Effects of 15 years of manure and mineral fertilizers on enzyme activities in particle-size fractions in a North China Plain soil

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Abstract
Soil organic matter (SOM) and enzymes are essential for nutrient cycling, and are considered as important indicators of soil quality. The effects of organic and mineral fertilization on soil organic carbon (SOC), total nitrogen (TN) and enzyme activities in bulk soil and particle-size fractions were investigated under a winter wheat/maize cropping system in the North China Plain. The experiment established in 1993 includes three treatments: (1) unfertilized control (CK); (2) mineral fertilizers (MF); and (3) farmyard manure (FYM). Application of FYM significantly increased SOC and TN contents and activities of six enzymes: invertase, β-glucosidase, urease, acid and alkaline phosphatases and dehydrogenase in bulk soil and in all particle-size fractions as compared to those in MF and CK. Highest contents of SOC and TN were found in coarse sand and lowest in the silt fraction. The C/N ratios decreased with decreasing particle-size fractions. β-Glucosidase and acid phosphatase activities predominated in coarse sand fraction, reflecting high substrate availability. The urease activity was highest in clay-size fractions, depending on mineral sorption processes. The SOM and enzyme activities in the coarse sand were the most sensitive to fertilization. The smallest response of SOM in the clay fraction to fertilization confirmed that SOM on clay is the most stable C pool. The 15-year fertilization experiment clearly showed that FYM represented the best management practice for improving soil quality and microbial activity. © 2013 Published by Elsevier Masson SAS.

1. Introduction
Soil organic matter (SOM) is considered as an important indicator of soil fertility and productivity because it determines physical, chemical and biological soil properties [1]. However, SOM losses or gains are difficult to measure directly in a short time because of the generally high background stocks, their very small annual changes and natural variability [2]. In contrast, the more dynamic characteristics such as enzyme activities respond much more quickly to changes in agricultural management practices or environmental stresses than total SOM [3–5]. Enzyme activities are crucial for biological and biochemical processes such as organic matter degradation, litter mineralization and recycling of such macronutrients as N, P, S and some microelements [6,7]. Furthermore, enzyme activities have been suggested as potential indicators of soil quality because they are sensitive, rapid, inexpensive and representative of the potential metabolic capacity of the soil [8]. The effect of fertilizer amendments on the activity of various enzymes has been intensively studied in recent years [9–11]. Farmyard manure and mineral fertilizers have been reported to have both positive and negative effects on soil enzymes [3,9,12,13]. Such inconsistencies reflect various factors such as the source and rate of fertilizers and site-specifics.

Particle-size fractionation is a valuable tool to study the forms and cycling of SOM [14]. Different forms of SOM might have contrast effects on soil quality and might respond differently to crop management. Although C and N turnover in particle-size fractions has attracted much attention, this is not the case for enzyme activities. The distribution of enzyme activities in particle-size fractions depends largely on the enzyme investigated. For example, invertase, acid and alkaline phosphatase, urease, and arylsulphatase were mainly located in the silt and clay fractions [9,15–17]. β-Glucosidase, α-glucosidase, cellobiohydrolase and β-xyllosidase were predominated in the sand fraction [16].

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Particularly scarce are the studies of long-term effects of fertilization on the distribution of enzymes in particle size fractions [9,18]. How and to what extent fertilization modify the enzymes in particle size fractions are still in debt. Long-term field experiments are the primary source of information to determine the effects of crop management on soil productivity. In the North China Plain (China Agricultural University’s Quzhou agricultural experimental station), a long-term fertilizer experiment was started in 1993. Effects of fertilization systems on crop growth and yields, soil labile C fractions, nitrogen mineralization, abundance and diversity of soil mite were investigated in detail [19–22]. However, the changes of biological soil properties, especially of enzymes remain unknown, despite these parameters reflect nutrient recycling. Therefore, the objectives of this study were to determine the effects of farmyard manure (FYM) and mineral fertilizers (MF) over 15 years on soil organic carbon (SOC) and total N (TN) contents, and enzyme activities involved in C-, N-, P-, and S cycling: invertase, β-glucosidase, urease, alkaline phosphatase, acid phosphatase, arylsulfatase and dehydrogenase in bulk soil and particle-size fractions. We hypothesized that 15-year application of FYM and MF would have distinct effects on SOM and enzymes in bulk soil and particle-size fractions. The results will help to select the best fertilization management practice for maintaining and improving soil health and quality under the most common cropping system in the North China Plain: winter wheat/maize.

