop straw and different N fertilizer application levels if straw and fertilization management practices are to improve on the North China Plain.

Soil organic matter is widely considered to be a key component of soil fertility and quality in agroecosystems. An increase in SOM could enhance soil aggregation, improve soil nutrient availability, and increase microbial activity, biomass, and diversity (Cookson et al. 2008; Kimetu and Lehmann 2010; Chen et al. 2017). However, a significant response by total SOM to changes in soil management practices is not easily detected over the short term because of its large pool size and spatial variability (Haynes 2005). Knowledge about dividing SOM into pools with different chemical and biological characteristics is important when attempting to understand the short-term turnover in SOM. Soil labile organic matter fractions, such as dissolved organic matter, hot-water extractable organic matter, and microbial biomass carbon, can act as important C and nutrient reservoirs for soil microbes and crop growth, and can be significantly influenced by short-term soil management approaches (Guo et al. 2015; Zhu et al. 2015; Chen et al. 2017). Accordingly, soil labile organic matter fractions are recognized as early indicators of changes in soil quality (Haynes 2005). Knorr et al. (2005) also suggested that measuring the sizes of the soil labile organic matter pools may allow fertilizer doses to be optimized and help predict SOM sensitivity to global warming.

Soil microbes have attracted special interest because they play a decisive role in many soil ecological processes, such as organic matter decomposition and nutrient cycling, and respond rapidly to soil environment changes (Nannipieri et al. 2003; Wall et al. 2013; Dong et al. 2014). Wall et al. (2013) suggested that the abundance and community composition of soil microorganisms and their interactions with environmental factors have important influences on soil organic carbon (SOC) dynamics. Dong et al. (2014) pointed out that the changes in microbial community composition could alter SOC availability and subsequently crop productivity in agroecosystems. Therefore, soil microbial properties, such as enzyme activities, microbial abundance, and community composition, have been widely used to assess the effects of crop straw management practices and/or nitrogen fertilizer addition levels (Li et al. 2012; Zhao et al. 2016b; Su et al. 2017; Chen et al. 2018). For example, Zhao et al. (2016b) reported that long-term crop straw inputs could increase fungal biomass, but it had no effect on bacterial biomass. This could lead to a significant change in microbial community composition. Chen et al. (2018) found that N addition significantly increased *N*-acetyl glucosaminidase and urease activities, but had a negative effect on glycine aminopeptidase activity and negligible effects on non-specific protease and leucine aminopeptidase activities. Several studies suggested that the responses of soil microbial properties to crop straw and fertilization management practices are complex and depend on the climate, soil type, and straw quality and quantity, etc. (Li et al. 2012; Chen et al. 2017).

On the North China Plain, burning crop straw has been banned and returning straw to soils is favored by the government. This means that more attention should be paid to investigating the response of soil properties to short-term straw return in this region. The hypothesis used in this study was that short-term straw return combined with N fertilizer input could improve soil fertility by increasing the contents of different soil organic matter fractions and by enhancing microbial activity. Therefore, the objectives of this study were to (1) investigate the effects of straw return on soil organic matter fractions and microbial properties and (2) assess the responses of soil organic matter fractions and microbial properties to N fertilizer input levels.

2 Materials and methods

2.1 Field site and experimental design

The field experiment took place in Pandian, Fengqiu County, Henan Province, China (114°34′ E, 35°01′ N, 67.5 m a.s.l.)

between October 2008 and September 2010. This region has a typical monsoon climate with an average annual precipitation of 597 mm and a mean annual temperature of 14.5 °C. The soil has a sandy loam texture and is classified as Typic Haplustepts according to the FAO. At the start of the experiment, the top soil (0–20 cm depth) had the following properties: pH 7.9, organic C 7.71 g kg^{-1}, total N 0.80 g kg^{-1}, total P_2O_5 1.58 g kg^{-1}, and total K_2O 23.5 g kg^{-1}.

The field experiment was established in October 2008 and was designed as a randomized complete block with three replicates. Each plot measured 44 m^2 (8 × 5.5 m). The two straw management practices were (1) S0: in which all previous aboveground crop straw was removed; and (2) S: in which all aboveground crop straw was returned after cutting it into ≤ 5 cm pieces. The total amount of aboveground straw, which consisted of maize and wheat straw, returned to soil in the straw return treatment was about 1.57 kg m^{-2} $year^{-1}$ (dry weight). Each straw management treatments were provided with 0 (N0), 270 (N270), 360 (N360), or 450 (N450) kg N ha^{-1} $year^{-1}$. The N fertilizer was applied as a split application. The wheat received 0, 150, 200, or 250 kg N ha^{-1} $year^{-1}$ in total, and the maize received 0, 120, 160, or 200 kg N ha^{-1} $year^{-1}$. Around 60 (wheat) or 40% (maize) urea was applied as a basal fertilizer, and the remaining 40 or 60% urea, respectively, was applied as topdressing. Calcium superphosphate (150 kg P_2O_5 ha^{-1} $year^{-1}$) and potassium sulfate (250 kg K_2O ha^{-1} $year^{-1}$) were applied to the eight treatments as basal fertilizers. After basal fertilizer application, all plots were cultivated to 20-cm depth using a spade so that the soil, chemical fertilizer, and crop straw were thoroughly mixed throughout the 0–20-cm layer. The maize was planted on the 10th of June and 8th of June, and harvested on the 30th of September and 27th of September in 2009 and 2010, respectively. The winter wheat was planted on the 15th of October and 13th of October in 2008 and 2009, respectively, and harvested on the 8th of June and 5th of June in 2009 and 2010, respectively.

