Tillage effects on phosphorus composition and phosphatase activities in soil aggregates

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A R T I C L E   I N F O

Article history:
Received 30 June 2012
Received in revised form 26 October 2013
Accepted 11 November 2013
Available online xxxx

Keywords:
Soil P composition
Phosphatase activities
31P NMR spectroscopy
Aggregate size fractions
Tillage

A B S T R A C T

Phosphorus (P) and phosphatase activities in soil aggregates affected by tillage under cold monsoon climate remain poorly understood. Based on the hypothesis that the distribution of P composition and phosphatase activities in soil aggregates should be affected by different tillage practices, a field experiment was conducted to study the effects of moldboard plow (MP), ridge tillage (RT), and no-tillage (NT) on the distribution of soil P composition determined by 31P nuclear magnetic resonance (NMR) and phosphatase activities in different size fractions of soil aggregates (>2, 1–2, 0.25–1, and <0.25 mm) at the 0 to 20 cm depth in northeastern China. NT treatment had significantly higher organic P proportion in total P and larger proportions of monomers and diesters in extracted total P than the MP treatment, whereas the MP treatment showed higher concentrations of total P, organic P, plant available P, NaOH-EDTA extracted total P, orthophosphate and monoesters. Soil alkaline phosphatase (AlP) and phosphodiesterase (PD) activities under NT were significantly higher than those under MP, and the responses of AlP in 0.25–1 mm size fraction and PD in <0.25 mm size fraction were more sensitive to tillage treatments. Overall, although NT facilitated more P stored in the organic P form and increased phosphatase activities, soil with NT had lower total and plant available P compared to traditional MP treatment and therefore, MP may be the right practice to conserve soil P under cold monsoon climate.

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1. Introduction

Tillage can affect the mineralization and decomposition of soil organic matter (SOM) by changing the physical and chemical properties of soils and altering the diversity and activity of the soil microbial community and enzymes, which in turn affects the concentration and composition of soil P (Redel et al., 2011; Selles et al., 1997; Wang et al., 2011). In temperate and tropical soils, numerous studies demonstrated that no-tillage (NT) increased the concentrations of total P, organic P, and plant available P compared with conventional tillage (CT) (López-Fando et al., 2007; Qin et al., 2010; Saavedra et al., 2007). In colder climates, the studies about the effect of tillage on soil P mostly concentrated on its runoff losses (Hansen et al., 2000; Tiessen et al., 2010).

Soil aggregates have been considered as the basic units of soil structure (Lynch and Bragg, 1985). Different aggregate size fractions have diverse effects on maintaining and supplying soil nutrients (Chen et al., 1994), and their stability as an indicator of vital soil functions can be used to assess soil quality (Seybold and Herrick, 2001). Previous studies have shown that tillage can speed microbial decomposition of fungal hyphae, roots, and other organic materials that bind microaggregates together to form macroaggregates (Tisdall and Oades, 1982) and can thereby affect the size distribution and stability of aggregates (Green et al., 2005; Helgason et al., 2010; Hernandez-Hernandez and Lopez-Hernandez, 2002). NT and ridge tillage (RT) soils exhibited higher amounts of >0.25 mm macroaggregates and greater aggregate stability compared to moldboard plow (MP) soils (Chung et al., 2008; Yang and Wander, 1998).

