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Changes in soil carbon and nitrogen pools after shifting from conventional cereal to greenhouse vegetable production

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ABSTRACT

In recent years large areas of conventional cereal production in China have been transferred to greenhouse production with huge excessive nitrogen (N) fertilizer application and massive irrigation. However, the effects of this change in land use on soil carbon and nitrogen pools remain to be explored. Here we report a comparative study in which paired soil samples were taken from four greenhouses and from adjacent conventional cereal fields. Soil organic carbon (SOC), carbonate carbon (IC), total nitrogen (TN) and mineral nitrogen (N_{min}) to 100 cm depth and the soil active organic pools, including particulate organic matter (POM), soil microbial biomass (SMB) and dissolved organic matter (DOM), to 0-40 cm depth were determined. The natural isotopic signatures of SOC, TN and POM were also analyzed. In both production systems all of the carbon and nitrogen pools in the surface soil (0-10 cm) were greater than deeper in the soil profile except for dissolved organic nitrogen (DON) and NH₄-N. SOC and TN and dissolved organic carbon (DOC) concentrations were higher in the greenhouse system than in conventional cereal soils (P > 0.05). A similar trend was found for POM (P < 0.05) and NO₃-N (P < 0.05, below 20 cm depth) pools but the opposite trend was found for soil IC, soil microbial biomass carbon (SMBC) and nitrogen (SMBN) (P > 0.05) and IC in the greenhouse system showed a dramatic decline. The SOC/TN ratios of different pools in the greenhouse soils were lower than in the conventional cereal system (P > 0.05). The SOC/TN ratio ranged from 8.4 to 10.0 in greenhouse soils and 8.5 to 11.7 in the cereal soils. At each depth POM content in the greenhouses $(1.5-7.1 \text{ g kg}^{-1})$ was significantly greater than that in the field soils (0.8–2.9 g kg⁻¹) (P < 0.05). Application of large amounts of manure increased SOC stocks, but the total carbon stock (SOC plus IC) was lower in the greenhouse system by tradeoff effect on declining IC in this calcareous soil due to soil acidification induced by excessive N fertilization plus massive irrigation. The higher nitrate concentrations found due to massive N fertilization combined with large amounts of irrigation water represent a considerable threat to groundwater quality and may be harmful to local residents.

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1. Introduction

Soils play an important role in the global carbon (C) and nitrogen (N) cycles. The amount of C stored in soils is about twice that stored in the atmosphere (Davidson et al., 2000). The functioning of soils as a C sink or source has moved into the focus of research within the scope of the scientific debate on global climate change (Lal et al., 1995; Schlesinger and Lichter, 2001; Huang and Sun, 2005; Knorr et al., 2005). Storage of soil organic carbon (SOC) and carbonate carbon (IC) in agricultural land can be influenced by management practices such as tillage, fertilizer N inputs and crop rotations (Halvorson et al., 2002; Russell et al., 2005). Optimizing fertilizer N inputs, for instance, may increase SOC content by increasing crop yields and hence the amount of residues returned to soils (Janzen et al., 1998; Halvorson et al., 1999; Huang and Sun, 2005). Excessive fertilizer N may block SOC sequestration through suppression of the microbial population or stimulation of mineralization of old native organic C (McCarty and Meisinger, 1997). Indeed, long-term over-use of fertilizer N in agricultural systems has resulted in substantial nitrate leaching (Ju et al., 2007) and soil carbonate has become depleted (Barak et al., 1997; Malhi et al., 1998; Ju et al., 2007). Hence, changes in SOC and carbonate content with N fertilization can affect soil C stability and soil SOC/TN ratio.

The natural abundance of stable isotopes can been used as a measure to reflect soil C and N cycling and storage (Clapp et al.,

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2000; Lynch et al., 2006; Watzka et al., 2006). Differences in plant δ^{13} C can be attributed to differences in photosynthesis between C₃ and C₄ plants (Clapp et al., 2000; Lynch et al., 2006) and differences in soil δ^{15} N can result from discrimination against ¹⁵N during N loss processes (Lynch et al., 2006; Watzka et al., 2006). Values of δ^{13} C of C₃ plants range between -23 and -30‰ and of C₄ plants from -9 to -17‰ (Mariotti and Peterschmitt, 1994; Lynch et al., 2006). Similarly, the δ^{15} N of chemical N fertilizers range between -3 and 2‰ and that of composted manure is larger than 8% (Choi et al., 2003). It is therefore possible to quantify changes in soil C and N pools after long-term changes in crop species and fertilizer regimes.