2. Materials and methods

2.1. Site description and soil sampling

A long-term fertilizer experiment has started in 1993 at China Agricultural University’s Quzhou agricultural experimental station (36°52’N, 115°01’E, 40 m a.s.l.) in Hebei province, Northern China. The experimental station has a continental temperate monsoon climate. The mean temperature and annual precipitation are 13.1 °C and 556 mm, respectively. The soil is classified as a Eutric Cambisol with silty loam texture[20]. The prevailing cropping system in this region is winter wheat (middle October to early June) and maize (early June to late September). Three treatments were selected for the study: (i) unfertilized control (CK); (ii) mineral fertilizers (MF: 362 kg N ha⁻¹ and 272 kg P ha⁻¹ for each crop); (iii) farmyard manure (FYM: 15 t ha⁻¹ for each crop). Each treatment was replicated three times (plot size 10.5 × 3 m²) in a randomized block design. The chemical N and P fertilizers used were CO(NH₂)₂, NH₄HCO₃ and Ca(H₂PO₄)₂·H₂O. The FYM contained 60% straw (wheat or maize straw), 30% live-stock dung, and 10% cottonseed-press mud. On average, it contained 228 g C kg⁻¹ and 6.7 g total N kg⁻¹ [20]. The entire doses of mineral fertilizers and manure were homogeneously surface broadcast by hand right before the wheat and maize sowing and immediately incorporated by a rotary cultivator to a depth of 18 cm. Three irrigation events for wheat (total 240 mm water) and two for maize (total 160 mm water) were applied annually, depending on precipitation. After harvest all the aboveground residues were pulverized and incorporated into soil in MF and FYM, while they were removed from CK.

Soil samples were collected at the depths of 0–10 and 10–20 cm from each plot with a 10 cm diameter soil corer after the maize harvest in October 2008. At each plot four soil cores were taken to make a composite sample representative of each plot and depth. Field-moist samples were passed through a 2 mm sieve and divided into two parts. One part was frozen at −20 °C for enzyme analysis. The remaining soil was stored at 4 °C for soil particle-size fractionation. Soil pH, SOC, TN and C/N are presented in Table 1 [22].

<table>
<thead>
<tr>
<th>Fertilization</th>
<th>pH(C₄H₄O₆, 1:2.5)</th>
<th>SOC (g kg⁻¹)</th>
<th>TN (g kg⁻¹)</th>
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<tbody>
<tr>
<td>0–10 cm</td>
<td></td>
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<tr>
<td>CK</td>
<td>7.63 (0.06) a</td>
<td>103.3 (1.02) b</td>
<td>1.46 (0.15) b</td>
<td>7.09 (0.01) b</td>
</tr>
<tr>
<td>MF</td>
<td>7.49 (0.06) ab</td>
<td>114.7 (0.33) ab</td>
<td>1.64 (0.02) b</td>
<td>7.01 (0.16) b</td>
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<tr>
<td>FYM</td>
<td>7.43 (0.10) b</td>
<td>16.14 (1.32) a</td>
<td>2.10 (0.19) a</td>
<td>7.71 (0.05) a</td>
</tr>
<tr>
<td>10–20 cm</td>
<td></td>
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</tr>
<tr>
<td>CK</td>
<td>7.75 (0.02) a</td>
<td>7.80 (0.38) b</td>
<td>1.18 (0.02) b</td>
<td>6.59 (0.34) b</td>
</tr>
<tr>
<td>MF</td>
<td>7.73 (0.04) a</td>
<td>7.75 (0.94) b</td>
<td>1.17 (0.12) b</td>
<td>6.63 (0.20) b</td>
</tr>
<tr>
<td>FYM</td>
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Values are means with the standard deviation in parenthesis (n = 3). Values within a column followed by different lowercase letters are significantly different (P < 0.05).

2.2. Soil particle-size fractionation

The soil-particle size fractionation procedure was based on the method developed by Stemmer et al. [23]. Field-moist soil samples were dispersed in distilled water using low-energy sonication [50 J s⁻¹ output energy for 120 s] and then fractionated by a combination of wet-sieving and repeated centrifuging. The coarse sand (2000–250 μm) and fine sand (250–53 μm) fractions were separated by manual wet sieving. The silt-sized particles (53–2 μm) were separated by centrifuging the remaining suspension at 150 g for 2 min. The pellets were re-suspended and centrifuged under the same conditions three-times to purify the silt fraction. The remaining supernatants were centrifuged at 3900 g for 30 min to yield the clay-sized particles (<2 μm). Samples for SOC measurements were pretreated with 0.5 M HCl to remove carbonate [24] and then ball-milled. SOC and TN contents in particle-size fractions were determined by dry combustion method using an elemental analyzer (Vario Macro CNS, Elementar, Germany).