Moreover, aggregate size fractions can also influence the concentration of P by acting on sorption and desorption of soil P (Linquist et al., 1997; Wang et al., 2001). Therefore, the distribution of P in different aggregate size fractions can be influenced by tillage directly through its effects on sorption and desorption of soil P by aggregates. The various P forms in soil aggregates affected by tillage, including total P, extractable P, and bound P, were extensively studied (Elliott, 1986; He et al., 1995; Maguire et al., 1998; Messiga et al., 2011; Urioste et al., 2006; Wright, 2009). In addition, the organic carbon and microbial biomass in soil aggregates affected by tillage were also investigated by some researchers (Liang et al., 2007; Zhang et al., 2012). However, the information about using 31P nuclear magnetic resonance (NMR) spectroscopy technique to study the effect of tillage on soil P composition in aggregates is still rare. The different P compounds in soil had variable abilities to provide P nutrient to crops (Dolette and Smernik, 2011), and the P compounds in soil could be identified successfully with 31P NMR spectroscopy when the soils were extracted with a NaOH-EDTA solution (Cade-Menun and Preston, 1996; Condron et al., 1990; Redel et al., 2011).
Soil phosphatases such as phosphomonoesterase (AcP and AIP), phosphodiesterase (PD) and inorganic pyrophosphatase (IPP) play critical roles in organic and condensed P hydrolysis and the plant available P supply to crops (Fox and Comerford, 1992; Tabatabai, 1994; Tarafdar and Jungk, 1987). Previous studies showed that the NT treatment increased phosphatase activities compared with the CT treatment in the surface soil (Deng and Tabatabai, 1997; Dick, 1984; Wang et al., 2011). The distribution of microorganisms (e.g., fungi, bacteria) differs among different soil aggregate size fractions (Gupta and Germida, 1988), and phosphatases may totally or partly derive from microorganisms (Tabatabai, 1994; Turner and Haygarth, 2005). Therefore, the activities of phosphatases among different aggregate size fractions can also be affected by tillage (Qin et al., 2010). Gupta and Germida (1988) showed that macroaggregates contained higher acid phosphatase activity than the corresponding microaggregates in both native and cultivated soils after 69 years of cultivation. Although the effect of tillage on phosphatase activities in aggregates was studied, previous researches mainly involved individual phosphatase (e.g., phosphomonoesterase) (Gupta and Germida, 1988; Kandeler et al., 1999; Marx et al., 2005; Mendes et al., 2003). Little is known about the effect of tillage on phosphodiesterase and inorganic pyrophosphatase activities in aggregates.

In northeastern China agroecosystems, MP has been the common practice for many years. Intensive tillage management without a cover of crop residues has caused a significant loss of SOM and serious soil degradation, and has threatened sustainable crop production and even national food security (Liu et al., 2010). To effectively stop and reverse the adverse trend, NT and RT practices have been proposed as an alternative agriculture option. In this study site, previous research has mainly focused on the effect of tillage on soil SOC in aggregates (Liang et al., 2011; Zhang et al., 2013). There have been few studies about the effects of various tillage practices on soil P in aggregates. Thus, the objectives of this study were to determine the effects of moldboard plow (MP), ridge tillage (RT), and no-tillage (NT) on (a) the distribution of P as well as P composition in aggregates and (b) the relative phosphatase activities in different aggregate size fractions at the 0 to 20 cm depth in northeastern China.

2. Materials and methods

2.1. Site description

The tillage experiment was initiated in fall 2001 at the experimental station (44°12’N, 125°33’E) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. The station is located in Daxing County, Jilin Province in the northeastern part of China, which has a continental monsoon climate. The mean annual temperature was 4.4 °C, and the mean annual precipitation varied from 500 to 600 mm with most of it occurring in June, July, and August. The type of soil used in this study was a clay loam soil, and it is classified as a phaeozem (FAO, 1998). The initial soil in this study site was slightly acidic with average pH of 6.5 (Liang et al., 2007) and has decreased to 5.7, 5.5, and 5.6 in MP, RT, and NT soils, respectively. Before the establishment of this tillage experiment, the field had undergone more than ten years of conventional tillage management for continuous maize cultivation (Liang et al., 2007).

The tillage experiment consisted of moldboard plow (MP), ridge tillage (RT), and no-tillage (NT) and was arranged in a randomized complete block design with four replicates. Each tillage plot was 5.2 m × 20 m and had a maize-soybean rotation with both crops present in each year. The MP treatment included one fall moldboard plowing (approximately 20 cm deep) after the crop harvest and spring disking (7.5 to 10 cm deep) before planting. The RT treatment included ridging in June for maize and soybean, chopping the crop stalk/roots in the fall (approximately 1/3 row width) and spring planting with a no-till planter. The NT treatment did not disturb the soil except for planting. All the treatments used a KINZE-3000 NT planter (Williamsburg, Iowa, USA) for the spring planting. Except for the plots under the MP treatment, all crop residues were retained on the soil surface directly after harvesting. Each year, 100 kg N ha⁻¹, 45.5 kg P ha⁻¹, and 78 kg K ha⁻¹ were applied to maize as basal fertilizer. An additional 50 kg N ha⁻¹ was applied as a top dressing at the V-6 stage (40 days after planting). For soybean, all fertilizers were applied as basal fertilizer, including 40 kg N ha⁻¹, 60 kg P ha⁻¹, and 80 kg K ha⁻¹. The basal fertilizers were applied simultaneously during the planting using the banding attachment on the KINZE-3000 NT planter (Williamsburg, Iowa, USA).