Soil organic matter (SOM) can be differentiated into active and passive pools. The active SOM fractions respond very sensitively to management practices and affect nutrient (including N) supply (Mrabet et al., 2001; Wander, 2004). The character of the active SOM can be denoted by particulate organic matter (POM), soil microbial biomass C and N and dissolved organic matter (DOM) because these pools bring together the physical, biological and chemical functions of SOM (Wander, 2004). POM is regarded as a nucleus for aggregates (Bongiovanni and Lobartini, 2006; Marriott and Wander, 2006a,b) and represents a substantial fraction of the fresh plant residues or those at various stages of decomposition (Lehmann et al., 2001). Soil microorganisms are the drivers of SOM turnover and soil microbial biomass (SMB) is the source and sink of nutrients (Perelo and Munch, 2005; Perelo et al., 2006). On one hand, microorganisms make full use of the energy provided by C sources to increase N immobilization (Zogg et al., 2000; Perelo et al., 2006) and on the other hand microbial decomposition and C turnover can also be activated by increasing N availability (Körner and Arnone, 1992; Hu et al., 2001). DOM is mainly derived from root excretion, plant debris, and living and dead microorganisms (Kalbitz et al., 2000; Park et al., 2002) and is greatly influenced by C or N addition (Park et al., 2002; Sinsabaugh et al., 2004) because microorganisms are able to assimilate DOM. In addition, POM provides a C source for microorganisms (Christensen, 2001; Marriott and Wander, 2006a,b) and may attach to microbial tissues (Mendham et al., 2004). There is thus a close connection among POM, SMB and DOM.

Greenhouse vegetable production has played an important role in increasing farming incomes during the last two decades. Accordingly, many farmers have shifted from conventional cereal cropping to greenhouse production systems. In Shouguang county, Shandong province, more than 65% of the arable land is now used for intensive greenhouse production, with 2220 kg fertilizer N ha⁻¹ and 1800 mm irrigation water per year applied for some crops (He et al., 2007). However, the effects of these excessive N application rates and massive irrigation on the C and N pools of the local soils, which are low in SOC, have not yet been determined. The present study was conducted to investigate changes in different soil C and N pools and their δ^{13} C and δ^{15} N signatures in greenhouse vegetable production compared with conventional cereal soils, to examine the stocks of C and N in the soil profile some years after the shift to greenhouse production, and to elucidate how soil management controls these changes.

2. Materials and methods

2.1. Site description

Shouguang county is located in Shandong province at 36°41′-37°19'N, 118°32'–119°10'E and has a typical continental monsoon climate. Annual average air temperature and precipitation (1993-2003) are 12.4 °C and 558 mm. Conventional maize/wheat rotations have been practiced since 1978 and greenhouse vegetable production has developed rapidly in place of the cereal rotation since the 1990s. In conventional cereal systems summer maize and winter wheat are sown in the middle of lune and the beginning of October of the same year and there are two fertilizer applications for each crop. Irrigation is combined with fertilizer N application during the wheat season but little irrigation is applied during the summer because there is sufficient rainfall. The wheat straw is returned to the soil under no-tillage conditions and the maize straw is harvested for silage. In greenhouse vegetable systems, from October to June of the next year double or triple cropping is practiced with up to 13 irrigation events and 7 fertilizer applications followed by a fallow period until October (Table 1).

2.2. Soil sampling

Paired soil samples were taken from four greenhouses and adjacent conventional cereal fields at the end of April 2007 for direct comparison of the two production systems (Table 1). Two pairs of samples were collected from Yingli village (118°48'N, 37°03′E) and the other two from Tianliu village (118°47′N, 36°59'E). The sampling distance between each greenhouse and the adjacent field was <50 m and was >200 m between greenhouses at each site. Samples were composites of at least 8 soil cores in each field of approximately 400 m^2 area. The 100 cm deep sample cores were divided into 0-10, 10-20, 20-40, 40-60, 60-80, 80-100 cm depth increments. A 100 cm deep soil profile under conventional cereals was selected at each village for the measurement of soil bulk density. As soil depth increased, soil bulk density was 1.42, 1.45, 1.47, 1.45, 1.43, 1.43 $g \text{ cm}^{-3}$ at the Yingli site and 1.48, 1.50, 1.46, 1.42, 1.41, 1.50 g cm⁻³ at Tianliu. Samples were passed through a 2 mm sieve and a portion of each sieved sample was stored at 4 °C for subsequent analysis for mineral nitrogen (N_{min}), SMBC, SMBN and DOM, and the remaining portion was air-dried for analysis of SOC, TN, IC and POM. The content and natural abundance of SOC and TN, and the content of