2.2 Soil sampling and analysis

In September 2010, soil samples (0–20 cm depth) were collected from each replicate plot after the maize harvest. One composite soil sample consisted of six soil cores that were randomly collected from each replicate plot. The samples were then transported to the laboratory on ice. After removing the stones, soil fauna, and visible plant residues, each soil sample was divided into two roughly equal parts. One part was air-dried and used to determine the soil chemical properties, and the other was stored at 4 °C after sieving and was used to measure the microbial properties.

Soil total organic carbon (TOC) and total nitrogen (TN) were determined by oxidation with potassium dichromate and Kjeldahl digestion (Lu 1999), respectively. These methods were also used to determine particulate organic carbon (POC) and nitrogen (PON), and occluded particulate organic carbon (OPOC) and nitrogen (OPON).

The POC and PON were determined according to Willson et al. (2001). Air-dried soils were dispersed in 5% sodium polyphosphate solution and shaken for 8 h. Then, the mixtures were passed through a 53-μm sieve using running distilled water to ensure separation. The material remaining on the 53-μm sieves were analyzed for total organic C and total nitrogen.

The OPOC and OPON were extracted from water-stable aggregates (53–2000 μm) (Six et al. 1998). Water-stable aggregates were mixed with 1.85 g mL^{-1} sodium polytungstate and slowly shaken. After centrifugation, the floating material was aspirated on to a 20-μm sieve and defined as free particulate organic matter (FPOM). The heavy fraction was rinsed with deionized water and dispersed in 0.5% sodium polytungstate by shaking for 18 h. The dispersed heavy fraction was passed through a 53-μm sieve. The material remaining on the sieve was used to determine OPOC and OPON. There was not enough FPOM collected in this study to measure its C and N contents.

Dissolved organic carbon (DOC) and nitrogen (DON) and hot-water extractable organic carbon (HEOC) and nitrogen (HEON) were measured according to Chodak et al. (2003) with the following modifications. Briefly, the soil samples were mixed with 0.5 mol L^{-1} K_2SO_4 solution at a ratio of 1:5 (*w*/*v*) at 25 °C for DOC and DON and 80 °C for HEOC and HEON (in a water bath maintained at a stable temperature) separately and shaken for 1 h. The mixtures then were centrifuged (4000 rpm, 20 min), and the suspensions were passed through a 0.45-μm filter. The organic C and total N contents in extracts were determined by a TOC analyzer (Multi N/C 3100 TOC/TN, Jena, Germany). The inorganic N content in the extracts was determined by a continuous-flow auto-analyzer (Auto Analyzer III, Bran+Luebbe GmbH, Norderstedt, Germany). The DON and HEON were calculated as the difference between the total N and inorganic N contents.

Microbial biomass carbon (MBC) was measured by the fumigation-extraction method as described by Vance et al. (1987). Briefly, fresh soil samples were placed in a vacuum desiccator and fumigated with ethanol-free chloroform for 24 h. The fumigated and non-fumigated soils were mixed with 0.5 mol L^{-1} K_2SO_4 solution at a ratio of 1:4 (*w*/*v*) at 25 °C. The organic C content in the suspensions was also determined by the same TOC analyzer as described above. The factor 2.64 was used to convert extracted C to MBC (Vance et al. 1987).

Soil NO_3-N was extracted with 2 mol L^{-1} KCl solution and determined by a segmented flow analyzer (Skalar, San Plus System, Breda, The Netherlands).

Soil enzyme activities were assessed according to Schinner et al. (1996). Enzyme activity assays without soil and substrates were performed at the same time as the controls.

Invertase activity was determined using sucrose as the substrate. Reducing sugar products were measured colorimetrically after reaction with a potassium ferric hexacyanide reagent. Polyphenol oxidase activity was determined using L-DOPA (L-3,4-dihydroxyphenylalanine) as the substrate, and the absorbance of the reaction product was measured at 460 nm. Protease activity was measured following incubation of the soils with casein and measurement of the formed tyrosine by Folin-Ciocalteu colorimetry. Urease activity was estimated following incubation of the soils with urea solution and measurement of released ammonium ion by indophenol blue colorimetry. Alkaline phosphatase activity was determined using *p*-nitrophenyl-phosphate as the substrate, and the absorbance of the reaction product was measured at 410 nm. Dehydrogenase activity was determined using 2,3,5-triphenyltetrazolium chloride (TTC) as the substrate. The produced triphenylformazan (TPP) was extracted with methanol and measured spectrophotometrically.

Community level physiological profiles (CLPP) of the soils were assessed using BIOLOG EcoPlate™ (BIOLOG Inc., Hayward, CA, USA) (Zhong et al. 2010). Briefly, fresh soil samples were shaken with sterile 0.85% NaCl solution on a reciprocating shaker (180 rev min^{-1}, 30 min) and diluted 250-fold using sterile 0.85% NaCl solution. Then, 150 μL of diluted solution was inoculated directly into each well and incubated at 25 °C in the dark. Substrate utilization was monitored every 12 h at 590 nm for 7 days with an Emax precision microplate reader (BIOLOG Inc.). The 96-h absorbance values were used to calculate average well color development (AWCD). The functional diversity indices (Shannon index, Simpson index, and McIntosh index) were calculated according to Zhong et al. (2010).