2.3. Enzyme assays

The activities of acid (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1), β-glucosidase (EC 3.2.1.21) and arylsulfatase (EC 3.1.6.1) were assayed on the basis of p-nitrophenol (pNP) release following the method described by Tabatabai [25]. In brief, a sample of 1 g fresh soil was placed in a 50 ml Erlenmeyer flask with 0.2 ml of toluene, 4 ml of buffer solution (modified universal buffer (MUB) at pH 6.5 for acid phosphatase, pH 11.0 for alkaline phosphatase, pH 6.0 for β-glucosidase, and acetate buffer at pH 5.8 for arylsulfatase) and 1 ml of desired substrate (p-nitrophenyl phosphate for phosphatase, p-nitrophenyl-β-D-glucopyranoside for β-glucosidase and p-nitrophenyl sul fate for arylsulfatase). The samples were mixed on a vortex and incubated at 37 °C for 1 h. Then, reactions were stopped by the addition of 1 ml of 0.5 M CaCl₂. The released pNP was extracted with 4 ml of 0.5 M NaOH (for phosphatase and arylsulfatase) or 4 ml of 0.1 M tris-hydroxymethylaminomethane (THAM, pH 12.0, for β-glucosidase). The suspensions were swirled for a few seconds, and filtered. Absorbance at 400 nm was determined against the reagent blank and pNP content was calculated by referring to a calibration curve.

Urease (EC 3.5.1.5) activities in soils were assayed by the method of Tabatabai [25]. A sample of 5 g fresh soil was placed in a 50 ml volumetric flask with 0.2 ml of toluene, 9 ml of THAM buffer (pH 9.0) and 1 ml of 0.5 M urea solution. The samples were mixed on a vortex and incubated at 37 °C for 2 h. Then 35 ml of KCl–Ag₂SO₄ solution was added, swirled for a few seconds, and cooled to room temperature. The NH₄⁺ in the soil suspension was determined by steam distillation of 20 ml aliquot with 0.2 g MgO for 4 min.

Table 1

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Controls were performed in each series of analyses to allow for NH4—N not derived from urea through urease activity.

Dehydrogenase (EC 1.1.1.1) activities in soils were assayed by the method of Tabatabai [25]. A sample of 6 g fresh soil and 60 mg CaCO3 were mixed thoroughly and then were transferred into each of three test tubes. To each tube with stopper, 1 ml of 3% 2,3,5-triphenyltetrazolium chloride (TTC) and 2.5 ml of distilled water were added. The samples were mixed on a vortex and incubated at 37 °C for 24 h. The trephosphorylformanzen (TPF), a product from the reduction of TTC, was extracted by adding 10 ml of methanol and shaking for 1 min. The suspension was filtered into a 100 ml volumetric flask through a glass funnel plugged with absorbent cotton. The tube and cotton were washed with methanol until the red color disappeared. The filtrate was then diluted to 100 ml with methanol. The color intensity was measured at 485 nm with methanol as a blank.

Invertase (EC 3.2.1.26) activities in soils were assayed by the method of Guan [26]. A sample of 5 g air-dried soil was placed in a 50 ml Erlenmeyer flask with 0.1 ml of toluene, 5 ml of phosphate buffer (pH 5.5) and 15 ml of 8% sucrose. The samples were mixed on a vortex and incubated at 37 °C for 24 h. The glucose released by invertase was then reacted with 3,5-dinitrosalicylic acid (DNS) and was measured by colorimetric method at 508 nm.

2.4. Statistical analyses

One-way ANOVA was used to identify differences of particle-size fraction weights, SOC and TN contents and enzyme activities in bulk soil and in each particle-size fraction among the fertilization treatments. The differences were considered significant at P < 0.05. Pearson linear correlation was used to evaluate the relationships between the parameters. All statistical analysis was performed with SPSS for windows, version 11.0.

