Soil organic carbon, nutrients and relevant enzyme activities in particle-size fractions under conservational versus traditional agricultural management

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Micro-scale investigation is helpful for better understanding of the relationships between organic matter, microorganisms and nutrients in soil, and for better interpretation of modifications induced by soil management. The soil particle-size fractions (2000–200, 200–63, 63–2, and 2–0.1 μm), contents of soil organic carbon (SOC), total N (STN), available P (SAP), dissolved organic C (DOC), light fraction organic C (LOC), microbial biomass N (MBN), basal respiration (SBR), and relevant enzyme activities of C, N and P transformations, such as β-glucosidase (β-G), N-acetyl-β-glucosaminidase (N-G), protease, urease and alkaline phosphomonoesterase (APH) were analyzed to study the effects of 8-year-period conservational (no-till with residue retention) (CAM) versus traditional agricultural management (moldboard plowing without residue retention) (TAM) to a Haplic Cambisol soil in the North China Plain (NCP). Our results showed that CAM significantly enlarged the stocks of SOC, DOC, LOC, STN and SAP in the 0–10 cm layer, increased the contents of SOC, STN and SAP in the sand fractions, and promoted all of the enzyme activities in the bulk soil and all of the four particle-size fractions. Our results suggested that CAM increased the nutrient contents in the sand fractions by both enlarging the content of particulate organic matter and enhancing the activities of enzymes involved in nutrient cycling in these fractions. On the contrary, the contents of SOC and nutrients in the silt and clay fractions were relatively resistant to the conversion from CAM to TAM, indicating the limitation of CAM for stable SOC sequestration.

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1. Introduction

Traditional agricultural management (moldboard plowing without residue retention) (TAM) is dominant in the North China Plain (NCP), an intensive agriculture area with 320,000 km² and over 200 million population. This agricultural strategy has resulted in serious environmental problems, such as groundwater contamination (Hu et al., 2006) and soil organic carbon (SOC) loss in the NCP (Wang et al., 2007). Conservational agricultural management (no-till with residue retention) (CAM) has recently been expanded in the NCP to increase crop yields whilst reducing soil erosion and environmental degradation (Wang et al., 2007), compared with TAM.

Tillage and residue management play an important role in SOC turnover, aggregate formation and microbial abundance in soil (Lagomarsino et al., 2009). No-till and crop residue retention tend to decrease the turnover rate of macro-aggregate (Six et al., 2000), resulting in SOC sequestration in the surface soil under CAM (Six et al., 2002; Pacala and Socolow, 2004).

Soil enzymes produced by microorganisms play a key role in the process of SOC mineralization (Ahn et al., 2009). They catalyze the decomposition of different organic substrates, release plant nutrients and influence whether SOC is depleted or sequestrated (Fansler et al., 2005). Enzyme activities are unequally distributed among soil particle-size fractions (Poll et al., 2003; Saviozzi et al., 2007). In general, the carbohydrases [β-glucosidase (β-G), β-celllobiohydrolase, N-acetyl-β-glucosaminidase (N-G) and β-xylanase] are reduced from the coarse sand to the clay fraction (Stemmer et al., 1998; Kandeler et al., 1999a; Marx et al., 2005), while the enzymes involved in nitrogen (urease and protease), in phosphorus (acid and alkaline phosphatase) and in sulphur (arylsulfatase) transformations, and the microbial biomass are predominant in both the silt and clay fractions (Kandeler et al., 1999b; Marx et al., 2005; Saviozzi et al., 2007). Similar to the enzyme activities, SOC is also heterogeneously distributed among particle-size fractions (Wu et al., 2006; Lagomarsino et al., 2009).

Mineral sorption is important to protect SOC from microbial attack (Allison and Jastrow, 2006). The quartz particles that are domi-
nant in the sand fractions have only weak bonding affinities to SOC, while the clay particles, such as sesquioxides and layer silicates, provide a large surface area and numerous reactive sites to adsorb SOC through strong ligand exchange and polyvalent cation bridges (Sposito et al., 1999). Consequently, SOC within the sand, silt and clay fractions are allocated into the active, intermediate and passive pool, respectively (Von Lützow et al., 2007). These organo-mineral pools have different microbial availability (Marx et al., 2005) and can be separated by the physical fractionations (Stemmer et al., 1998), such as wet-sieving and centrifugation.