Table 1

Farm	management.	CLOD	vields and	DН	distribution	in t	the soil	profiles of	the	sampling sites.	
	,							P			

Cropping	Site	te Year started	Crop rotation	Fertilizer/manure ^b (kg N ha ⁻¹ yr ⁻¹)	Irrigation ^b (mm yr ⁻¹)	Yield ^b (t ha ⁻¹ yr ⁻¹)	pH at different depths (cm) in the soil profile						
system"							0-10	10–20	20-40	40-60	60-80	80-100	
G1 ^c	Yingli	1996	Tomato/cucumber	1634/1300	1400-1700	171.0	7.71	7.87	8.07	8.13	8.05	8.14	
C1	Yingli	1978	Maize/wheat	600/0	<300	16.5	7.97	8.15	8.22	8.28	8.51	8.55	
G2	Yingli	1996	Tomato/cucumber	1572/1246	1500-1700	175.6	7.38	7.67	8.20	8.17	8.15	8.11	
C2	Yingli	1978	Maize/wheat	600/0	<300	17.0	7.84	7.97	8.18	8.22	8.23	8.35	
G3	Tianliu	1993	Tomato/cowpea	1829/1318	1300-1700	168.4	5.59	5.53	5.58	7.21	7.31	7.60	
C3	Tianliu	1978	Maize/wheat	500/100	<300	18.0	7.89	8.22	8.30	8.22	8.02	8.02	
G4	Tianliu	1993	Tomato/sweet pepper	1620/1866	1400-1600	150.0	6.87	6.90	7.26	7.45	7.56	7.56	
C4	Tianliu	1978	Maize/wheat	500/100	<300	18.0	7.89	7.99	8.11	8.24	7.96	7.83	

^a G, greenhouse system; C, conventional cereal system.

^b Data acquisition: April 2007.

^c Yield in greenhouse system refers to fresh weight and that in conventional cereal system to air-dried weight.

 N_{min} , IC and soil bulk density were determined at each depth increment and the content and natural abundance of POM, and the content of SMBC, SMBN and DOM were determined in 0–10, 10–20 and 20–40 cm depth categories.

2.3. Measurements

Soil samples for SOC analysis were soaked for 24 h in excess 0.3 mol L^{-1} HCl solution to remove calcium carbonate (CaCO₃). The wet soil was cleaned with deionized water until the solution pH was above 6 and oven-dried at 60 °C. POM was determined as described by Bronson et al. (2004). Briefly, 25 g air-dried soil was dispersed in 100 ml of sodium hexametaphosphate solution (5 g L^{-1}) for 1 h on a reciprocal shaker and the suspension was washed over a 53 µm sieve until the rinsing water was clear. The remaining material (except for visible stones and roots on the sieve) was oven-dried in a beaker at 60 °C. TN, SOC, POM-C and POM-N of soil passed through a 0.15 mm mesh were determined with a CN analyzer (Vario Max CN, Elementar, Germany). Their δ^{13} C and δ^{15} N were determined with a mass spectrometer (Delta Plus XP, Thermo Finnigan, Germany). δ^{13} C values were expressed relative to Pee Dee Belemnite and $\delta^{15} N$ to atmospheric N₂ for N in terms of ‰ value according to the equation:

$$\delta X(\infty) = 1000 \times \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right)$$

where X is 13 C or 15 N and R is the molar ratio of the ${}^{13}C/{}^{12}$ C or ${}^{15}N/{}^{14}$ N working standard defined as 0.011237 or 0, respectively.