Microbial community structure was determined by phospholipid fatty acid (PLFA) analysis as described by Brant et al. (2006). The lipids were extracted from fresh soils (3 g, oven-dried) using a mixture of chloroform, methanol, and phosphate buffer (pH 7.0) at a ratio of 1.0:2.0:0.8. The phospholipids were separated from glycolipids and neutral lipids on a silicic acid column. They were saponified and methylated to fatty acid methyl esters (FAMEs) using 0.2 mol L^{-1} methanolic KOH and then dissolved in hexane containing a 19:0 FAME standard (60 μg mL^{-1} methyl nonadecanoate fatty acid). The FAMEs were determined by a gas chromatograph mass spectrometer (GC-MS) QP 2010 PLUS (Shimadzu, Kyoto, Japan). The fatty acids (15:0, 16:0, 17:0, and 18:0) were presumed to represent general bacteria. The a13:0, i15:0, a15:0, and a17:0 PLFAs were chosen to represent Gram-positive bacteria (G^+), and Gram-negative bacteria (G^-) were represented by the sum of cy17:0, 16:1ω7c, and 19:1ω9t (Zelles 1999). Fungal biomass was estimated from the 18:1ω9c, 18:2ω6,9c, and 18:2ω9,12t markers (Frostegård et al. 1993), and 10me18:0 was used as the marker for actinomycetes (Zelles 1999).

2.3 Statistical analysis

All results reported are expressed on an oven-dried basis (105 °C for 24 h). Statistical analyses were carried out by using SPSS 17.0 software package for Windows (SPSS Inc., Chicago, IL, USA). The main effects and interactions between straw management and N fertilizer rate were determined by two-way ANOVA. Differences were considered significant at $p < 0.05$. Tukey's HSD test for multiple comparisons was run if significant differences were found ($p < 0.05$). Principal component analysis (PCA) was used to evaluate the changes in the composition of the microbial communities.

3 Results

3.1 Soil organic matter fractions

Crop straw return increased TOC, TN, POC, PON, OPOC, and HEOC by 11, 7, 22, 29, 19, and 27%, respectively ($p < 0.05$) compared to straw removal (Table 1), but it had no significant effects on the OPON, DOC, DON, HEON, MBC, and NO_3-N contents. The fertilizer N rates had no significant effects on the TOC, TN, POC, PON, OPOC, OPON, DOC, DON, HEON, and MBC contents. However, N fertilizer addition significantly increased the HEOC and NO_3-N contents compared to the N0 treatment. There were no interaction effects between straw return and N application rates for soil organic matter pools and NO_3-N.

3.2 Soil enzyme activities

Crop straw return led to 37, 40, 22, and 39% increases in invertase, protease, urease, and dehydrogenase activities, respectively, compared to straw removal ($p < 0.05$) (Table 2). The invertase, polyphenol oxidase, protease, alkaline phosphatase, and dehydrogenase activities were not significantly affected by N application rate. Urease activity was significantly lower in the N0 treatment than in the N270, N360, and N450 treatments. There were no interaction effects between straw return and N application rate for soil enzyme activities.

3.3 Community level physiological profiles

The value of average well color development (AWCD), and the Shannon, Simpson, and McIntosh indexes were not significantly influenced by crop straw return or N fertilizer application rate (Table 3). The same trend was also observed for the utilization intensity of the six carbon sources. There were no interaction effects between straw return and N application rate for the community level physiological profile indexes.

Table 1 The contents of soil organic matter pools and NO_3-N under different crop residue management and N fertilizer rate

Labile SOM pools	Crop straw treatment			Fertilizer N rate					Interactions
	S0	S	Significance	N0	N270	N360	N450	Significance	
TOC (g kg^{-1})	7.92 ± 0.76	8.77 ± 0.38	**	8.05 ± 0.69	8.26 ± 0.77	8.53 ± 0.81	8.55 ± 0.73	ns	ns
TN (g kg^{-1})	0.86 ± 0.07	0.92 ± 0.06	*	0.84 ± 0.07	0.90 ± 0.06	0.90 ± 0.06	0.92 ± 0.07	ns	ns
POC (g kg^{-1})	1.62 ± 0.17	1.98 ± 0.24	**	1.67 ± 0.24	1.93 ± 0.34	1.74 ± 0.18	1.85 ± 0.30	ns	ns
PON (g kg^{-1})	0.14 ± 0.02	0.18 ± 0.02	**	0.14 ± 0.02	0.17 ± 0.03	0.17 ± 0.02	0.18 ± 0.03	ns	ns
OPOC (g kg^{-1})	1.39 ± 0.13	1.66 ± 0.28	*	1.58 ± 0.27	1.53 ± 0.10	1.54 ± 0.37	1.45 ± 0.26	ns	ns
OPON (g kg^{-1})	0.12 ± 0.02	0.13 ± 0.02	ns	0.13 ± 0.03	0.12 ± 0.01	0.12 ± 0.03	0.12 ± 0.02	ns	ns
DOC (mg kg^{-1})	202 ± 32	194 ± 21	ns	179 ± 26	200 ± 33	200 ± 19	212 ± 22	ns	ns
DON (mg kg^{-1})	5.33 ± 1.32	6.47 ± 1.59	ns	4.78 ± 0.87	5.70 ± 2.07	6.51 ± 1.51	6.61 ± 1.03	ns	ns
HEOC (mg kg^{-1})	654 ± 152	831 ± 184	**	545 ± 86b	809 ± 218a	857 ± 111a	758 ± 166a	**	ns
HEON (mg kg^{-1})	13.7 ± 5.0	17.4 ± 4.3	ns	14.0 ± 5.13	14.9 ± 4.54	15.2 ± 3.8	18.2 ± 6.2	ns	ns
MBC (mg kg^{-1})	143 ± 31	151 ± 29	ns	150 ± 39	154 ± 15	146 ± 37	137 ± 27	ns	ns
NO_3-N (mg kg^{-1})	24.9 ± 10.7	25.9 ± 11.5	ns	10.0 ± 2.6c	27.2 ± 7.2b	29.2 ± 5.2ab	35.2 ± 6.4a	**	ns