2.2. Soil sampling

Soil sampling was carried out in April 2010 after the snow melted. In the central rows of each tillage plot, three undisturbed soil samples were random taken from 0 to 20 cm (≈2000 cm³) after the soil floor materials were removed. Then, the undisturbed soil samples were placed in hard plastic containers to maintain their primary structures. After transportation to the laboratory, the three undisturbed soil samples were composited for soil aggregate fractionation.

To acquire the aggregate-size fractions, the soils were sieved according to the methods described by Schutter and Dick (2002) and Sainju et al. (2003). After sampling, large soil clods were gently broken by hand, and then soils were laid out on brown paper to dry slowly for several days. This process was conducted at 4 °C to minimize the impact of air drying on the microbial communities and activities (Schutter and Dick, 2002) until a gravimetric water content of approximately 80 g kg⁻¹ soil was reached so that dry sieving method could be effectively implemented at this moisture level. The soil was fractionated into aggregates by a dry-sieving method because dry-sieving the soil would disrupt the physical habitat of microbial communities to lesser degrees than wet-sieving would (Schutter and Dick, 2002). Before sieving, visible plant residues were removed, and then, cold air-dried soils were passed through a 5-mm sieve, and large particles retained in the sieve were gently crushed by hand to pass through it. The particles that did not pass through the 5-mm sieve contained mostly stone and plant fragments and were discarded (Sainju et al., 2003). Fractionation was achieved by placing 100 g of cold air-dried, sieved soils (<5 mm) on nested sieves mounted on Retsch AS200 Control (Retsch Technology, Düsseldorf, Germany). The sieves were mechanically shaken (amplitude 1.5 mm) for 2 min to separate the soil into the following aggregate-size classes: >2 (large macroaggregates), 1–2, 0.25–1 (small macroaggregates), and <0.25 mm (microaggregates, silt + clay size fraction). Preliminary experiments showed that shaking for 2 min at 1.5 mm amplitude was adequate for separating the soil aggregates without causing the mechanical destruction of large aggregates (data not shown).

The fractionated samples were later combined to make composite samples for each aggregate-size class. The aggregate distribution was determined by weighing soil from each aggregate size class. The bulk soil and all the soil aggregate samples were stored in polyethylene bags at 4 °C until they were analyzed for their chemical and biological properties.

2.3. Soil P analysis

With the exception of samples for total P analysis, which required the samples to go through a 100-mesh (0.15 mm) sieve, the bulk soil and all the aggregate samples were air-dried, sieved (<2 mm), and stored at ambient laboratory temperature before other P analysis. The plant available P was determined by the molybdenum blue colorimetric method (Murphy and Riley, 1962) after extraction by 0.5 M NaHCO₃ (Olsen et al., 1954). The total P was determined by the same method following perchloric acid (HClO₄) digestion (Kuo, 1996). The inorganic P was extracted with 0.5 M H₂SO₄ (1:25 soil-to-solution ratio for 16 h) and measured by the method of Kuo (1996). The organic P was
calculated as the difference between total P and inorganic P (Gupta and Germida, 1988).

2.4. Phosphatase activity assays

The field-moist samples of bulk soil and all the aggregates were analyzed for phosphatase activities. The activities of alkaline phosphatase (AlP), acid phosphatase (AcP), phosphodiesterase (PD) and inorganic pyrophosphatase (IPP) were determined as described by Tabbatabai (1994). In brief, AlP and AcP were assayed using p-nitrophenyl phosphate as the substrate with the buffer adjusted to pH 11.0 and 6.5, respectively. PD was assayed using bis-p-nitrophenyl phosphate as the substrate with the buffer adjusted to pH 8.0. IPP was assayed using sodium pyrophosphate decahydrate as the substrate. The activities of AlP, AcP, and PD were expressed as mg p-nitrophenol kg⁻¹ soil h⁻¹, and IPP activity was expressed as mg PO₄³⁻P kg⁻¹ soil 5 h⁻¹.