3. Results

3.1. Enzyme activities in bulk soil

The activities of invertase, β-glucosidase, urease, acid phosphatase and dehydrogenase in bulk soil were significantly higher in the FYM treatment than those in MF or CK in both 0–10 and 10–20 cm depths (Fig. 1). FYM also had significantly higher alkaline phosphatase activity than those in MF or CK at 0–10 cm, but not at 10–20 cm (Fig. 1). In both 0–10 and 10–20 cm depths, there were no significant differences between MF and CK in invertase, alkaline phosphatase, acid phosphatase and dehydrogenase activities. However, MF significantly increased urease activity compared to CK in both soil layers (Fig. 1). No significant difference was detected in arylsulfatase activity among the three fertilization treatments in the top 20 cm (Fig. 1). Activities of all enzymes were higher in surface soil (0–10 cm) as compared to subsurface soil (10–20 cm).

3.2. Soil organic C and total N in particle-size fractions

Particle-size fractionation was processed for the 0–10 cm layer. The soil weight recovery by the fractionation procedure was about 97–100% (Table 2). The content of coarse sand (2000–250 μm) was very low (around 2% of the soil weight); Silt (53–2 μm) represented the greatest portions, accounting for about 80% of whole soil. As expected, fertilizer additions did not affect the particle-size distribution (Table 2).

Contents of SOC and TN were highest in coarse sand, and lowest in the silt fraction (Fig. 2). Application of FYM significantly increased SOC and TN contents in all of the four particle-size fractions as compared to those in MF and CK. The greatest FYM effect was observed in coarse sand, with an increase of 97% for SOC and 128% for TN as compared to CK, respectively (Fig. 2). No differences were detected in SOC and TN contents between MF and CK in all particle-size fractions, except for SOC content in silt, which was significantly higher in MF treatment than that in CK (Fig. 2).

The most parts of SOC and TN were located in the silt-sized (34–40% for SOC, 38–44% for TN) and clay-sized (24–34% for SOC, 32–40% for TN) fractions (Fig. 3). FYM had significantly higher SOC and TN amounts than those in either MF or CK in all particle-size fractions. MF only had significantly higher SOC amount than CK in the silt fraction. There was no difference in TN amount between MF and CK (Fig. 3). The C/N ratio in coarse sand was significantly higher than that in fine sand, and both of which were significantly higher than those in silt-sized and clay-sized fractions (Fig. 4).

3.3. Enzyme activities in particle-size fractions

The urease activity was highest in clay followed by coarse sand, and lowest in silt-sized fraction (Fig. 5). Both β-glucosidase and acid phosphatase showed the highest activities in coarse sand, and lowest in silt-sized fraction (Fig. 5). The activities of all three enzymes were significantly higher in the FYM treatment compared to those in CK in all particle-size fractions. These effects were especially strong in the coarse sand fraction, where FYM increased β-glucosidase, urease and acid phosphatase activities by 138, 141 and 70% compared to those in CK, respectively (Fig. 5). MF significantly increased urease activity in fine sand, silt-sized and clay-sized fractions, and β-glucosidase activity in coarse sand, as compared to those in CK (Fig. 5). However, MF showed no effect on acid phosphatase activity in all particle-size fractions as compared to CK (Fig. 5).

4. Discussion

4.1. Effects of fertilization on enzyme activities in bulk soil

With respect to the C cycle related enzymes, invertase and β-glucosidase play critical role in releasing low molecular weight sugars that are important as energy sources for microorganisms [8,27]. Urease catalyzes the hydrolysis of urea to carbon dioxide and ammonia, and plays an important role in the N cycling [28]. Phosphatase plays an essential role in the mineralization of organic P. Dehydrogenase is only present in viable cells, and it is thought to reflect the total range of oxidative activity of soil microflora [29]. The increase in invertase, β-glucosidase, urease, phosphatase and dehydrogenase with application of FYM compared to MF and CK (Fig. 1E and F) is attributed to higher content of organic matter and enhanced microbial activity. The positive effect of FYM addition on enzyme activities has been demonstrated in several studies [9,30–32]. Our results suggest that application of FYM enhances soil quality by improving the ability of soils to perform nutrient cycling and transformation (as reflected by hydrolytic enzyme activities). The absence of effects of mineral fertilizers on invertase, β-glucosidase, phosphatase and dehydrogenase is indirectly connected with the absence of SOC and TN increase compared to control (Table 1). Similar results were also found by Sahar et al. [10,11], Liu et al. [31], and Mandal et al. [29]. In contrast, Böhm and Böhm [32] reported that addition of MF increased β-glucosidase activity as a result of the improved nutrient condition and enhanced root biomass by fertilizers.