The distributions of SOC and microbial activities in particle-size fractions are important for determining how agricultural practices influence soil nutrient release and SOC balance (Kandeler et al., 1999a; Saviozzi et al., 2007; Lagomarsino et al., 2009). A limited influence soil nutrient release and SOC balance (Kandeler et al., 1999b; Poll et al., 1999a; Saviozzi et al., 2007; Lagomarsino et al., 2009). However, few of them linked the micro-scale enzyme activities to soil nutrient contents and SOC balance in response to both tillage and residue management.

By investigating the soil enzyme activities involved in C, N, and P transformations [β-G, N-G, protease, urease and alkaline phosphomonoesterase (APH)], and the major nutrient contents [SOC, total N (STN) and available P (SAP)] in the particle-size fractions of a Haplic Cambisol in the NCP, our objective was to test whether the different tillage and residue management modified the microbial activities associated with the nutrient release and SOC balance at the primary particle scale.

2. Materials and methods
2.1. Description of field site

This study was conducted on an 8-year conservational agriculture experimental field (37°90′ N, 114°67′ E, elevation 50 m), which is located at the piedmont of the Taihang Mountains, in the NCP and belongs to the Luancheng Agro-ecosystem Station, the Chinese Academy of Sciences. The annual mean solar radiation, temperature, precipitation and frost-free period in the station is 547 kl cm⁻², 12°C, 539 mm and 200 days, respectively. The soil is classified as a silt loam Haplic Cambisol (IUSS Working Group WRB, 2006). In this NCP region, the prevalent cropping system is winter wheat (middle October to early June) with summer corn (early June to late September).

2.2. Experiment design and soil sampling

Two contrary agricultural management systems were established in this study since 2001: (1) CAM [chopping and spreading maize stalk (mean 11.26 Mg ha⁻¹ year⁻¹) after harvest, following by wheat seeding with no-till seeders]; (2) TAM (removing out maize stalk from field after harvest, following by 20 cm moldboard plowing and wheat seeding with traditional seeders). Each management treatment had three replicated plots. The size of each plot was 30.0 m × 45.0 m. Fertilizer and irrigation were identical for both management systems. Before winter wheat sowing, 177 kg ha⁻¹ urea and 326 kg ha⁻¹ diammonium hydrogen phosphate were applied. Shortly after jointing, both wheat and corn were fertilized at the rate of 193 kg urea ha⁻¹ by surface broadcast. All plots were irrigated at sowing. Depending on rainfall, additional three or four irrigations for wheat and two or three irrigations for corn were applied using a sprinkler system. The irrigation schedule was determined by soil moisture. Generally 40–50 mm was applied for each irrigation when the soil moisture of root zone declined to 60–65% of the field water-holding capacity (FWC).

Soil samples for SOC, STN, SAP and microbial activity assays were collected on October 6, 2008. Additionally, another soil sampling was conducted on February 20, 2010 for soil basal respiration (SBR), dissolved organic C (DOC) and light fraction organic C (LOC) measurements. Five surface soil cores (0–10 cm) were collected randomly from each plot after removing surface litters. This sampling depth was used for the reason that the tillage effects on soil microbial activities tend to be more significant near the surface than those in the deeper layers (Muruganandam et al., 2009). The five soil cores were mixed into one sample, stored in an ice box and then in a cold room. The soil samples were ground through a 2-mm sieve and divided into two sub-samples. One sub-sample was stored at field moisture (75% of FWC) at 4°C for chemical and biochemical analyses and the other was dried at 105°C for gravimetric water content.

