The soil organic carbon (SOC) pool is a major part of the global carbon cycle and plays an important role in the process of global carbon change. Its dynamic balance is an important indicator of soil fertility (Lefroy et al. 1993). A high SOC content can effectively improve the crop nutrient supply and soil structure to increase biodiversity and enhance microbial activity (Pospíšilová et al. 2011, Yang et al. 2012). Soil biochemical properties, e.g., microbial biomass and enzyme activities, are important indicators of soil quality because of their roles in carbon sequestration, SOC decomposition and nutrient cycling and availability (Alvaro-Fuentes et al. 2008). Soil enzyme activity is an important biological indicator of soil fertility. Many studies have shown that the application of organic fertilizers can significantly increase soil enzyme activity (Zhang et al. 2015).

However, long-term application of mineral fertilizers has been heavily used and has been a central issue in the green revolution worldwide (Abdoulaye and Sanders 2005). Furthermore, although the use of excessive quantities of mineral fertilizer can increase crop productivity, it also leads to pollution and degradation of the agricultural environment (Mandal...
Therefore, it is essential to seek other methods of fertilization. Crop straw can affect the balance of SOC accumulation and decomposition and enhance soil fertility and microbial biomass (Zhao et al. 2015, Torma et al. 2018). There are direct and indirect ways to reuse straw. Several studies investigated the direct return of straw in field, but fewer studies examined indirect ways to reuse straw, e.g., biogas slurry and manure. The use of biogas slurry (digestate) can improve soil N uptake because it has plenty of nitrogen (Sheets et al. 2015, Tan et al. 2016), reduce pests and increase crop yields (Cao et al. 2013, Lošák et al. 2016), and can also enhance soil microbial biomass and diversity (Abubaker et al. 2013). Manure was used as the main amendment to improve soil fertility (Liang et al. 2012). Manna et al. (2007) reported that the combined application of organic and inorganic fertilizers can increase soil invertase activity and available nutrients in the north-western India. Although straw, biogas slurry, manure and other organic amendments are used directly as fertilizers on farmlands, there are few reports regarding the effect of straw recycling on soil quality, crop yield and water use efficiency.

Input of organic matter, e.g., straw, biogas slurry and manure, is a major way to improve soil fertility. However, which of these organic matter inputs is better for soil fertility and crop productivity remains poorly understood. Therefore, this study attempts to analyse soil chemical properties, microbial biomass, enzyme activities, and crop yield under four different fertilizer treatments, including soil amendments with straw, biogas slurry, cow manure and a mineral fertilizer, to explore the most rational straw utilization method.

MATERIAL AND METHODS

Site description. This experiment was conducted in the Pingyuan County on the Huang-Huai-Hai Plain of China (116°26'E, 37°09'N) from 2012 to 2015. The local area has a warm temperate continental monsoon climate with about 25 m a.s.l., an annual mean temperature of 14.55°C and an annual rainfall of 516 mm. The experimental soil is a typical winter wheat-summer maize (cv. Gaoyou 5580) cropping system used in this study. It was designed as a randomized block with three replicates of four treatments. Each of the 12 plots was 80 × 4 m², and equal quantities of nitrogen were used in each plot. The four different fertilization treatments were set up as follows: (i) mineral fertilization (CK); (ii) after the maize harvest, the straw was returned directly to the field (S); (iii) after the maize harvest, the straw was transported to the biogas pool and fermented and the remaining biogas slurry was used as a fertilizer for the farmland (B); (iv) after the maize harvest, the straw was transported to the dairy farm and processed into silage to feed the dairy cows, and the cow manure was then used as a fertilizer for the farmland (M). The annual application rates of these organic and mineral fertilizers are shown in Table 1.

Soil sampling and laboratory determinations. Soil samples were collected at 0–20, 20–40 and 40–60 cm with three repeats per experimental plot during the harvest stage of the summer maize growing season each year. After returning to the laboratory, some soil samples were passed through a 2 mm mesh screen. Some soil samples were stored in a refrigerator at 4°C till the determinations of soil microbial biomass carbon (MBC); microbial biomass nitrogen (MBN) and soil enzyme activities were performed and the remaining samples were air-dried for the analysis of soil chemical properties.