2.5. NaOH-EDTA extraction and solution ³¹P NMR spectroscopy

The soil P was extracted by shaking 5 g of soil with 100 mL of a solution containing 0.25 M NaOH and 0.05 M EDTA for 16 h at 20 °C (Cade-Menun and Preston, 1996). The NaOH-EDTA extracts were centrifuged at 10,000 × g for 30 min, and the pH of the extracts was rapidly adjusted to between approximately 6.6 and 7.0 by HCl solution, which could reduce the hydrolysis of organic P composition during the freeze-dried process (Cade-Menun et al., 2006). The extracts were then immediately frozen at −40 °C and subsequently freeze-dried over several days. Before the total P concentrations were determined by ICP-OES, the extracts were diluted 25-fold to prevent interference over several days. Before the total P concentrations were determined by ICP-OES, the extracts were diluted 25-fold to prevent interference from EDTA during the analysis (Turner et al., 2003b).

The freeze-dried NaOH-EDTA extracts (200 mg) were re-dissolved in 0.1 mL of 10 M NaOH and 0.5 mL D₂O (for signal lock) and were transferred to 5 mm diameter NMR tubes. The solution ³¹P NMR spectra were obtained using a JEOL ECA 600 spectrometer (Tokyo, Japan) operating at 243 MHz, 45° pulse, 1.077-s acquisition time, and 1.0-s delay time were used. Approximately 26,000 scans were performed for all samples, and the P composition was identified by ICP-OES, the extracts were diluted 25-fold to prevent interference from EDTA during the analysis (Turner et al., 2003a, b) and Cade-Menun (2005). The spectra were plotted using a line broadening of 10 Hz and processed using NMR Utility Transform Software (NUTS) for Windows (Acorn NMR, Livermore, CA).

2.6. Statistical analysis

All soil results were calculated based on oven-dried (105 °C) weight. The soil data were the mean value of four replicates with their standard deviation. The Duncan test at the P = 0.05 level and one-way ANOVA were used to analyze the effect of tillage on soil parameters in aggregates. The effects of tillage and aggregate-size on soil parameters were tested by two-way ANOVA. The correlation of soil parameters was based on the Pearson correlation coefficients. All statistical analyses were conducted with the software SPSS 16.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. P forms in aggregate size fractions

Tillage significantly affected the concentrations of total P, organic P, and plant available P, but aggregate-size and its interaction with tillage had no significant effects on total P, organic P and plant available P of soils (Table 1). The MP soils had significantly higher total P and plant available P concentrations than the NT soils in bulk soil. However, the organic P in bulk soils of the three treatments, equivalent to 57.1 to 63.0% of total P, did not differ significantly between the treatments. The concentrations of total P and organic P differed among the three tillage systems only in the >2 mm aggregate size fraction, where total P and organic P concentrations were significantly higher in MP soil than those in NT and RT soils (Table 2). The plant available P concentration, however, was significantly higher in the >0.25 mm size fractions in MP soil than that in NT and RT soils.

The only significant difference in concentrations of soil P in aggregates was found in MP soil for organic P concentration (Table 2), where the organic P concentration was the highest in >2 mm size fraction, and other size fractions did not have significant differences.

3.2. Phosphatase activities in aggregate size fractions

Tillage significantly affected the activity of soil AlP and PD (Table 1). The AlP activity only differed among the three tillage systems in the 0.25–1 mm aggregate size fraction, whereas the PD activity only differed in the microaggregate (<0.25 mm) size fraction, where the AlP and PD activities were significantly higher in NT soils than in MP soils (Fig. 1a, c). Although the AlP activity was not affected significantly by aggregate-size (Table 1), it showed significant higher activity in the microaggregate (<0.25 mm) size fraction than in the >2 mm size fraction (Fig. 1a).