Mineral N was extracted with 1 mol L^{-1} KCl at a soil:water ratio of 1:5 (w/v) and measured with a continuous flow analyzer (TRAACS 2000, Bran and Luebbe, Germany). SMBC and SMBN were determined by the CHCl₃ fumigation-extraction (FE) method (Brookes et al., 1985). The C in fumigated and unfumigated samples in 0.5 M K₂SO₄ solution (1:4, w/v) were determined with a TOC analyzer (Phoenix 8000, Tekmar, USA), and their N contents were determined by continuous flow analyzer (FIA Star 5000, Foss, Sweden) following Kjeldahl digestion. SMBC and SMBN were calculated as: SMBC or SMBN = (total C or N in fumigated extracts-total C or N in unfumigated extracts)/ K_E , where $K_{\rm E}$ = 0.45 (Wu et al., 1990; Jenkinson, 1988). DOC was the amount of TOC in the unfumigated extract (Cookson et al., 2007; Ghani et al., 2007) and DON was the difference between N and NH₄-N in unfumigated extracts (Cookson et al., 2007; Ghani et al., 2007). Soil carbonate C was determined by the pressure calcimeter method (Loeppert and Suarez, 1996). Soil pH was measured by electrode using a soil:water ratio of 1:2.5. Soil bulk density was calculated using dry weights and the volumes of soil sampled.

2.4. Statistical analysis

Data are expressed on oven-dried soil basis. Statistical analysis was performed using the SPSS 11.0 for Windows software package. Student's *t*-test was used to assess differences at the 5% level between greenhouse and conventional cereal systems. Data are reported as mean \pm one standard error of the mean (SEM).

3. Results

3.1. SOC and TN and their natural abundance

In both greenhouse soil and cereal soil profiles, SOC and TN decreased in the surface soil above 40 cm depth. Below 40 cm only little change was observed (Fig. 1a and b). In the profile of the greenhouse soils, for example, the SOC content declined from 1.4% in the 0–10 cm layer to 0.5% at 80–100 cm depth. The concentra-

tions of SOC and TN in the greenhouse soils tended to be higher than in the cereal soils (Fig. 1a and b). Due to the large variation between the two sites, however, statistically significant differences between the two production systems were only found in SOC in the 20–40 and 40–60 cm layers and in TN in the 10–20 cm layer (P < 0.05). In both production systems the SOC/TN ratio increased with soil depth and was slightly higher in the conventional cereal system than in the greenhouse production system (Fig. 1c). In the greenhouse soil profiles the SOC/TN ratio ranged between 8.4 and 10.0 and in the cereal system between 8.5 and 11.7. Similarly, because of the high variability between replicates, differences in SOC/TN ratio between the two production systems were not significant at the 5% level.

 $δ^{13}$ C of SOC and $δ^{15}$ N of TN increased with soil depth above 40 cm depth (Fig. 1d and e) with little change below 40 cm. The differences in both $δ^{13}$ C and $δ^{15}$ N between the two production systems were largest in topsoil but were not significant (*P* > 0.05). A similar trend was found in $δ^{13}$ C/ $δ^{15}$ N ratio (Fig. 1f). $δ^{13}$ C ranged from -17.8 to -20.9% in the greenhouse soil profiles and from -17.9 to -21.9‰ in the cereal rotations (Fig. 1d). Similarly, $δ^{15}$ N varied between 6.3 and 6.9% in the greenhouses and 5.6 and 7.2‰ in the cereal soils (Fig. 1e).

3.2. Storage of SOC, IC, total carbon (TC) and TN

The storage of TC in the greenhouse soils was less than in the conventional cereal system except for the 4th pair of samples because of the distinct difference in IC content between the Yingli and Tianliu sites (Table 2). IC showed a dramatic decline in the greenhouse system. At site G4, for example, more than 78% of the former IC had been lost by acidification. Over the four pairs of samples the SOC summed up over the whole profile was significantly different between the two production systems (P < 0.05; greenhouses: 101.0 ± 9.9 t C ha⁻¹; cereals: 83.0 ± 9.8 t C ha⁻¹). No significant difference in TN was observed between the production systems (P > 0.05; greenhouses: 11.6 ± 2.4 t N ha⁻¹; cereal rotation: 8.5 ± 1.2 t N ha⁻¹).