N0, N270, N360, and N450 total annual N fertilizer input to wheat and maize crops at 0, 270, 360, and 450 kg N ha^{-1} $year^{-1}$. Different letters in a row indicate significant difference at $p < 0.05$

S0 straw removal, *S* straw returning, *TOC* total organic carbon, *TN* total nitrogen, *POC* particulate organic carbon, *PON* particulate organic nitrogen, *OPOC* occluded particulate organic carbon, *OPON* occluded particulate organic nitrogen, *DOC* dissolved organic carbon, *DON* dissolved organic nitrogen, *HEOC* hot-water extractable organic carbon, *HEON* hot-water extractable organic nitrogen, *MBC* microbial biomass carbon, *ns* no significant

*Significant at the 0.05 probability level

**Significant at the 0.01 probability level

3.4 Microbial biomass and community composition

Crop straw return increased the biomasses of Gram-positive bacteria, and fungi, and the total PLFA by 38, 120, and 23%, respectively, compared to straw removal ($p < 0.05$) (Table 4). There were no significant N application rate effects and no significant interactions between the biomasses of the general bacteria, Gram-positive bacteria, Gram-negative bacteria, actinomycetes, and total PLFA. Fungal biomass was significantly lower in the N0 treatment than in the N270, N360, and N450 treatments. Furthermore, straw return significantly increased the ratio of Gram-positive bacteria to Gram-negative bacteria (G^+/G^-) and the ratio of fungi to bacteria (F/B) compared to straw removal. There were no significant N application rate or straw–N fertilizer interaction effects on the G^+/G^- value. The N270, N360, and N450 treatments increased the F/

Table 2 Soil enzyme activities under different crop residue management and N fertilizer rate

Enzyme activity	Crop straw treatment			Fertilizer N rate					Interactions
	S0	S	Significance	N0	N270	N360	N450	Significance	
Invertase (mg g^{-1} day^{-1})	16.5 ± 6.1	22.6 ± 4.4	*	18.6 ± 5.1	21.1 ± 6.8	18.0 ± 7.7	20.5 ± 5.6	ns	ns
Polyphenol oxidase (mmol kg^{-1} h^{-1})	0.18 ± 0.01	0.18 ± 0.01	ns	0.18 ± 0.01	0.18 ± 0.00	0.17 ± 0.01	0.18 ± 0.02	ns	ns
Protease (mg kg^{-1} day^{-1})	13.6 ± 3.2	19.0 ± 2.4	**	15.7 ± 2.0	17.0 ± 4.3	15.9 ± 4.5	16.6 ± 5.2	ns	ns
Urease (mg kg^{-1} day^{-1})	144 ± 11	176 ± 37	**	134 ± 12b	174 ± 35a	161 ± 17a	171 ± 40a	**	ns
Alkaline phosphatase (mg kg^{-1} h^{-1})	144 ± 10	148 ± 18	ns	142 ± 12	141 ± 14	147 ± 9	155 ± 19	ns	ns
Dehydrogenase (mg kg^{-1} day^{-1})	14.2 ± 3.4	19.8 ± 2.5	**	16.3 ± 2.1	17.7 ± 4.5	16.5 ± 4.7	17.3 ± 5.4	ns	ns

N0, N270, N360, and N450 total annual N fertilizer input to wheat and maize crops at 0, 270, 360, and 450 kg N ha^{-1} $year^{-1}$ Different letters in a row indicate significantly different at $p < 0.05$

S0 straw removal, *S* straw returning, *ns* no significant

*Significant at the 0.05 probability level

**Significant at the 0.01 probability level

Table 3 Changes in community level physiological profile under different crop residue management and N fertilizer rate