Table 1

<table>
<thead>
<tr>
<th>Factors</th>
<th>Tillage (T)</th>
<th>Aggregate-size (A)</th>
<th>T × A</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F value</td>
<td>P value</td>
<td>F value</td>
</tr>
<tr>
<td>Total P</td>
<td>5.304</td>
<td>0.010</td>
<td>0.801</td>
</tr>
<tr>
<td>Organic P</td>
<td>5.338</td>
<td>0.010</td>
<td>2.103</td>
</tr>
<tr>
<td>Plant available P</td>
<td>11.534</td>
<td>0.000</td>
<td>1.061</td>
</tr>
<tr>
<td>AlP</td>
<td>8.417</td>
<td>0.001</td>
<td>2.760</td>
</tr>
<tr>
<td>AcP</td>
<td>1.681</td>
<td>0.203</td>
<td>27.671</td>
</tr>
<tr>
<td>PD</td>
<td>8.897</td>
<td>0.001</td>
<td>1.170</td>
</tr>
<tr>
<td>IPP</td>
<td>1.496</td>
<td>0.240</td>
<td>2.341</td>
</tr>
<tr>
<td>IC-P total P</td>
<td>4.936</td>
<td>0.014</td>
<td>2.219</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>10.529</td>
<td>0.000</td>
<td>4.788</td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>0.717</td>
<td>0.496</td>
<td>1.393</td>
</tr>
<tr>
<td>Monesters</td>
<td>0.335</td>
<td>0.150</td>
<td>0.411</td>
</tr>
<tr>
<td>Diesters</td>
<td>6.16</td>
<td>0.005</td>
<td>10.665</td>
</tr>
</tbody>
</table>

AIP: alkaline phosphomonoesterase activity; AcP: acid phosphomonoesterase activity; PD: phosphodiesterase activity; IPP: inorganic pyrophosphatase activity; IC-P: total P; NaOH-EDTA extracted total P.

Values followed by different uppercase letters within a column indicate difference (P < 0.05) among tillage systems. Values followed by different lowercase letters within a column indicate difference (P < 0.05) in aggregate size fractions in each tillage system. Columns without letters indicate no significant difference (P > 0.05).
The aggregate-size only significantly affected the activity of soil AcP (Table 1). Among the three tillage systems, the AcP activity significantly increased with decreasing aggregate size from >2 mm to 0.25–1 mm with the exception of MP soil (Fig. 1b). The lowest AcP activity was found in the microaggregate (<0.25 mm) size fraction.

In contrast to AlP, PD, and AcP, the activity of inorganic pyrophosphatase (IPP) was not affected by either tillage or aggregate-size and their interaction (Table 1). A homogeneous distribution of IPP activity in aggregates was found in the three tillage systems (Fig. 1d).

3.3. P composition determined by $^{31}$P NMR in aggregates

The concentration of total P extracted by NaOH-EDTA in the bulk soil ranged from 197 to 323 mg kg$^{-1}$ (Table 3), equivalent to 38.8 to 53.6% of total P in the soil. The extracted total P concentration was affected significantly by tillage (Table 1) and showed a higher value in MP soil compared to NT and RT soils (Table 3).

3.3.1. Inorganic P composition in aggregate size fractions

The NaOH-EDTA extracts were dominated by inorganic orthophosphate, which accounted for 54.4–66.2% of the extracted total P in bulk soil (Table 3, Fig. 2). Inorganic orthophosphate was affected by both tillage and aggregate-size significantly (Table 1). The concentration and proportion of orthophosphate in the extracted total P were higher in MP soils compared to NT soils, and RT soils had intermediate values. In the three tillage systems, the concentration and proportion of inorganic orthophosphate in aggregates represented a similar tendency: it increased with decreasing aggregate size fractions, and the highest value was found in the microaggregate (<0.25 mm) size fraction.