2.3. Soil particle-size fractionation

Soil particle-size fractions were obtained by a combination of wet-sieving and centrifugation after dispersion by the low-energy sonication (Stemmer et al., 1998). This fractionation procedure is less destructive to SOC and enzyme activities than the chemical procedures (Stemmer et al., 1998). Field moist soil samples (35 g equivalent dry weight) were dispersed in 100 ml of cooled distilled water (4°C) by a probe-type ultrasonic disaggregator (50 W, 120 s, probed at 15 mm depth). The coarse sand (2000–200 μm) and fine sand (200–63 μm) fractions were recovered by wet-sieving. The silt-size particles (63–2 μm) were separated by centrifuging the remaining suspension at 150 × g for 2 min, and the pellet was re-suspended and centrifuged again under the same condition. This procedure was repeated twice to purify the silt fraction. The remaining supernatants were centrifuged at 3900 × g for 30 min to separate the clay-size particles (2–0.1 μm). The separated fractions were divided into two sub-samples. One was dried at 105°C for 24 h to calculate the recovery of fractionation. The other was freeze-dried and stored at 4°C for chemical and biochemical analyses within 4 weeks. In order to test the validity of this fractionation method, a standard sedimentation method (Rowell, 1994) was conducted on the same soil.

2.4. Soil chemical and biochemical assays

SOC (mg g⁻¹ dry soil) was determined by the dichromate digestion method (Kalmbassa and Jenkinson, 1973), STN (mg g⁻¹ dry soil) by the semi-microkjeldahl method (Nelson and Sommers, 1980), MBN (μg g⁻¹ dry soil) by Kandeler et al. (1999b), and SAP (μg P g⁻¹ dry soil) by Olsen et al. (1954).

SBR (mg CO₂-C kg⁻¹ dry soil) and LOC (mg C kg⁻¹ dry soil) were determined by the method of Cookson et al. (2008). The majority (more than 80%) of DOC and LOC were lost during the wet-sieving procedure. Consequently, we determined the bulk stocks of DOC (kg ha⁻¹) and LOC (Mg ha⁻¹) in the 0–10 cm layer using the method of Cookson et al. (2008). The activities of APH (μmol p-nitrophenol h⁻¹ g⁻¹ dry soil), N-G (μmol p-nitrophenol h⁻¹ g⁻¹ dry soil), β-G (μmol p-nitrophenol h⁻¹ g⁻¹ dry soil), protease (μg tyrosine h⁻¹ g⁻¹ dry soil) and urease (μg NH₄⁺ h⁻¹ g⁻¹ dry soil) were determined. The methods used to determine these biochemical parameters were shown in Table 1. All enzyme activities were determined in triplicate. The percentage contribution of each parameter in each particle-size fraction to the bulk soil was calculated based on the method of Steimer et al. (1998). The ratios of microbial parameters to MBN were calculated based on the method of Landi et al. (2000).
2.5. Statistical analysis

Data (means ± SE) were compared by the LSD test at P < 0.05. All statistical analyses were conducted with the software SPSS 13.0 (SPSS, 2004).

3. Results

3.1. Particle-size fractionation

The sonication method and the classical sedimentation method yielded similar particle-size distributions (Table 2). The proportions of particle-size fractions were not significantly different between CAM and TAM (Table 2). The recovery rate of the sonication fractionation procedure exceeded 99% (Table 2).

3.2. SOC, STN, SAP and C/N ratio in bulk soil and particle-size fractions

The contents of SOC and SAP in the bulk soil were significantly greater under CAM than those under TAM (Fig. 1). On the contrary, STN content in the bulk soil was not significantly affected by different managements (Fig. 1). The contents of SOC, STN, and SAP increased with the reduction of particle size (Table 2). The C/N ratio was similar in the bulk soil, in the silt and the clay fractions between the two management treatments, but it was significantly greater in the coarse and fine sand fractions under CAM than that under TAM (Table 2).

3.3. Soil microbial properties in bulk soil and particle-size fractions

In the bulk soil, MBN and SBR, and the activities of APH, N-G, β-G, protease, and urease were significantly greater under CAM than those under TAM (Figs. 1 and 2). These parameters were also significantly higher in all of the four soil particle-size fractions under CAM than those under TAM, except for MBN in the clay fraction and SBR in the coarse sand, clay and silt fractions (Figs. 1 and 2). Under the same management, the contents of SOC, STN, and SAP increased with the reduction of particle size (Figs. 1 and 2). The average recovery rates of APH, SBR, MBN, N-G, β-G, protease, and urease were 105.0%, 98.0%, 99.6%, 72.9%, 88.1%, 104.6% and 103.2%, respectively (Table 3). The correlation coefficients between SOC and microbial parameters were more remarkable in coarse and fine sand fractions than those in silt and clay fractions (Table 4). CAM significantly promoted the ratios of SBR and enzyme activities to MBN in the sand fractions, but not in the bulk soil and the silt and clay fractions (Table 5).