3.3. N_{min}

The concentration of NO₃-N in the greenhouse soil was much higher than in the conventional system and the difference was significant below 20 cm soil depth (P < 0.05; Fig. 2a). The concentration of NO₃-N in the greenhouse system ranged from 51.9 to 130.8 mg kg⁻¹, and that in the cereal system varied between 6.0 and 110.7 mg kg⁻¹. NO₃-N summed up over the whole profile in the greenhouse and conventional cereal systems was 933 ± 265 and 339 ± 192 kg N ha⁻¹, respectively. The concentration of NH₄-N was very low in all soil profiles. The NH₄-N concentration ranged from 0.9 to 2.2 mg kg⁻¹ and no significant differences were observed between the two systems (P > 0.05; Fig. 2 b).

3.4. Active C and N pools

POM-C, POM-N, POM-C/SOC and POM-N/TN in the top 40 cm of the soil profile were significantly higher in the greenhouse soils than the cereal soils and decreased with soil depth in both production systems (P < 0.05; Table 3). POM-C expressed as a percentage of SOC in the greenhouse system ranged between 24.3 and 52.4% and in the conventional system between 16.0 and 27.2%. Similarly, POM-N as a percentage of TN ranged from 14.0 to 31.7% and from 8.3 to 14.6%, respectively. The POM-C/POM-N ratio in the cereal system tended to be higher than in the greenhouses (18.6 versus 13.5) but the difference between production systems was not significant (P > 0.05). Although δ^{13} C of POM-C did not differ significantly between the two systems, δ^{15} N of POM-N and δ^{13} C/



Fig. 1. Soil organic carbon (SOC) and total nitrogen (TN) content, C/N ratio of soil organic matter, and the respective $\delta^{13}C$ and $\delta^{15}N$ abundances in the soil profiles of greenhouse (G) and a conventional (C) production systems. Values at each depth are the means of four replicates. An asterisk represents a significant difference (P < 0.05) between greenhouse and conventional production systems.

Table 2

Storage of soil organic carbon (SOC), soil inorganic carbon (IC), total carbon (TC) and total nitrogen (TN) in the soil profile (0–100 cm) of greenhouse (G) and conventional cereal (C) production systems.

Site	te SOC (t C ha ⁻¹)		ΔSOC (t C ha ⁻¹ yr ⁻¹)	IC (t C ha ⁻¹)		$\frac{\Delta IC}{(tCha^{-1}yr^{-1})}$	TC (t C ha ⁻¹)		ΔTC (t C ha ⁻¹ yr ⁻¹)	TN (t N ha ⁻¹)		Δ TN (t N ha ⁻¹ yr ⁻¹)
	G	С	G–C	G	С	G–C	G	С	G–C	G	С	G–C
Yingli 1 Yingli 2 Tianliu 1 Tianliu 2	95.5 109.5 89.8 109.1	68.6 88.9 89.3 85.4	2.46 1.87 0.07 1.69	360.7 343.2 15.1 5.0	399.3 433.8 34.7 23.1	-3.51 -8.24 -1.40 -1.30	456.2 452.7 104.9 114.1	467.8 522.7 124.0 108.6	-1.05 -6.37 -1.33 0.39	14.6 12.4 9.9 9.4	8.8 9.1 9.2 6.7	0.53 0.12 0.08 0.10



Fig. 2. Concentrations of NO₃-N and NH₄-N in the soil profiles of greenhouse (G) and conventional (C) production systems. Values at each depth are the mean of four replicates. An asterisk represents a significant difference (*P* < 0.05) between greenhouse and conventional production systems.

 δ^{15} N ratio of POM were significantly higher in the greenhouses than in the cereal soils at 0–20 cm depth (*P* < 0.05).

In both production systems the concentrations of SMBC and SMBN decreased with soil depth (Table 4). Both SMBC and SMBN were higher in the conventional system than in the greenhouses (P > 0.05). The concentration of SMBC ranged between 84.1 and

284.8 mg kg⁻¹, and that of SMBN ranged between 12.4 and 44.8 mg kg⁻¹ across both production systems. The percentage of SMBC to SOC and SMBN to TN ranged from 1.3% to 2.8% and 1.6% to 4.6%, respectively. Below 10 cm soil depth, SMBC/SOC and SMBN/TN in the conventional system were significantly higher than in the greenhouses (P < 0.05). SMBC/SMBN ranged from 5.8 to 7.1, and

Table 3

Concentration of particulate organic matter C (POM-C) and N (POM-N), natural abundance and their ratio as well as their respective percentage of SOC and TN between greenhouse (G) and conventional cereal (C) systems; values are the means of four replicates; numbers in parentheses indicate standard error.