Soil total nitrogen (TN) and total organic carbon (TOC) were determined using the semi-micro Kjeldahl method and an Elementar vario TOC (Elementar Analysensysteme, Hanau, Germany), respectively. Soil MBC and MBN were determined by chloroform fumigation extraction (Joergensen 1996). Urease (UA), invertase (IA) and hydrogen peroxidase (HA) activities were determined by indophenol blue colorimetry, Na₂S₂O₃ titration and KMnO₄ titration, respectively (Parthasarathi and Ranganathan 2000).

Grain yield and water use efficiency. After the wheat and summer maize was mature, 1 m² wheat grains were collected with three replicates. Six rows of maize were grown in each plot, 5 m double rows maize were harvested from the centre of each plot.
in triplicate; the samples were air dried and threshed and then weighed to calculate the yield.

According to the definition of Jones (2004), water use efficiency (WUE) of crops has been defined as the ratio of harvested yield or aboveground biomass or total biomass to plant transpiration or evapotranspiration or total available water. WUE was calculated as the percent of grain yield divided by the quantity of evapotranspiration (ET) during the growing season (Hussain and Al-Jaloud 1995) using the following equation (Huang et al. 2005):

\[ \text{ET} = P + C + (SW_1 - SW_2) - D - R \]

\[ \text{WUE} = \text{yield/ET} \]

Where: ET – evapotranspiration (mm); P – effective precipitation during the growing season (mm); C – upward flow into the root zone; SW1 – soil water content at the time the crop was sown (mm); SW2 – soil water content at the time the crop was harvested (mm); D – downward drainage from the root zone, and R – surface runoff. In this study area, the ground was flat and the visual surface runoff can be regarded as zero. The groundwater depth was below 4 m; the quantity of groundwater recharge can therefore be regarded as negligible. The depth of infiltration was not greater than 2 m; thus, the depth of the leakage can be regarded as zero. Therefore, the terms C, D and R can be ignored.

**Statistical analysis.** Statistical analyses were conducted using SPSS 18 (Chicago, USA) and Sigmaplot 10.0 (Chicago, USA). Significant differences between treatments were assessed using the Duncan’s test at \( P < 0.05 \). Principal component analysis (PCA) was carried out using Canoco 4.5 (Ithaca, USA) to determine the relationships between TN, TOC, MBN, MBC and enzyme activities. The equivalent ratio values of nine indicators are standardized. The values of CK treatment are considered as 1. A spider plot was created in the Microsoft Office Excel (Redmond, USA).

**RESULTS AND DISCUSSION**

**Soil TN, TOC, MBN and MBC.** M treatment was taken as the standard value of 1, and each index was analyzed (Table 2). In the 0–20 cm soil layer, compared with CK, S and B, the TN equivalent ratio in the M treatment increased by 25, 23 and 20%, the MBN increased by 32, 22 and 14%, and the MBC increased by 57, 14 and 5%, respectively. Yet, the TOC of the S treatment was the highest. This indicates straw mulch is an effective way for straw disposal that can provide more SOC (Soon and Lupwayi 2012). Compared with the 0–20 cm layer, organic matter application had little effect on the soil TN, TOC, MBN and MBC contents in the 20–40 cm and 40–60 cm soil layers.

Biogas slurry is a water-soluble, quick-acting fertilizer, and fertilizer efficiency results in the rapid release of carbon. Weiland (2000) suggested that the product of biogas fermentation is a valuable resource for plant nutrients and can be recycled as a fertilizer. However, the results of this experiment showed that biogas slurry recycling did not show a significant effect on soil fertility, relative to the S and M treatments. A portion of the carbon and nitrogen contained in manure can be released gradually during the crop growth stage for...
crop absorption, while the remainder is transformed into stable carbon and nitrogen, which promotes the formation of soil organic-inorganic complexes and micro-aggregates and thus increases the soil nitrogen content (Jannoura et al. 2014). In this experiment, the sources of soil carbon and nitrogen were broadened, and the loss of carbon and nitrogen was avoided. Although the overall effect of M treatment was the best, some indicators such as TN, TOC and MBN show that the overall effect of S treatment was higher. The assimilation of carbon and nitrogen in the straw and the improvement of the straw returning amount were conducive to an increase in MBN and MBC.