3.4. Soil nutrients stock

The stocks of SOC, STN, SAP, DOC and LOC in the surface soil (0–10 cm) were significantly greater under CAM than those under TAM (Table 6).

4. Discussion

Compared with the chemical fractionation procedure (chemical dispersion + sedimentation), the ultrasonic procedure (ultrasonic dispersion + centrifugation) has less effect on soil biochemical properties (Stemmer et al., 1998). However, this ultrasonic procedure is not able to completely disperse soil aggregates (Marx et al., 2005; Von Lützow et al., 2007). This was proven by the result that the percentages of the sand fractions obtained by the ultrasonic procedure were slightly higher than those obtained by the chemical procedure (Table 2). The high recovery rates of SOC, STN, and SAP indicated the validation of the method of Stemmer et al. (1998). The recovery rates of β-G (88.1%) and N-G (72.9%) were relatively lower in the chemical dispersion + sedimentation procedure; this may be related to the fact that the contents of SOC and SAP exceeded 99% (Table 3). The C/N ratio was similar in the bulk soil, in the silt and the clay fractions under the two management treatments (Table 2). Under the same management, the C/N ratio significantly decreased with the reduction of particle size (Table 2).
ment (Koarashi et al., 2009). The increase in bulk SOC content under CAM tends to increase due to crop residue retention, and quality of organic matter returned into soil might determine its labile SOC sequestration. The quantity of SOC under CAM was probably resulted from the residue returned into soil (Arshad et al., 1990). This was true for our study that the SOC content in the surface layer (0–10 cm) was significantly increased under 15-year CAM in the northern China. The increase of SOC under CAM was probably resulted from the residue returned into soil (Arshad et al., 1990). This was true for our study that the SOC content in the surface layer (0–10 cm) in Western Australia. These results indicated that CAM had the potential to increase labile SOC sequestration. The quantity and quality of organic matter returned into soil might determine its distribution in different soil particle-size fractions (Lagomarsino et al., 2009). The crop residues generally have a high availability to soil microorganisms and consequently can be easily incorporated into macro-aggregate (2000–250 μm) fractions during their decom-

Table 2
Soil particle-size distributions (percent of bulk soil) determined by the ultrasonic method (ultrasonic dispersion + centrifugation) and the standard chemical method (chemical dispersion + Sedimentation) and C/N ratio (soil organic C/total N) under the conservation (CAM) versus traditional agricultural management (TAM).

<table>
<thead>
<tr>
<th>Managements</th>
<th>Fractionation method</th>
<th>Particle size distribution</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coarse sand</td>
<td>Fine sand</td>
<td>Silt</td>
</tr>
<tr>
<td>CAM</td>
<td>Ultrasonic dispersion + centrifugation</td>
<td>3.8 ± 1.2a, δ</td>
<td>10.3 ± 1.5a, γ</td>
</tr>
<tr>
<td></td>
<td>Chemical dispersion + Sedimentation</td>
<td>3.0 ± 1.0a, δ</td>
<td>9.6 ± 1.2a, γ</td>
</tr>
<tr>
<td>TAM</td>
<td>Ultrasonic dispersion + centrifugation</td>
<td>3.2 ± 1.0a, δ</td>
<td>10.9 ± 0.7a, γ</td>
</tr>
<tr>
<td></td>
<td>Chemical dispersion + Sedimentation</td>
<td>3.8 ± 1.5a, δ</td>
<td>9.1 ± 0.7a, γ</td>
</tr>
</tbody>
</table>

Managements | Fractionation method | Particle size fraction | In bulk soil |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coarse sand</td>
<td>Fine sand</td>
</tr>
<tr>
<td>CAM</td>
<td>Ultrasonic dispersion + centrifugation</td>
<td>15.0 ± 1.1a, α</td>
</tr>
<tr>
<td></td>
<td>Chemical dispersion + Sedimentation</td>
<td>12.2 ± 1.9b, β</td>
</tr>
</tbody>
</table>

Data (means ± SE, n = 3) followed by the different letters in columns (a, b) and in rows (α, β, γ, δ) indicate significant difference (P<0.05).