Cropping system	POM-C (g kg ⁻¹)	POM-δ ¹³ C (‰)	POM-N (×10 ⁻³ g kg ⁻¹)	POM-δ ¹⁵ N (‰)	POM-C/SOC (%)	POM-N/TN (%)	POM-C/POM-N	$\delta^{13}C/\delta^{15}N$
0–10 cm G C	7.1(2.0) 2.9(1.3)	-21.5(0.9) -21.5(1.4)	511.4(113.9) 168.9(47.6)	6.2(0.8) 2.6(1.0)	52.4(6.1) 27.2(6.7)	31.7(6.0) 14.4(8.2)	13.8(1.8) 16.7(3.5)	-3.5(0.6) -9.3(4.4)
<i>t</i> -Test 10–20 cm	*	ns	**	**	**	*	ns	**
G C <i>t</i> -Test	5.2(1.6) 2.0(0.4)	-21.5(1.4) -20.9(0.4) ns	385.2(92.7) 118.9(27.1)	6.2(0.8) 3.0(0.2)	44.4(1.6) 24.7(4.1)	28.7(4.5) 14.6(2.2)	13.5(1.5) 17.2(3.1) ns	-3.5(0.7) -6.9(0.3)
20–40 cm	15(02)	10.9(1.6)	105 7(28 6)	71(15)	242(22)	140(24)	148(22)	20(0.7)
C <i>t</i> -Test	0.8(0.3)	-17.6(1.2) ns	44.9(16.4)	6.2(0.6) ns	16.0(4.1)	8.3(2.2)	14.6(2.5) 18.6(0.6) ns	-2.9(0.7) -2.9(0.4) ns

Significant at P < 0.05.

^{*} Significant at P < 0.01; ns, not significant at P > 0.05.

Table 4

Soil microbial biomass (SMBC and SMBN) concentrations, SMBC/SMBN and their respective percentages in SOC or TN in greenhouse and conventional production systems. Values are the means of four replicates; numbers in parentheses indicate standard error.

Cropping system	SMBC (mg kg $^{-1}$)	SMBN (mg kg ⁻¹)	SMBC/SOC (%)	SMBN/TN (%)	SMBC/SMBN
0–10 cm					
G	215.0(82.8)	31.6(12.5)	1.60.6)	1.9(0.7)	6.8(1.2)
С	284.8(70.1)	44.8(22.6)	2.8(0.3)	3.4(1.3)	7.0(1.7)
t-Test	ns	ns	*	ns	ns
10–20 cm					
G	163.5(90.6)	27.2(11.6)	1.3(0.5)	2.0(0.7)	5.8(1.6)
С	227.1(40.2)	36.7(3.2)	2.8(0.5)	4.6(1.0)	6.2(1.2)
t-Test	ns	ns	**	**	ns
20–40 cm					
G	84.1(34.5)	12.4(2.4)	1.4(0.6)	1.6(0.2)	6.8(2.8)
С	118.4(23.8)	16.9(3.4)	2.3(0.3)	3.2(0.5)	7.1(1.2)
t-Test	ns	ns	*	**	ns

* Significant at P<0.05.

* Significant at P < 0.01; ns, not significant at P > 0.05.

Table 5

Dissolved organic matter (DOC and DON) concentration, DOC/DON and their respective percentages in SOC and TN in greenhouse and conventional production systems. Values are the means of four replicates; numbers in parentheses indicate standard error.