### Table 3

<table>
<thead>
<tr>
<th>Tillage systems</th>
<th>Aggregate size (mm)</th>
<th>NaOH-EDTA extracted total P</th>
<th>Inorganic phosphorus</th>
<th>Organic phosphorus</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Orthophosphate</td>
<td>Monoesters</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pyrophosphate</td>
<td>Diesters</td>
</tr>
<tr>
<td>MP</td>
<td>Bulk soil</td>
<td>323 (53.6)</td>
<td>214 (66.2)</td>
<td>7.7 (2.4)</td>
</tr>
<tr>
<td></td>
<td>&gt;2</td>
<td>265 (42.2)</td>
<td>179 (67.5)</td>
<td>8.8 (3.3)</td>
</tr>
<tr>
<td></td>
<td>1–2</td>
<td>270 (46.4)</td>
<td>175 (64.8)</td>
<td>8.0 (3.0)</td>
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<tr>
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<td>0.25–1</td>
<td>294 (49.4)</td>
<td>187 (63.7)</td>
<td>7.1 (2.4)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.25</td>
<td>315 (52.3)</td>
<td>225 (71.4)</td>
<td>6.3 (2.0)</td>
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<td></td>
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<td>141 (40.5)</td>
<td>8.7 (3.7)</td>
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<td></td>
<td></td>
<td></td>
<td>101 (56.1)</td>
<td>8.8 (4.9)</td>
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<td>121 (55.3)</td>
<td>9.6 (4.4)</td>
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<td>139 (60.0)</td>
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<td>107 (54.5)</td>
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<td>93 (50.3)</td>
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<td></td>
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<td></td>
<td>170 (63.9)</td>
<td>9.1 (3.4)</td>
</tr>
<tr>
<td>RT</td>
<td>Bulk soil</td>
<td>233 (42.3)</td>
<td>232 (43.1)</td>
<td>101 (56.1)</td>
</tr>
<tr>
<td></td>
<td>&gt;2</td>
<td>180 (34.4)</td>
<td>121 (55.3)</td>
<td>9.6 (4.4)</td>
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<td>139 (60.0)</td>
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<tr>
<td></td>
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<td>169 (65.7)</td>
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<tr>
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<td></td>
<td></td>
<td>170 (63.9)</td>
<td>9.1 (3.4)</td>
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Values in the column represent mean concentration of NaOH-EDTA extracted P and the proportion of total P (in parentheses) recovered by NaOH-EDTA in bulk soil and aggregate size fractions.
The remaining inorganic P composition in the NaOH-EDTA extracts was pyrophosphate, and it only accounted for 2.4–3.7% of extracted total P (Table 3, Fig. 2). In contrast to inorganic orthophosphate, pyrophosphate was not significantly affected by either tillage or aggregate-size and their interaction (Table 1).

3.3.2. Organic P composition in aggregate size fractions

The organic P composition determined by 31P NMR mainly consisted of orthophosphate monoesters and diesters (Table 3, Fig. 2). Monoesters accounted for a large proportion (30.4–40.4%) of the extracted total P, whereas diesters only accounted for a small proportion (1.1–1.5%).

Diesters were the only P compounds that were affected significantly by tillage, aggregate-size, and their interaction (Table 1), whereas monoesters were not significantly affected. The proportion of diesters in the extracted total P among the three tillage systems showed a similar distribution trend as that of monoesters; both decreased as follows: NT > RT > MP (Table 3). Moreover, the proportion of monoesters in the extracted total P in aggregates was lowest in the microaggregates (<0.25 mm) size fraction. In contrast to monoesters, the proportion of diesters in the extracted total P was highest in the 0.25–1 mm size fraction in MP and RT soils and in the microaggregates (<0.25 mm) size fraction in NT soils (Table 3).

3.4. Correlations between soil chemical and biological characteristics

Both AlP and PD activities had significant negative correlations with total P, organic P, plant available P, monoesters and orthophosphate concentrations (Table 4). The concentrations of monoesters and orthophosphate showed significant positive correlations with total P, organic P, and plant available P concentrations. With respect to monoesters, the positive correlations with orthophosphate and pyrophosphate concentrations were also found. The concentration of diesters presented significant positive correlations with total P, plant available P, orthophosphate, pyrophosphate and monoesters concentrations (Table 4).

4. Discussions

4.1. P in aggregate size fractions

Previous research reported that soils with NT often had higher concentrations of total P and plant available P than soils with CT (Qin et al., 2010; Saavedra et al., 2007; Selles et al., 1997). These results were attributed to minimal soil disturbance and the management of crop residues at the surface of soil under NT treatment (Redel et al., 2007; Wang et al., 2011) and to increased contact between fertilizer-derived...
P and soil particles in MP soil, which thereby enhanced the formation of stable insoluble P compounds in soils (Djodjic et al., 2002). However, our study found that total P and plant available P concentrations were significantly lower in NT soil compared to MP soil at 0–20 cm of depth (Table 2). Because no significant difference in crop yield among the tillage systems was found (Fan et al., 2012), the reason that NT soil had lower total P concentration than MP soil could be attributed to higher leaching of P from top 20 cm into deep soil profile under NT treatment. It has been widely reported that the soil P leaching could be affected by tillage. Gaynor and Findlay (1995) found that zero tillage had higher dissolved reactive P concentrations in the leachate than conventional tillage. Hangen et al. (2002) found that conventional tillage destroyed macro-pores and could reduce the dissolved P loss through leaching. In our study site, Fan et al. (2013) found that NT soil had higher infiltration rate and infiltrated water amount than MP soil due to more biological macro-pores that occur. By using dye tracer to observe the infiltration depth, the authors also found that the dye reached a deeper depth in NT soil (>25 cm) compared with that in MP soil (<25 cm) (Fan et al., 2013). Because leaching of soil P was affected by infiltration rate and infiltration amount (Schatonius et al., 2013; Sharpley et al., 2001), NT soil with the higher amount of infiltrated water and deeper penetration depth might increase the leaching of P from top 20 cm into deep soil profile.