Our result showed that MF caused a significant increase in urease activity in the top 20 cm compared to CK (Fig. 1C). This finding is in contrast to the results of Dick et al. [12] and Bandick and Dick [8], who reported that urease activity was decreased with application of inorganic N. They hypothesized that the addition of
the end product of the enzymatic reaction (NH$_4^+$) could suppress enzyme synthesis [10]. The positive effect of MF on urease activity in our study is because the MF treatment contained not only ammonia-based N fertilizer (ammonium bicarbonate) but also urea as N source. In a 10-year fertilizer experiment in India, Saha et al. [11] also observed that urease activity was significantly higher in the plots received urea as N fertilizer compared to that in unfertilized treatment.

Arylsulfatase is important in nutrient cycling because it releases plant available SO$_4^{2-}$ [8]. Knauff et al. [33] reported that arylsulfatase activity was correlated with the amount of organic carbon and can be increased by long-term amendment of organic manure. However, the activity of arylsulfatase in the 0–20 cm depth was not sensitive to fertilization present (Fig. 1D). Mijangos et al. [34] also found that arylsulfatase activity did not respond to the addition of organic and mineral fertilizers in a clay loam in northern Spain.

The Pearson correlation matrix revealed that all the selected enzyme (except arylsulfatase) activities were positively correlated with SOC and TN contents in the 0–10 and 10–20 cm layers obtained after low-energy sonication.

<table>
<thead>
<tr>
<th>Fertilization</th>
<th>Particle-size distribution (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000–250 µm</td>
<td>250–53 µm</td>
</tr>
<tr>
<td>CK</td>
<td>1.58 (0.27)</td>
<td>6.20 (1.33)</td>
</tr>
<tr>
<td>MF</td>
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</tr>
<tr>
<td>FYM</td>
<td>1.70 (0.19)</td>
<td>7.50 (0.36)</td>
</tr>
</tbody>
</table>

Values are means with the standard deviation in parenthesis (n = 3). CK: unfertilized control; MF: mineral fertilizers; FYM: farmyard manure.
Table 3, indicating the SOM as major determinant. SOM not only provides substrates for enzymes, but also plays a vital role in protecting soil enzymes by forming complexes with clay and humus [10]. Invertase, β-glucosidase, urease, acid phosphatase and dehydrogenase activities were significantly and positively correlated with each other in the upper 20 cm (Table 3). These suggested that they were closely interrelated and could be potential indicators for the fertilization effects on soil quality.

4.2. Effects of fertilization on soil organic C and total N in particle-size fractions

The coarse sand fraction had much higher SOC and TN contents than the other fractions in all treatments, which was in accordance with those reported by Chen et al. [24] and Conant et al. [35]. On the contrary, increasing in SOC and TN contents with diminishing particle size has been reported by several authors [18,36,37]. Fertilization affected SOC and TN not only in bulk soil but also in particle-size fractions (Table 1, Figs. 2 and 3). Application of FYM over the 15 years significantly increased SOC and TN contents and amounts in all particle-size fractions (2000–250, 250–53, 53–2, and <2 µm) as compared to those in MF and CK (Figs. 2 and 3). The increase in SOM amount in particle-size fractions with addition of organic manure was supported by the studies of Kandeler et al. [9] in Germany and Gerzabek et al. [38,39] in central Sweden. The amounts of SOC and TN in particle-size fractions responded distinctly to fertilization. Compared to CK, the largest increase in SOC amount by FYM application occurred in the coarse sand fractions (123%), followed by fine sand (101%), silt (58%) and clay (18%). For TN amount, the magnitude of changes between FYM and CK also ranked in the order: coarse sand (159%) > fine sand (110%) > silt (48%) > clay (24%) (Fig. 3). These results indicated that
SOC and TN associated with sand fractions were more sensitive to fertilization management than those associated with silt-sized and clay-sized fractions. In the sand fractions, the SOC and TN are present as unprotected SOM pools and could be highly influenced by management practices [40,41]. The large increase of SOC and TN amounts in sand with FYM application might be attributed to the increased particulate organic matter derived from manure, since the organic material added to the soil was initially located in the coarser fractions [42]. On the contrary, the SOM in clay is absorbed and protected by clay particles and forms stabilized organo-mineral complexes [17]. The smallest response of SOM in the clay fraction to fertilization management confirmed that SOM on clay is the most stable.