	Crop straw treatment			Fertilizer N rate					Interactions
	S0	S	Significance	N0	N270	N360	N450	Significance	
AWCD	0.67 ± 0.12	0.69 ± 0.06	ns	0.71 ± 0.06	0.64 ± 0.15	0.68 ± 0.11	0.69 ± 0.04	ns	ns
Shannon index	3.07 ± 0.12	3.12 ± 0.05	ns	3.13 ± 0.10	3.05 ± 0.15	3.09 ± 0.08	3.10 ± 0.04	ns	ns
Simpson index	0.96 ± 0.01	0.96 ± 0.00	ns	0.96 ± 0.00	0.95 ± 0.01	0.95 ± 0.00	0.96 ± 0.00	ns	ns
McIntosh index	4.46 ± 0.57	4.50 ± 0.33	ns	4.56 ± 0.31	4.32 ± 0.63	4.48 ± 0.61	4.56 ± 0.22	ns	ns
Utilization intensity									
Carboxylic acids	5.51 ± 0.56	5.30 ± 1.14	ns	4.98 ± 1.31	5.66 ± 0.71	5.98 ± 0.45	4.99 ± 0.53	ns	ns
Carbohydrates	6.35 ± 0.83	6.85 ± 0.80	ns	6.92 ± 1.09	6.66 ± 0.87	6.62 ± 0.16	6.19 ± 0.96	ns	ns
Amino acids	4.78 ± 0.35	4.25 ± 0.86	ns	4.22 ± 1.23	4.87 ± 0.37	4.54 ± 0.25	4.43 ± 0.50	ns	ns
Miscellaneous	1.30 ± 0.07	1.37 ± 0.31	ns	1.52 ± 0.26	1.28 ± 0.05	1.36 ± 0.23	1.19 ± 0.19	ns	ns
Polymers	3.31 ± 0.45	3.41 ± 0.53	ns	3.25 ± 0.36	3.39 ± 0.42	3.72 ± 0.60	3.08 ± 0.38	ns	ns
Amines	0.94 ± 0.21	0.99 ± 0.30	ns	0.93 ± 0.34	1.14 ± 0.17	0.95 ± 0.23	0.85 ± 0.21	ns	ns

N0, N270, N360, and N450 total annual N fertilizer input to wheat and maize crops at 0, 270, 360, and 450 kg N ha^{-1} $year^{-1}$

S0 straw removal, *S* straw returning, *AWCD* average well color development, *ns* no significant

B value by 100, 118, and 73%, respectively ($p < 0.05$) compared to N0. There were significant interactions between straw treatment and N application rate for 19:1ω9t, 18:2ω9,12t, total fungi, and the F/B value.

The effects of the different straw management treatments and the N application rates on microbial community composition were evaluated by principal component analysis (PCA) (Fig. 1). The first principal component (PC1) explained 38.4% of the variance and separated the straw return treatment from the straw removal treatment (Fig. 1a). The effects of N application rate on microbial community composition showed obvious sequential shifting along the second principal component (PC2), which explained 19.5% of the variance. The treatment order was N0–N270–N360 and N450. The significant differences in PLFA 15:0, a17:0, 19:1ω9t, and 18:2ω6,9c may define the differences in microbial community composition along PC1 (Table 1). The PLFA biomarkers a13:0, i15:0, and 17:0 may be the main drivers of PC2.

3.5 Relationships between SOM fractions and microbial properties

There were significantly positive correlations between the TOC, TN, and PON contents and urease and invertase activities (Table 5). The DON and NO_3-N contents were positively correlated with urease activity, and the POC and HEOC contents were positively correlated with protease, urease, and dehydrogenase activities. The POC content was also positively correlated with invertase activity.

The TOC, POC, and PON contents were significantly positively correlated with fungal biomass, G^+/G^- and F/B values (Table 5). The POC content was also positively correlated with total PLFA biomass. The HEOC, DON, and NO_3-N contents were positively correlated with fungal biomass and the F/B value, and TN content was positively correlated with fungal biomass and the G^+/G^- value.

4 Discussion

4.1 Effects of crop straw on soil biochemical properties

Crop straw plays an important role in increasing soil organic matter sequestration in agroecosystems. This study showed that short-term crop straw inputs over 2 years significantly increased POC, PON, and HEOC contents, which suggested that the labile organic matter fractions could respond rapidly to changes in straw management practices. Similar results have also been reported by other studies (Guo et al. 2015; Zhao et al. 2016a). Crop straw decomposition and turnover may contribute to the increase in the contents of the different labile organic matter fractions in this study. The results also showed that short-term crop straw inputs could significantly increase TOC and TN contents, which was consistent with Zhu et al. (2015) who reported that short-term wheat or rice straw inputs could significantly increase TOC in a wheat-rice rotation system. The improvement recorded in this study may be due to the low organic carbon (OC) content of the original soil (about 7.7 g kg^{-1}). The OC-poor soil could supply abundant sorption sites on mineral surfaces (Kaiser and Guggenberger 2003). The existence of unsaturated mineral surfaces may provide capacity for the sequestration of SOM either by the direct adsorption of organic molecules onto sorption sites or through the occlusion of coarse straw components (Kolbl et al. 2007; Kimetu and Lehmann 2010).