**Soil enzyme activities.** Urease activity decreased with increasing soil depth and the invertase activity first decreased and then increased (Table 2). In the 0–20 cm soil layer, the M treatment showed the greatest urease and invertase activities, compared with CK, S and B; the UA equivalent ratio in the M treatment increased by 52, 12 and 21%, and the IA equivalent ratio increased by 21, 20 and 26%, respectively, while the IA equivalent ratio in the B treatment was decreased by 5% compared with that in the CK treatment. In the 20–40 cm soil layer, the urease activity in the S treatment was significantly lower than in the other treatments ($P < 0.05$). In the 40–60 cm soil layer, the B treatment had the lowest IA, the HA equivalent ratio was significantly higher in the M treatment than that in the other treatments ($P < 0.05$). In the 40–60 cm soil layer, however, the M treatment showed the lowest levels of HA.

Straw, biogas slurry and cow manure themselves contain a certain quantity of enzymes, which enhance soil enzyme activity. The input of organic materials increases the level of soil organic matter, thereby increasing the protective environment for soil enzymes (Martens et al. 1992), while simultaneously increasing the carbon source for soil microbes, promoting microbial reproduction, and stimulating enzyme activity (Gianfreda and Ruggiero 2006). This process indicates that there is an important link between soil nutrient, microbial biomass and enzyme activity.

**Crop grain yield and WUE.** In order to reduce the environmental pressure and the amount of fertilizer and improve agricultural sustainability, cow manure and biogas slurry can be recycled in farmland (Liu et al. 2017). Other studies have shown that animal manure can be used as fertilizer to reduce environmental problems and improve crop yield (Cerutti et al. 2011). From 2012–2015, the average wheat grain yield of the CK, S and B treatments was significantly ($P < 0.05$) lower than that in the M treatment, i.e., by 13.22, 5.46 and 7.13%, respectively (Figure 1). The average maize grain yield of the CK, S and B

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**Table 2. Effects of incorporated organic materials on soil properties, microbial biomass and enzyme activities (2012–2015)**

<table>
<thead>
<tr>
<th>Soil layer (cm)</th>
<th>Treatment</th>
<th>TN</th>
<th>TOC</th>
<th>MBN</th>
<th>MBC</th>
<th>UA</th>
<th>IA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20</td>
<td>CK</td>
<td>0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.68&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.77&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.88&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>20–40</td>
<td>CK</td>
<td>1.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.17&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.42&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>S</td>
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<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>40–60</td>
<td>CK</td>
<td>0.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.53&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>B</td>
<td>0.96&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.41&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters in each column indicate significant differences between different fertilizer applications ($P < 0.05$; Duncan’s test). M treatment was expressed as 1 and CK, S, B as a percentage of the M treatment. CK – conventional fertilization; S – straw, B – biogas slurry, M – manure incorporation; TN – total nitrogen; TOC – total organic carbon; MBN – microbial biomass nitrogen; MBC – microbial biomass carbon; UA – urease activity; IA – invertase activity; HA – hydrogen peroxidase activity.
The average WUE of the CK, S and B treatments was significantly ($P < 0.05$) lower than that in the M treatment by 10.04, 4.31 and 6.15%, respectively, during the wheat season and by 24.68, 2.80 and 12.57%, respectively, during the maize season (Figure 1). The study has also shown that the use of animal manure can improve crop yields and WUE (Wang et al. 2017). Overall, the average annual WUE of the CK, S and B treatments was significantly ($P < 0.05$) lower than that in the M treatment, i.e., by 19.83, 3.30 and 10.44%, respectively. The results of the present study also showed that regardless of the yield and WUE of a single crop or the annual yield and WUE, the overall sequence of the treatments was M > S > B > CK; the M treatment had the highest yield and WUE.