Compared with MP soil, the lower plant available P concentration in NT and RT soil could be attributed to the immobilization of P by soil microorganisms under NT and RT treatments, where the activities of microorganisms increased because of a higher rate of organic matter input. Similar result was also reported by Omidi et al. (2008).

The concentrations of total P and organic P differed among the three tillage systems only in the >2 mm size fraction. This indicates that the differences in total P and organic P concentration among different tillage systems may be highly related to the >2 mm size fraction. Unlike total P and organic P, plant available P concentration in macroaggregates in MP soil was significantly higher than that in NT and RT soils (Table 2). This suggests that plant available P in macroaggregates may be more likely to be affected by tillage systems.

The organic P concentration in MP soil was significantly higher in the >2 mm size fraction than in the 1–2 mm size fraction (Table 2). This could be attributed to lower phosphatase activities in the >2 mm size fraction (Fig. 1a) of MP soil, which could catalyze the hydrolysis of organic P (Tabatabai, 1994). Actually, significant and negative correlations between AlP and PD activities and organic P concentration were obtained (Table 4), which also indicated that AlP and PD activities may play a critical role in the hydrolysis of organic P.

4.2. Phosphatase activities in aggregate size fractions

Soil enzyme activities could be used as a very good indicator of changes in soil properties induced by tillage because of their higher sensitivity to soil management practices (Roldan et al., 2005). Our results indicated that AlP and PD activity was significantly higher in NT soil compared to MP soil (Fig. 1a, c), which was in accordance with other studies (Deng and Tabatabai, 1997; Dick, 1984; Mina et al., 2008). According to Deng and Tabatabai (1997), the result was caused by the differences in the origin, states, and/or persistence of the different groups of enzymes in soils. In our study, it could be because NT with a cover of residues can supply more substrate available for phosphatases and hence promote their activities. Moreover, we found that AlP activity differed among the three tillage systems only in the 0.25–1 mm aggregate size fraction, whereas PD activity differed only in the microaggregate (~0.25 mm) size fraction (Fig. 1a, c). This suggests that the response of AlP activity to tillage may be more sensitive in the 0.25–1 mm size fraction, whereas PD activity was more sensitive in the microaggregate (~0.25 mm) size fraction.

Gupta and Germida (1988) found that macroaggregates contained higher AcP activity in both native and cultivated soils than in their respective microaggregates, which coincides with our results. The reason was ascribed to higher microbial biomass in macroaggregates. However, in our study, the microaggregate (~0.25 mm) size fraction showed higher microbial biomass compared to the macroaggregates (Zhang et al., 2012), which conflicts with Gupta and Germida (1988). We hypothesized that the difference could be because of differences in the composition of the microbial community in aggregates as AcP mainly derived from fungi in acidic soils (Turner and Haygarth, 2005). Actually, Sessitsch et al. (2001) reported that the microbial community structure was significantly affected by particle size, and small size fractions showed higher diversity of microbes than coarse size fractions did. Moreover, it had been shown that bacteria may be preferentially associated with smaller size fractions (Sessitsch et al., 2001) because of higher nutrient availability in smaller size fractions, whereas fungi were abundant in macroaggregates (Gupta and Germida, 1988; Tisdall and Oades, 1982).

Although AlP activity was not affected significantly by aggregate-size, AlP activity in MP soil was significantly higher in the microaggregate (~0.25 mm) size fraction than in the >2 mm size fraction (Fig. 1a). This could be attributed to higher microbial biomass in microaggregates than that in the >2 mm size fraction because AlP was considered to be totally derived from microorganisms (Dick et al., 1983; Tabatabai, 1994).