Table 4 Change in microbial community composition under different crop residue management and N fertilizer rate

	Crop Straw treatment			Fertilizer N rate					
	S0	S	Significance	N0	N270	N360	N450	Significance	Interactions
	nmol g^{-1}			nmol g^{-1}					
15:0	2.94±0.95	5.01±1.56		3.91±1.94	4.09±1.28	4.18±2.48	3.72±0.86	ns	ns
16:0	11.5±2.0	11.9±3.3	ns	11.9±1.6	12.0±2.1	10.1±3.1	12.6±3.5	ns	ns
17:0	0.60±0.34	0.55±0.37	ns	0.92±0.43a	0.68±0.16ab	0.39±0.09b	0.31±0.27b	**	ns
18:0	2.36±0.78	2.53±0.78	ns	2.43±1.22	2.40±0.39	2.41±0.60	2.54±0.83	ns	ns
General bacteria	17.3±2.9	19.95±3.2	ns	19.1±3.1	19.2±3.2	17.1±2.5	19.1±4.3	ns	ns
a13:0	0.40±0.24	0.41±0.25	ns	0.67±0.29a	0.47±0.07ab	0.19±0.04b	0.28±0.09b	**	ns
i15:0	0.09±0.07	0.11±0.12	ns	0.23±0.11a	0.09±0.02b	0.04±0.01b	0.05±0.02b	**	ns
a15:0	1.64±1.39	2.02±0.55	ns	1.39±0.35	1.81±0.61	2.55±1.88	1.56±0.27	ns	ns
a17:0	1.80±0.58	2.86±0.85	**	2.02±0.71	2.57±0.94	2.59±1.26	2.13±0.63	ns	ns
Gram-positive	3.93±1.82	5.39±1.17	**	4.30±0.93	4.94±1.56	5.38±2.74	4.02±0.88	ns	ns
cy17:0	1.61±0.70	1.95±0.51	ns	1.35±0.38	2.00±0.36	2.06±0.95	1.73±0.50	ns	ns
16:1ω7c	4.31±1.88	2.03±0.66	**	3.47±1.09	3.24±2.01	3.18±2.23	2.78±2.12	ns	ns
19:1ω9t	0.00±0.00	1.25±0.93	**	0±0c	0.88±0.99ab	1.07±1.27a	0.55±0.62b	**	**
Gram-negative	5.93±2.43	5.23±1.61	ns	4.82±0.87	6.12±1.57	6.31±3.09	5.06±2.15	ns	ns
Total Bacteria	27.2±6.5	30.6±3.6	ns	28.3±3.9	30.3±5.56	28.8±7.7	28.2±5.1	ns	ns
18:1ω9c	1.77±0.57	2.08±0.67	ns	1.57±0.33	2.28±0.53	2.13±0.76	1.73±0.67	ns	ns
18:2ω6,9c	1.71±0.55	2.29±0.88	*	1.53±0.25b	2.02±0.52ab	2.63±1.22a	1.82±0.40ab	*	ns
18:2ω9,12t	0.00±0.00	3.34±2.14	**	0±0b	2.33±2.68a	2.37±2.61a	1.98±2.21a	**	**
Fungi	3.49±1.09	7.72±3.10	**	3.10±0.37b	6.63±3.31a	7.13±3.96a	5.54±2.62a	**	**
10me18:0	1.80±0.91	1.68±0.55	ns	1.98±0.87	1.84±0.55	1.96±0.92	1.20±0.29	ns	ns
Total PLFA	32.5±7.6	40.0±6.1	**	33.3±4.4	38.7±8.4	37.9±106	34.9±7.2	ns	ns
G^+/G^-	0.69±0.21	1.06±0.14	**	0.92±0.28	0.83±0.28	0.86±0.15	0.90±0.35	ns	ns
F/B	0.13±0.02	0.25±0.09	**	0.11±0.02b	0.22±0.09a	0.24±0.11a	0.19±0.08a	**	**

S0 straw removal, *S* straw returning. *N0*, *N270*, *N360* and *N450* total annual N fertilizer input to wheat and maize crops at 0, 270, 360 and 450 kg N ha^{-1} $year^{-1}$

G^+/G^- the ratio of gram-positive bacteria to gram-negative bacteria, F/B the ratio of fungi to bacteria

* and ** indicate significant at the 0.05 and 0.01 probability level, respectively. ns no significant.

Different letters in a row indicate significantly different at $p < 0.05$.

Crop straw return significantly increased invertase, protease, and urease activities, which suggested that crop straw plays an important role in controlling hydrolytic enzyme activities. Substrate concentrations are an important influencing factor on hydrolytic enzyme activity (Allison et al. 2014), and the release of carbohydrate and protein components from crop straw may stimulate these enzymes. In addition, it is well established that fungi play a crucial role in controlling soil enzyme levels because soil enzymes are mainly synthesized and secreted by fungi (Kluber et al. 2010). The significant increase in fungal abundance with crop straw return (Table 4) could promote enzyme activity. Phospholipid fatty acids are only present in living microbial cells. Therefore, the increase in total PLFA content (Table 4) suggested that there were more living microbes in the straw return treatment than in the straw removal treatment. This may explain the increase in dehydrogenase activity because dehydrogenase activity only occurs in viable microorganisms.

The ability of microbes to utilize a range of C substrates is fundamental to all ecological functions in soils. This study showed that crop straw return had no significant effects on the microbial functional diversity index and carbon source utilization intensity. Konopka et al. (1998) suggested that the conditions of the BIOLOG plate environment may be biased towards fast growing bacteria suited to growth in high nutrient conditions. Therefore, the biology profile may become dominated by these species. However, in this study, crop straw return only increased the biomasses of Gram-positive bacteria and fungi, which are characterized by slow growth rates (Chen et al. 2016). The limitations of this technique may have contributed to the similar CLPP between the straw return and straw removal treatments. On the other hand, the metabolic redundancy that is many species have genetic potential to use a specific substrate may also contribute to the similar