The C/N ratios generally decreased with decreasing particle-size fractions (Fig. 4) [23,39]. The C/N ratio could reflect the degree of decomposition and humification of SOM. The high C/N ratios in coarse sand suggested that the SOM associated with this fraction is less decomposed material, which originated from organic matter input such as crop residues [38]. In comparison, the SOM complexed with clay particles is dominated by microbially derived metabolites and has a low C/N ratio, reflecting a high degree in humification of SOM [14,43].

4.3. Effects of fertilization on enzyme activities in particle-size fractions

The distribution of β-glucosidase, urease and acid phosphatase activities in particle-size fractions varied with enzyme assayed. β-Glucosidase activity generally decreased from coarser to finer size fraction and followed the distribution pattern of SOC content (Fig. 5). This is a clear indicator for a close relationship between β-glucosidase activity and organic C. Similarly, Allison and Jastrow [44] found that the coarse and fine POM had higher β-glucosidase activity than silt- and clay-sized fractions in a restored grassland soils in Batavia, USA. The high β-glucosidase activity in coarse sand is explained by the abundant polymeric material in this fraction [17]. The large amount of labile C in coarse sand could be easily used by microorganisms for growth and enzyme production, and subsequently induced an increase of enzyme activities [44]. Therefore, the high activity of β-glucosidase in coarse sand reflects the availability of its substrate and indicates a high C turnover rate in this fraction.

Urease activity was mainly located in the clay-sized fraction, which was in accordance to Kandeler et al. [9]. However, the pattern of urease activity did not completely match the distribution of SOC and TN contents in particle-size fractions (Figs. 2 and 5). This result suggested that the urease activity in the clay fraction depended on mineral sorption processes rather than on substrate availability [9,44].

Acid phosphatase activity was found highest in coarse sand (Fig. 5). This result was supported by Rojo et al. [45] who found that phosphatase activity was mainly associated with larger soil fractions (2000–100 μm) containing plant debris and less humified organic matter. Lagomarsino et al. [16] also found highest acid phosphatase activity in coarse sand of a Calcaric Gleyic Cambisol after 13 years’ tillage and fertilizer experiment in central Italy. However, contrasting result was reported by Marx et al. [17] who showed that the acid phosphatase activity was predominated in the clay-sized fractions under grassland in a silty clay loam in North Wyke, UK. These discrepancies suggested that the distribution of phosphatase may be influenced by various physical and chemical soil properties [15]. Continuous application of FYM significantly increased β-glucosidase, urease and acid phosphatase activities in all particle-size fractions compared to those in MF and CK treatment (Fig. 5), indicating an acceleration of C, N and P cycling by adding FYM in these fractions. Farmyard manure delivered large amount of organic materials into the soil. These increased the amount of substrates firstly in the sand fractions and subsequently in the finer fractions (silt and clay) for enzymes to act on, thereby stimulating the enzymes activities in these fractions. The effect of FYM on increasing enzyme activities in particle-size fractions was also found by Kandeler et al. [9], who reported that 95-year application of FYM increased xylanase and urease activities in all particle-size fractions as compared to those in CK in a Haplic Phaeocem in Germany. FYM addition increased xylanase activity in all particle-size fractions and promoted invertase activity in finer fractions compared to unfertilized control in a long-term field experiment since 1878 in northeast Germany [18]. These studies have documented an increase in enzymes in particle-size fractions with increase availability of organic substrates. In the present study, the most increased enzyme activities in the coarse sand fractions

![Fig. 5. β-Glucosidase, urease and acid phosphatase activities in particle-size fractions at the depth of 0–10 cm depending on different fertilization. Error bars represent standard deviation (n = 3). Values followed by different lowercase letters within particle-size fractions are significantly different between fertilization treatments (P < 0.05).](image-url)
are attributed to the concentrated substrates in this fraction by FYM application.

5. Conclusions

Continuous application of FYM during 15 years significantly increased SOC and TN contents and enzyme activities (except arylsulfatase) in the bulk soil and in all particle-size fractions as compared to those under MF and CK in a winter wheat/maize cropping system in the North China Plain. Application of MF alone did not lead to significant increase in SOM and enzyme (except urease) activities over those in CK. Soil organic matter and enzyme activities in the coarse sand fraction were more sensitive to fertilization than those in finer fractions. We concluded that long-term application of FYM is the best management option for improving soil quality and microbial functioning.

Acknowledgments

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