Zhang et al. (2015) reported positive effects of straw incorporation on crop yields and soil nutrients. Straw incorporation can significantly improve crop yield.

Figure 1. Crop grain yields (left) and water use efficiency (WUE) (right) of wheat and maize with different straw incorporation treatments during the period 2012–2015. Different letters in the same year indicate significant differences between different fertilizer applications ($P < 0.05$; Duncan’s test). CK – conventional fertilization; S – straw, B – biogas slurry, M – manure incorporation.
and WUE. Therefore, in most cases, straw returning was the best alternative to cow manure.

Principal component analysis. In the three soil layers, there was a clear separation among CK, S, B and M treatments by PC1 and PC2 (Figure 2). In the 0–20 cm soil layer, PC1 and PC2 explained 60.48% and 27.24% of the total variation, respectively. Four treatments were dispersed in the principal component diagram and the cow manure treatment handled the maximum projection on the positive direction of the x axis. IA, HA, TN, MBC and MBN had a positive effect on cow ma-

Figure 2. Principal component analysis (PCA) of the fertilization methods (left) and the loading values (right) of soil total nitrogen (TN), total organic carbon (TOC), microbial biomass and enzyme activities for different incorporation treatments and three soil layers. The horizontal and vertical bars were standard deviation of each treatment of the PC1 and PC2, respectively. CK – conventional fertilization; S – straw, B – biogas slurry, M – manure incorporation; MBN – microbial biomass nitrogen; MBC – microbial biomass carbon; UA – urease activity; IA – invertase activity; HA – hydrogen peroxidase activity
nure treatment, and made them differentiate from other treatments. The loading values of the soil properties and enzyme activities showed that PC1 was closely related to MBC, MBN, TN, hydrogen peroxidase and invertase and that PC2 was closely related to TOC and urease activity (Figure 2a,b). In the 20–40 cm soil layer, PC1 and PC2 explained 58.87% and 27.06% of the total variation, respectively. The cow manure treatment was distributed in the first quadrant. Compared with other treatments, the projection in the connecting line between the environmental factors (MBC, MBN, IA, HA and UA) and the origin was closest to the arrow, so MBC, MBN, IA, HA and UA had a positive effect on cow manure treatment, while S treatment was the most affected by TOC. The loading values of the soil properties and enzyme activities showed that PC1 was closely related to hydrogen peroxidase, MBN, MBC and invertase and that PC2 was closely related to TOC, urease and TN (Figure 2c,d). In the 40–60 cm soil layer, PC1 and PC2 explained 55.67% and 31.63% of the total variation, respectively. The difference between the cow manure treatment and the other three treatments was obvious. Compared with other treatments, the projection in the connecting line between the environmental factors (MBC, IA, HA, UA) and the origin was closest to the arrow; MBC, IA, HA and UA had thus a positive effect on cow manure treatment. Similarly, TOC and TN had a greater impact on CK. MBN had the greatest impact on S treatment. It eventually led to differences between the treatments. At this depth, PC1 was closely related to TOC, urease, invertase and MBC and PC2 was closely related to MBN, hydrogen peroxidase and TN (Figure 2e,f).

**Spider plot of equivalent ratios.** In general, the equivalent ratio values for the M treatment were higher than for other treatments (Figure 3). However, the equivalent ratio for TOC in the S treatment was higher than in the other treatments. The average equivalent ratio values of the M treatment were 1.07-, 1.15- and 1.32-fold higher than those of the S, B and CK treatments during 2012–2015, respectively.

In conclusion, taken together, compared with the other treatments, the manure incorporation treatment was the most effective in improving the soil fertility, microbial biomass, enzyme activities and crop grain yield, with the exception of the organic carbon content; it was followed by straw, biogas slurry and mineral fertilizer. Straw returning was the best substitute for cow manure. The three types of organic material represented different ways to reuse straw waste. Their reuse can prevent a considerable waste of resources, reduce environmental pressure, and provide great ecological benefits; therefore, the use of such cycles in agriculture should be vigorously developed to contribute to agricultural sustainable development.

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Received on July 12, 2018
Accepted on October 18, 2018
Published online on November 2, 2018