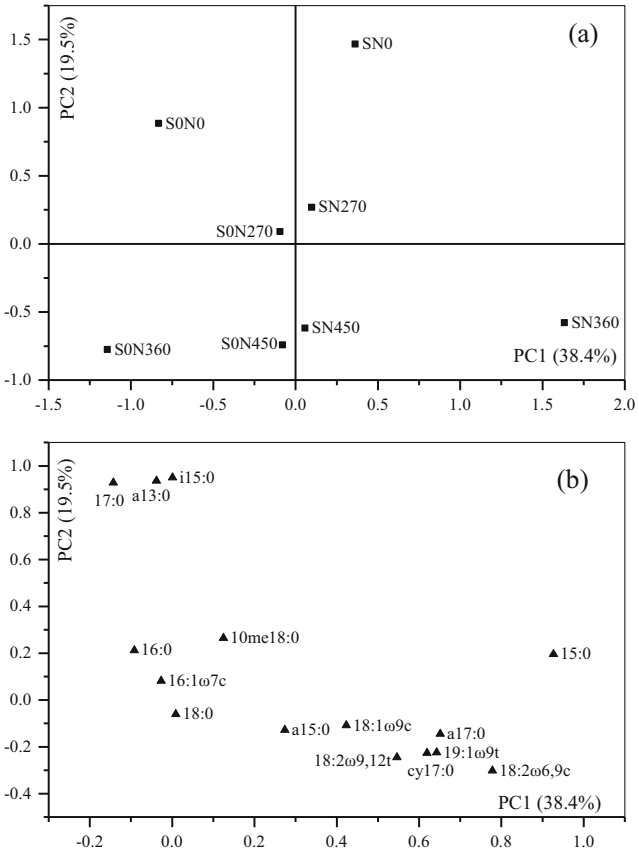

Fig. 1 Principal component analysis of the PLFA pattern from eight treatments. **a** Score plot for the eight treatments. **b** Loading plot with all individual fatty acids. Treatments are explained in Sect. 2.1

functional diversity as assayed by carbon substrate utilization patterns (Konopka et al. 1998; Rousk et al. 2009).

Crop straw return significantly increased the biomasses of Gram-positive bacteria and fungi, which led to a significant increase in total PLFA biomass. Zhao et al. (2016b) also reported that long-term crop straw inputs could increase fungal biomass. The growth of soil microbes is limited by carbon source availability (Schimel and Weintraub 2003). Therefore, the significant increase in the contents of the different soil organic carbon fractions in the straw return treatment (Table 1) may have stimulated microbial growth. Kramer and Gleixner (2008) suggested that Gram-negative bacteria preferentially decomposed easily degradable straw components, whereas Gram-positive bacteria used more complex components. In the 4 months following straw return (from June to September), the Gram-positive bacteria may have been able to outcompete Gram-negative bacteria by adapting to the relatively recalcitrant straw component. In addition, crop straw return could increase the soil C:N ratio, which may also have contributed to the increase in fungal biomass because fungi have a low N demand and use C more efficiency than bacteria (Fierer et al. 2003). In addition, straw return also changed the microbial community composition, which was consistent with other studies (Guo et al. 2015; Chen et al. 2017). Previous studies have shown that soil TOC content is the main factor driving changes in microbial community composition (Chen et al. 2017). The TOC content increase in the crop straw return treatment (Table 1) may also have contributed to the microbial community composition changes seen in this study.

4.2 Effects of N application rate on soil biochemical properties

In this study, N fertilizer addition had no significant effects on soil organic matter pools compared to the no N addition treatment, with the exceptions of HEOC and NO_3-N, which suggested that C and N fractions with different physical and chemical compositions and qualities respond differently to N addition. Theoretically, N addition could increase soil organic matter fraction sequestration by enhancing net primary production (Nave et al. 2009). However, N addition could also increase microbial activities by mitigating N limitation and

Table 5 Correlation coefficients between soil organic matter fractions and microbial properties in a northern China soil

	Protease	Urease	Invertase	Dehydrogenase	Fungi	Total PLFA	G^+/G^-	F/B
TOC	ns	0.436*	0.586**	ns	0.460*	ns	0.426*	0.462*
TN	ns	0.519**	0.591**	ns	0.441*	ns	0.424*	ns
POC	0.560**	0.653**	0.585**	0.560**	0.559**	0.477*	0.589**	0.528**
PON	ns	0.722**	0.573**	ns	0.595**	ns	0.544**	0.627**
DON	ns	0.500*	ns	ns	0.577**	ns	ns	0.574**
HEOC	0.553**	0.633**	ns	0.553**	0.724**	ns	ns	0.758**
HEON	ns	ns	0.497*	ns	ns	ns	ns	ns
NO_3-N	ns	0.482*	ns	ns	0.414*	ns	ns	0.405*

TOC total organic carbon, *TN* total nitrogen, *POC* particulate organic carbon, *PON* particulate organic nitrogen, *DON* dissolved organic nitrogen, *HEOC* hot-water extractable organic carbon, *HEON* hot-water extractable organic nitrogen, G^+/G^- the ratio of gram-positive bacteria to gram-negative bacteria, *F/B* the ratio of fungi to bacteria, *ns* no significant

*$p < 0.05$; **$p < 0.01$

decrease the C:N ratio of the organic materials derived from living plants. These effects would lead to fast decomposition and turnover rates (Fornara and Tilman 2008), which have the potential to limit soil organic matter storage capacity. However, the change in HEOC may be controlled by other mechanisms and needs further study. The increase in NO_3-N content as the N input level rose indicated that the highest N input level may be more than the crop can assimilate, which could potentially lead to an increased risk of N leaching. Similar results have also been reported by other studies (Zhang et al. 2008; Zhao et al. 2016a).