Cropping system	DOC $(mg kg^{-1})$	DON $(mg kg^{-1})$	DOC/SOC (%)	DON/SON (%)	DOC/DON
0–10 cm					
G	50.0(20.5)	9.3(4.0)	1.9(0.7)	0.4(0.09)	5.9(2.0)
С	35.2(2.1)	7.9(4.3)	3.4(1.3)	0.4(0.07)	6.0(4.1)
<i>t</i> -Test	ns	ns	ns	ns	ns
10–20 cm					
G	45.5(19.7)	9.5(4.4)	2.0(0.7)	0.4(0.13)	4.9(0.5)
С	31.1(1.8)	10.8(1.1)	4.6(1.0)	0.4(0.05)	2.9(0.1)
<i>t</i> -Test	ns	ns	**	ns	**
20–40 cm					
G	25.0(4.1)	8.4(3.8)	1.6(0.2)	0.4(0.09)	3.4(1.3)
С	20.5(4.1)	8.3(1.3)	3.2(0.5)	0.4(0.04)	2.5(0.6)
<i>t</i> -Test	ns	ns	**	ns	ns

^{**} Significant at P < 0.01; ns, not significant at P > 0.05.

the ratio in the conventional system was greater than in the greenhouses, though the difference was not significant (P > 0.05).

The concentration of DOC at 0–40 cm soil depth was higher in the greenhouse system than that in the conventional system and the concentration of DON in both production systems was largest at 10–20 cm soil depth (Table 5). Differences in DOC or DON were not significant in either production system (P > 0.05). DOC as a percentage of SOC and DON as a percentage of TN were 1.6 to 4.6% and 0.4%, respectively. DOC/DON ratio ranged between 2.5 and 6.0. At 10–20 cm depth DOC/DON was significantly higher in the greenhouse system than in the cereal rotation (P < 0.01).

4. Discussion

4.1. SOC, IC and TN

The higher SOC concentration in the greenhouse system (Fig. 1a) may be attributable to the numerous different types of manure incorporated into the soil to maintain high vegetable yields. The amount of N applied in manures in the greenhouse system at Shouguang was found by He et al. (2007) to be up to 936 kg N ha⁻¹ per year. Higher yields increase root exudation and exudates and dissolved organic matter from manures can move deep down the soil profile with excessive irrigation (Brye et al., 2001). Microorganisms may also make some contribution to SOC in the soil profile. These factors may account for the significant difference in SOC between the two production systems at 20-60 cm depth (P < 0.05). The higher concentrations of TN and NO₃-N in the greenhouse system (Figs. 1b and 2a) may also have resulted from excessive N fertilizer and manure applications (Zhu and Chen, 2002; He et al., 2007). NO₃-N as a percentage of TN was 6.0–10.0% at 0– 100 cm depth in the greenhouse system and 1.2-8.1% in the conventional system. Excessive NO₃⁻ leaching in the highly irrigated greenhouse system may have resulted in heavy pollution of the groundwater (Li et al., 2007), with the irrigation rate reaching 1800 mm per year in the greenhouse system (He et al., 2007).

Discrimination against the heavier C isotope can result in less negative values in C₄ plants (e.g. maize) than in C₃ plants (e.g. vegetables) and a similar effect may have occurred during the decomposition of the applied manures. Excessive manure application in the greenhouses may have been mainly responsible for the difference in δ^{13} C in the surface soil but little change was found below 10 cm depth in both production systems (Fig. 1d). The difference at 10–40 cm depth might be related to root distribution and maize/wheat rotation because the root distribution of vegetables is much more restricted than that of maize or wheat (Zotarelli et al., 2009; Nakamoto, 2000). δ^{13} C at 40–100 cm may

reflect the soil background because the δ^{13} C values in the two production systems were almost equivalent (Fig. 1d). Mineral N fertilizers are originally derived from atmospheric N₂ and their δ^{15} N is close to 0‰. In contrast, composts become enriched in ¹⁵N by a series of biological and chemical processes (Kerley and Jarvis, 1996). Watzka et al. (2006) found that the δ^{15} N of various organic fertilizers and composts varied within the range 6.0–14.3‰. We also measured the δ^{15} N of different fertilizers and manures and obtained values of: (NH₂)₂CO 0.2%, NH₄HCO₃ 2.4%, (NH₄)₂HPO₄ 0.6‰, cattle manure 7.9‰, pig manure 11.9‰ and chicken manure 16.8‰. Thus, the δ^{15} N of surface soils in the conventional system with chemical fertilizer was less dominant than that in the greenhouses with large manure inputs (Fig. 1e). The variation in δ^{15} N below 20 cm soil depth may be related to the accumulation of NO₃-N (Fig. 2a) in the profile after long-term excessive fertilizer N application.