Table 3
The percentage contribution (%) of the parameters in each particle-size fraction to the bulk soil and the total recovery rates of parameters under CAM and TAM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAM Mean SE</th>
<th>TAM Mean SE</th>
<th>Particle-size fraction</th>
<th>Parameter</th>
<th>CAM Mean SE</th>
<th>TAM Mean SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC</td>
<td>2.1 0.4</td>
<td>1.0 0.1</td>
<td>2000–200 μm</td>
<td>APH</td>
<td>2.2 0.2</td>
<td>1.79 0.3</td>
</tr>
<tr>
<td>STN</td>
<td>1.1 0.3</td>
<td>0.7 0.1</td>
<td>2000–200 μm</td>
<td>β-G</td>
<td>5.48 0.4</td>
<td>5.5 0.5</td>
</tr>
<tr>
<td>SAP</td>
<td>0.7 0.1</td>
<td>0.35 0.1</td>
<td>2000–200 μm</td>
<td>N-G</td>
<td>4.0 0.2</td>
<td>3.7 0.2</td>
</tr>
<tr>
<td>MBN</td>
<td>0.85 0.3</td>
<td>0.5 0.0</td>
<td>2000–200 μm</td>
<td>Protease</td>
<td>3.61 0.1</td>
<td>2.5 0.3</td>
</tr>
<tr>
<td>SBR</td>
<td>2.2 0.2</td>
<td>2.1 0.3</td>
<td>2000–200 μm</td>
<td>Urease</td>
<td>2.2 0.1</td>
<td>2.1 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.23 0.5</td>
<td>10.1 0.7</td>
</tr>
</tbody>
</table>

Abbreviations: SOC, soil organic C; STN, soil total N; SAP, soil available P; MBN, microbial biomass N; SBR, soil basal respiration; APH, alkaline phosphomonoesterase; β-G, β-glucosidase; N-G, N-acetyl-β-glucosaminidase.
Table 4
Correlation coefficients between properties in the particle-size fractions.

<table>
<thead>
<tr>
<th></th>
<th>SOC</th>
<th>STN</th>
<th>SAP</th>
<th>MBN</th>
<th>APH</th>
<th>β-G</th>
<th>N-G</th>
<th>Protease</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In the 2000–200 μm fraction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOC</td>
<td>1</td>
<td>0.72</td>
<td>0.81</td>
<td>0.81</td>
<td>0.75</td>
<td>0.83</td>
<td>0.86</td>
<td>0.87</td>
<td>0.86</td>
</tr>
<tr>
<td>STN</td>
<td>1</td>
<td>0.70</td>
<td>0.67</td>
<td>0.75</td>
<td>0.83</td>
<td>0.83</td>
<td>0.67</td>
<td>0.81</td>
<td>0.85</td>
</tr>
<tr>
<td>SAP</td>
<td>1</td>
<td>0.91</td>
<td>0.90</td>
<td>0.91</td>
<td>0.90</td>
<td>0.90</td>
<td>0.96</td>
<td>0.87</td>
<td>0.87</td>
</tr>
<tr>
<td>MBN</td>
<td>1</td>
<td>0.85</td>
<td>0.83</td>
<td>0.92</td>
<td>0.92</td>
<td>0.91</td>
<td>0.91</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>APH</td>
<td>1</td>
<td>0.88</td>
<td>0.86</td>
<td>0.76</td>
<td>0.86</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>β-G</td>
<td>1</td>
<td>0.84</td>
<td>0.87</td>
<td>0.79</td>
<td>0.84</td>
<td>0.87</td>
<td>0.87</td>
<td>0.87</td>
<td>0.87</td>
</tr>
<tr>
<td>N-G</td>
<td>1</td>
<td>0.94</td>
<td>0.91</td>
<td>0.94</td>
<td>0.94</td>
<td>0.91</td>
<td>0.91</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>Protease</td>
<td>1</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>Urease</td>
<td>1</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
<td>0.84</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 3. Numbers in bold are significant (P < 0.05) correlations, n = 9.

Table 5
Ratios of soil basal respiration (SBR) and enzyme activities to microbial biomass N (MBN) in bulk soil and particle-size fractions under conservational (CAM) and traditional agricultural management (TAM).