This study showed that, with the exception of urease, N addition had no significant effect on enzyme activities. Different enzyme activity responses to N addition have been reported in previous studies (Shen et al. 2010; Zhou et al. 2012; Chen et al. 2018). For example, Zhou et al. (2012) found that invertase and alkaline phosphatase activities increased at low N addition levels, but decreased at high N addition levels, and that polyphenol oxidase activity stayed unchanged at low N addition rates, but decreased at high N addition rates. Therefore, N addition could directly affect the microbial production of enzymes through the increase in N availability, but the effect varies with the type of soil and enzyme (Chen et al. 2018). In this study, N addition levels had no significant effects on microbial biomass according to the total PLFA levels (Table 4). This may partly explain the similar enzyme activities for the different N addition treatments. Dick and Tabatabai (1992) reported that dehydrogenase activity can increase as microbial population levels rise because dehydrogenase occurs only within living cells. However, N addition also had negative effects on enzyme activities because it decreased pH and increased soil salinity (Shen et al. 2010). Urease is involved in the hydrolysis of urea type substrates, and its activity is mainly controlled by substrate concentration. In this study, the N fertilizer was added as urea, which may have contributed to the increase in urease activity. However, the rise in the hydrolytic production of urea (NH_4^+) with increasing urea addition could repress urease activity (Ajwa et al. 1999). This may be an important explanation for the similar urease activities among the different N addition treatments (N270, N360, and N450).

In this study, N fertilizer addition had no significant influence on the microbial community level physiological profile (CLPP), which suggested that maintaining microbial community functional stability may be a general response to changes in N fertilizer addition over a short period of time. Zhou et al. (2012) also found no significant effects of N addition on the CLPP. Although N addition increases N availability, it also causes the acidification and secondary salination of soils, which have negative effects on microbes (Shen et al. 2010). On the other hand, as the availability of organic C is a crucial role in influencing CLPP (Cookson et al. 2008), the similar contents of SOC fractions in the different N addition treatments (Table 1) may have also contributed to the similar CLPP in this study.

Nitrogen availability has an important influence on the growth and proliferation of soil microbes. This study showed that fertilizer N input significantly increased fungal biomass, but had no significant effects on bacterial biomass, which led to a higher F/B ratio. However, there were no significant differences in fungal biomass between the N270, N360, and N450 treatments. Zhao et al. (2014) also reported that fertilizer N input did not influence bacterial biomass, but did increase fungal biomass. Zhong et al. (2010) indicated that soil bacteria were sensitive to organic manure applications and that soil fungi were sensitive to mineral fertilizer. N addition could directly alter the biomass of soil microbes or indirectly alter them by decreasing soil pH and/or by altering the organic carbon sources (Allison et al. 2008). As fungi growth needs less N compared to bacteria (Fierer et al. 2003), the soil acidification caused by N fertilizer inputs may be an important factor contributing to the increase in fungal biomass. Rousk et al. (2009) reported that fungal growth was favored at low pH, while bacterial growth was favored at high pH in a century old experiment using arable soil. Furthermore, this study showed that the microbial community composition was similar in the high N addition treatments (N360 and N450), but there was a difference in community composition between the high N addition treatments and the low N addition (N270) and control (N0) treatments. The similar fungal biomass and microbial community compositions indicated that N input level had a non-linear effect on microbial properties. Similar results were also reported by other studies (Zhang et al. 2008; Li et al. 2012). Zhang et al. (2008) suggested that there is a threshold for N addition at which microbial response shifted from positive to negative. Low N addition levels might change microbial community composition by alleviating soil N availability and C limitation through the stimulation of plant growth and litter decomposition. In contrast, excess N fertilizer addition can limit C availability to microbes and lower the soil pH, which may have a deleterious effect on soil microbial communities. There were also significant interaction effects between straw return and N application rates for fungi biomass and the F/B ratio. Soil fungi prefer an acid soil environment and have a lower N demand and higher C use efficiency than bacteria (Fierer et al. 2003; Rousk et al. 2009). Therefore, N fertilizer input may change the availability of soil nitrogen and/or the soil pH, which could influence fungal growth, leading to the change in F/B ratio after crop straw return to the soil.

5 Conclusions

The results suggest that straw return could significantly increase TOC, TN, POC, PON, OPOC, and HEOC contents, and enhance invertase, protease, urease, and dehydrogenase

activities. It may also increase Gram-positive bacterial and fungal biomasses and change microbial community composition. These findings suggest that straw return can efficiently increase SOM, which may help to replenish soil fertility in this region, even over a short period of time. In addition, the nitrogen fertilizer input treatments led to significantly higher HEOC and NO_3-N contents, urease activity, and fungal biomass than the non-N addition treatment, but there were no significant differences between the different N input levels, which indicated that there was a non-linear response of soil properties to N application rates. This study was only conducted at one site. Further studies that include more sites are needed to verify the responses of soil properties to short-term crop straw and N fertilizer management practices under a winter wheat-summer maize cropping system.

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