4.3. P composition determined by 31P NMR in aggregate size fractions

4.3.1. Inorganic P composition in aggregate size fractions

The extracts were dominated by inorganic orthophosphate. Similar results were also reported by Solomon and Lehman (2000) for chromic lusisols in Tanzania.

Inorganic orthophosphate showed higher concentration in MP soil than that in NT and RT soil. The reason could be attributed to higher leaching of orthophosphate in NT soil. Similar result was also reported by Gaynor and Findlay (1995).

Moreover, the concentration and proportion of orthophosphate increased with decreasing size fraction in the three tillage systems, and there was the highest value in the microaggregate (~0.25 mm) size fraction (Table 3). This could be attributed to enhanced mineralization and hydrolysis of monoesters in the microaggregate (~0.25 mm) size fraction by phosphatases (Tabatabai, 1994). Indeed, our results showed that there was significant negative correlation between orthophosphate concentration and AlP activity (Table 4).

Pyrophosphate as the substrate in soil could be rapidly hydrolyzed to produce orthophosphate by IPP (Dai et al., 1996; Tabatabai, 1994), thus the homogeneous distribution of IPP activity in different tillage and in aggregates may be the reason why the concentration of pyrophosphate was not affected by either tillage or aggregate size (Table 1).

4.3.2. Organic P composition in aggregate size fractions

Monoesters accounted for a large proportion of extracted total P. In contrast to orthophosphate, the proportion of monoesters in extracted total P among the three tillage systems decreased as followed: NT > RT > MP. Diesters only accounted for a small proportion. Our results are similar to the results observed by Cardoso et al. (2003) for Oxisols and Makarov et al. (2004) for umbric leptosols. The proportion of diesters among the three tillage systems showed a similar distribution trend to that of monoesters, which was also in the order of NT > RT > MP. The decrease of the proportion under the MP treatment could be attributed to a lack of crop residue (Redel et al., 2011) and to the enhanced mineralization of organic P caused by tillage (Solomon and Lehman, 2000; Wright, 2009).

Our study also indicated that the proportion of monoesters in aggregates was lowest in the microaggregate (~0.25 mm) size fraction of the three tillage systems. This was in accordance with the observation reported by Makarov et al. (2004). We assumed that it may be related to microbial attack and enzymatic hydrolysis (Tabatabai, 1994). Indeed,
the microbial biomass and the activity of phosphomonoesterases may be higher in the microaggregate (<0.25 mm) size fraction (Fig. 1a). Moreover, the significant and negative correlations between monooesters and AIP activity had also confirmed the assumption (Table 4).

Unlike monooesters, the proportion of diesters in aggregates were highest in the 0.25–1 mm size fraction in MP and RT soils and in the microaggregate (<0.25 mm) size fraction in NT soil. The reason could be attributed to the higher microbial biomass and wetter and cooler circumstances in small aggregates. This could be supported by the observation that DNA mainly accumulated in the cold, wet and acidic soils, and phospholipids and teichoic acids were mainly in the more microbially active soils (Makarov et al., 2002). Moreover, in uncultivated soils of North American grasslands, Summan et al. (1998) also found that diesters were enriched in the clay fraction of soil because of increased microbial activity and physical protection from degradation. The difference between the relationships of monooesters and diesters with soil aggregate size suggests that the process in soil controlling their abundance was different, which was in agreement with that reported by Turner et al. (2003b).

5. Conclusions

In this paper, the NT treatment showed significantly higher soil AIP and PD activities and larger proportions of monooesters and diesters in extracted total P than the MP treatment, whereas the MP treatment had higher concentrations of total P, organic P, plant available P, NaOH-EDTA extracted total P, orthophosphate and monooesters. Overall, although NT facilitated more P stored in the organic P form and led to higher phosphatase activities, soil with NT had lower total and plant available P compared to traditional MP treatment and therefore, MP may be the right practice to conserve soil phosphorus under cold monsoon climate.

Acknowledgments

This study was supported by the National Basic Research Program of China (973 Program) (Nos. 2011CB100506, 2011CB100504), the National Natural Science Foundation of China (41171241) and the National Key Technology R&D Program of China (2012BAD14B04).

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