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Bulk</th>
<th>2000–200 μm</th>
<th>200–63 μm</th>
<th>63–2 μm</th>
<th>2–0.1 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBR:MBN (g CO₂-C g⁻¹ MBN d⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAM</td>
<td>0.5x, b</td>
<td>0.5 x, c</td>
<td>1.2 y, a</td>
<td>2.7 x, a</td>
<td>1.3 y, a</td>
</tr>
<tr>
<td>TAM</td>
<td>0.5x, b</td>
<td>0.5 x, c</td>
<td>1.2 y, a</td>
<td>2.7 x, a</td>
<td>1.3 y, a</td>
</tr>
<tr>
<td><strong>APH:MBN (μmol PNP g⁻¹ MBN h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAM</td>
<td>7.4 x, c</td>
<td>7.0 x, c</td>
<td>15.9 y, a</td>
<td>27.2 x, a</td>
<td>13.6 y, b</td>
</tr>
<tr>
<td>TAM</td>
<td>7.4 x, c</td>
<td>7.0 x, c</td>
<td>15.9 y, a</td>
<td>27.2 x, a</td>
<td>13.6 y, b</td>
</tr>
<tr>
<td><strong>β-G:MBN (μmol PNP g⁻¹ MBN h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAM</td>
<td>11.2 x, b</td>
<td>11.4 x, b</td>
<td>59.32 y, a</td>
<td>102.4 x, a</td>
<td>54.3 y, a</td>
</tr>
<tr>
<td>TAM</td>
<td>11.2 x, b</td>
<td>11.4 x, b</td>
<td>59.32 y, a</td>
<td>102.4 x, a</td>
<td>54.3 y, a</td>
</tr>
<tr>
<td><strong>N-G:MBN (μmol PNP g⁻¹ MBN h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAM</td>
<td>20.7 y, b</td>
<td>26.9 x, c</td>
<td>74.3 y, a</td>
<td>108.0 x, a</td>
<td>26.9 y, b</td>
</tr>
<tr>
<td>TAM</td>
<td>20.7 y, b</td>
<td>26.9 x, c</td>
<td>74.3 y, a</td>
<td>108.0 x, a</td>
<td>26.9 y, b</td>
</tr>
<tr>
<td><strong>Protease:MBN (μg tyrosine g⁻¹ MBN h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAM</td>
<td>0.6 x, b</td>
<td>0.8 x, c</td>
<td>1.3 y, a</td>
<td>3.3 x, a</td>
<td>1.4 y, a</td>
</tr>
<tr>
<td>TAM</td>
<td>0.6 x, b</td>
<td>0.8 x, c</td>
<td>1.3 y, a</td>
<td>3.3 x, a</td>
<td>1.4 y, a</td>
</tr>
<tr>
<td><strong>Urease:MBN (g NH₄⁺ g⁻¹ MBN h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAM</td>
<td>0.6 x, b</td>
<td>0.8 x, c</td>
<td>1.3 y, a</td>
<td>3.3 x, a</td>
<td>1.4 y, a</td>
</tr>
<tr>
<td>TAM</td>
<td>0.6 x, b</td>
<td>0.8 x, c</td>
<td>1.3 y, a</td>
<td>3.3 x, a</td>
<td>1.4 y, a</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 3. Data (means ± SE, n = 3) followed by the different letters indicate significant difference between CAM and TAM (x, y) for each fraction and among different particle-size fractions (a, b, c, d and e) for each management, respectively.

Table 6
Bulk density and C, N and P stocks in the surface soil (0–10 cm) under the conservational (CAM) and traditional agricultural management (TAM).

<table>
<thead>
<tr>
<th>Managements</th>
<th>Bulk density (Mg m⁻³)</th>
<th>SOC (Mg ha⁻¹)</th>
<th>DOC (kg ha⁻¹)</th>
<th>LOC (Mg ha⁻¹)</th>
<th>STN (Mg ha⁻¹)</th>
<th>SAP (kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>1.45 ± 0.02 a</td>
<td>15.14 ± 1.1 a</td>
<td>76.82 ± 8.4 a</td>
<td>0.45 ± 0.12 a</td>
<td>1.89 ± 0.11 a</td>
<td>61.3 ± 3.8 a</td>
</tr>
<tr>
<td>TAM</td>
<td>1.45 ± 0.05 b</td>
<td>13.93 ± 1.6 b</td>
<td>54.52 ± 2.1 b</td>
<td>0.33 ± 0.05 b</td>
<td>1.53 ± 0.08 b</td>
<td>49.6 ± 5.2 b</td>
</tr>
</tbody>
</table>

Abbreviations: SOC, soil organic C; DOC, dissolved organic C; LOC, light fraction organic C; STN, soil total N; SAP, soil available P. Data (means ± SE, n = 3) followed by the different letters in columns (a, b) are significantly different (P < 0.05).
sition (Stemmer et al., 1998), contributing to the high SOC contents and C/N ratios in the coarse and fine sand fractions under CAM (Table 2). Moreover, 68% and 22% of the total SOC were existed in the silt and clay-size fraction, respectively (Table 3). However, the SOC contents in these fractions were not significantly different between CAM and TAM (Fig. 1). These results indicated the resistance of SOC in these fractions to management modifications. The silt and clay fractions had rather low C/N ratios (average 7) (Table 2). This suggested the high degree of SOC humification in these fractions (Stemmer et al., 1998).

Besides the carbon input, the SOC decomposition rate is another important factor influencing SOC balance. SBR directly indicates the SOC decomposition rate. Some enzymes involved in soil C cycling, such as β-G and N-G, can provide the information on SOC decomposition potential (Allison and Jastrow, 2006). The β-G catalyzes the final step of cellulose degradation and N-G hydrolyzes the glucosamine (Reboreda and Caçador, 2008). The activities of both β-G and N-G tended to decrease (Fig. 2), while the MBN tended to increased, from the coarse sand to the clay fraction (Fig. 1). These trends agreed with Marx et al. (2005) who explained that the large amount of polymeric material in the sand fractions could account for the high carbohydrate activities in these fractions. The higher content of MBN in the finer fractions was probably due to the microorganism protection from faunal predation (Poll et al., 2003). CAM significantly increased SBR in the bulk soil and the fine sand fraction, and increased MBN and the activities of N-G and β-G in all of the four particle-size fractions, especially in the sand fractions (Figs. 1 and 2). These results indicated that the accumulated SOC in the sand fractions under CAM had a high decomposition potential.

To sum up, our results showed that CAM increased the SOC stock in the surface soil layer (0–10 cm) (Table 6). However, the accumulated SOC was mainly derived from the coarse and fine sand fractions rather than from the silt and clay fractions (Fig. 1), suggesting the limitation of CAM for SOC sequestration. Furthermore, the C/N ratios and enzyme activities in the sand fractions were higher under CAM than those under TAM. These results indicate that the accumulated C under CAM was easy to be decomposed, which further limited the potential of CAM for stable SOC sequestration.

4.2. Management effects on the nutrient contents and relevant enzyme activities in soil particle-size fractions

SOC is the major source of plant nutrients (Bauer and Black, 1994). Its quantity and quality directly influence the availability of soil nutrients, such as nitrogen and phosphorus. The increases of STN and SAP in the sand fractions (2000–200 and 200–63 μm) under CAM (Fig. 1) were probably resulted from the residue retention (Arshad et al., 1990). The increasing trend of STN from coarse sand to clay fractions (Fig. 1) agreed with Marx et al. (2005). The SAP determined by the Olsen method is a valid parameter for estimating the P availability in soil (Demetz and Insam, 1999). The SAP content tended to increase and be less susceptible to management modifications from coarse sand to clay fraction (Fig. 1). These results suggested that CAM increased P availability in the sand fractions, which could increase the crop yields. On the contrary, phosphorus in the silt and clay fractions was associated with mineral particles and hardly modified by management (Fig. 1). This kind of phosphorus had poor availability to plants and soil microorganisms. The processes of nutrient release from SOC are regulated by soil enzyme activities (Dorondikov et al., 2009). Therefore, soil enzymes involved in nutrient cycling have been suggested as potential indicators of soil quality (Cardelli et al., 2004). Urease and protease play important roles in organic N mineralization since they hydrolyze urea into ammonium and hydrolyze protein into amino acid, respectively. Phosphomonoesterase is important to degrade organic phosphorus. Acid phosphomonoesterase dominates in acid soils, while APH dominates in alkaline soils (Eivazi and Tabatabai, 1977). In the present study, the soil is weak alkaline (pH 7.6). So we determined the APH activities in particle-size fractions. In the sand fractions, activities of urease, protease and APH were significantly higher under CAM than those under TAM (Fig. 2), indicating the acceleration of nutrient cycling in these frac-
tions. This accounted for the larger stocks of SAP and STN in the surface layer (0–10 cm) of soil under CAM, compared with those under TAM (Table 6). On the contrary, the increase of these enzyme activities in the silt and clay fractions did not significantly increase the nutrient contents in these fractions (Fig. 1), probably due to the fact that some minerals, such as allophane, may sorb and stabilize soil enzymes (Allison, 2006). These stabilized enzymes are considered to have potential rather than in situ activities (Burns, 1982). These results indicated that the enzyme activities in the silt and clay fractions had little direct effects on nutrient cycling.

In a word, CAM promoted the microbial activities in all of the four particle-size fractions. However, nutrient cycling was increased only in sand fractions. It seemed that the increased enzyme activities in the sand fractions contributed to the nutrient release, while those in the silt and clay fractions did not.

4.3. Correlation between soil chemical and biochemical properties in bulk soil and particle-size fractions

The Pearson correlation coefficients were analyzed between the soil chemical and the microbial properties in different particle-size fractions (Table 4). Significant liner correlations between chemical and microbial parameters were widely reported in bulk soil (Cookson et al., 2008). In the particle-size fractions, however, the correlation coefficients varied with both the fraction and parameter. In general, significant correlations with all tested microbial properties were observed for SOC in the coarse sand (2000–200 µm) fraction, for STN in the coarse (2000–200 µm) and fine (200–63 µm) sand fractions (Table 4). On the contrary, SOC and STN were sometimes not significantly correlated with microbial parameters in the silt and clay fractions (Table 4). These results indicated that SOC in the sand fractions had high microbial availability, while that in the finer fractions were protected by mineral particles and consequently had a low microbial availability. The correlation coefficients between microbial parameters were significant in all of the four fractions (Table 4). This was probably attributed to the fact that extracellular enzymes accounted for considerable part of enzyme activities in the silt and clay fractions. Simmons and Coleman (2008) reported that soil nutrient concentrations were one of the factors that most heavily influenced the abundance and diversity of soil microbes. Those significant correlations in the sand fractions indicated the dependence of soil C, N and P transformations on soil enzymes and the availability of substrates for soil microbes to excrete such soil enzyme activities in these fractions (Lagomarsino et al., 2009).

4.4. Ratios of enzyme activities to microbial biomass in bulk soil and in the particle-size fractions

The ratios of SBR and enzyme activities to microbial biomass (MBN) were significantly higher under CAM than those under TAM in the coarse and fine sand fractions, though no significant difference existed in the bulk soil (Table 5). This result indicated that the increases in enzyme activities under CAM were mostly originated from the enhancement of enzyme production rather than the increase of microbial biomass. In general, the ratios decreased with the decline of particle size (Table 5). This suggested that microorganisms in the coarse and fine sand fractions were more active in SOC decomposition than those in the silt and clay fractions.

5. Conclusions

Enzyme production by microorganisms and stabilization by physical processes influence SOC decomposition and nutrient cycling (Martens, 2000). The separation of differently sized particle fractions provided SOC and enzyme pools which differed significantly in size and stability. Microorganisms within sand fractions were active in SOC decomposition. Therefore, the accumulated particular organic carbon in the sand fractions under CAM significantly increased the enzyme activities and consequently accelerated the nutrient cycling in the sand fractions, resulting in the increase of nutrient contents in these fractions. On the contrary, SOC and enzymes within the silt and clay fractions were stabilized by mineral particles and consequently had limited capacity to release plant nutrients. Although the enzyme activities in the silt and clay fractions were significantly increased by CAM, this increase did not bring greater contents of plant nutrients in these fractions. We concluded that the conversion from TAM to CAM accumulated the SOC content, prompted the microbial activities, and subsequently increased the nutrient contents in the sand fractions. The 8-year period of CAM was too short to induce modifications to the contents of SOC and plant nutrients in the silt and clay fractions, though the enzyme activities were significantly increased in these fractions.

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