The SOC/TN ratio reflects the stability of soil organic matter and relative decomposition stage and the age of the humus (Vejre et al., 2003; Russell et al., 2005). As Fig. 1f shows, at 0–20 cm depth the regenerative rate of SOM in the greenhouse system is faster than in the conventional system because the fresh SOM formed from manures is less stable than the native SOM (Springob and Kirchmann, 2003). Below 20 cm depth the SOC/TN ratio was lower in the greenhouse soil than in the cereal areas (Fig. 1c), due mainly to N-rich organic material deposition (e.g. POM-C & POM-N in Table 3), the accumulation of excessive NO₃-N (Fig. 2a) and the massive irrigation, with NO₃⁻ facilitating soil H₂O hydrolysis for the charge balance with further decline in soil carbonate (Table 2) as a result of soil acidification (Table 1) (Barak et al., 1997; Malhi et al., 1998; Ju et al., 2007).

The contents of SOC and TN were no more than 110 t C ha⁻¹ and 15 t N ha⁻¹ at 0–100 cm depth in the soil profile in the two studies systems, respectively (Table 2). There could be considerable potential for C and N sequestration in the two systems in the study area if protective management practices were adopted (Batjes, 2002). The main component of soil total C (TC) is soil inorganic C (IC) at the Yingli site and SOC at the Tianliu site (Table 2). IC content in the greenhouse system at the four sites was lower than in the conventional system, and this may be attributed to decreasing soil pH in the greenhouse system as shown in Table 1. Soil acidification was likely the result of the nitrification of excessive applied N and NO_3^- leaching under the unrestricted irrigation regime (Ju et al., 2007).

4.2. Soil active C and N pools

POM can respond sensitively to management practices (Mrabet et al., 2001) and can enhance soil aggregation by different binding

agents such as fungal hyphae, fine roots, bacterial cells and polysaccharides (Jastrow and Miller, 1997). The increase in C and N concentrations of POM (Table 3) in the greenhouse system can be attributed to excessive manure and fertilizer applications. Compared to the conventional system, δ^{13} C and δ^{15} N of POM at 0-20 cm depth indicate that the contribution of manure to POM-N was significantly greater (P < 0.05) than that to POM-C in the greenhouse system. Mendham et al. (2004) reported that a higher quality POM could lead to a decline in net N mineralization rate: POM-C as a percentage of SOC and POM-N as a percentage of TN were significantly higher in the greenhouse soils (P < 0.05). According to Marriott and Wander (2006a,b), this would indicate that manure application facilitated an increase in POM concentration and that soil aggregation would increase. However, in the greenhouse system in Shouguang county soil carbonate collapse (Table 2) indicates that the original soil structure was undergoing deterioration.

The POM-C/POM-N ratio can reflect the original C/N ratio of organic materials incorporated into the soil and their degree of decomposition (Gregorich et al., 1996; Christensen, 2001). The POM-C/POM-N ratio in the greenhouse system (13–15) was reduced compared to the conventional counterparts (16–19) and the ratio of POM- δ^{13} C to POM- δ^{15} N was significantly different in the two systems (P < 0.05; Table 3). These results reflect the significant alteration of the original organic materials of POM after over one decade of excessive N-rich and low C:N manure amendment in the greenhouses. Cereal (both wheat and maize) residues have high C/N ratios which are reflected in the higher POM-C/POM-N ratio in the conventional system.

Soil microbial biomass accounts for 1–5% of the SOC and TN pools (Perelo et al., 2006) and our results are in agreement with this (Table 4). At different soil depths the higher proportion of SMBC to SOC and SMBN to TN in the conventional system indicate that there were more complex microbial communities for substrate-use efficiency with inputs of exogenous materials (Moore et al., 2000). In general, manures markedly increase soil microbial biomass (Masto et al., 2006) but the higher SOC and TN content with the lower concentrations of SMBC and SMBN in the greenhouse system (Fig. 1a and b, Table 4) indicate that the capacity of nutrient cycling in the greenhouse system decreased.

The ratio of SMBC/SMBN can partly reflect soil microbial community structure and status. Paul and Clark (1989) reported that the C/N ratios for bacteria ranged from 3 to 5 and those for fungi from 4 to 15. In our study the SMBC/SMBN values were in the range 5.7–7.1. SMBC/SMBN ratio can be impacted by soil quality properties such as pH, texture, structure, microbial diversity, SMBC/SOC and SMBN/TN (Moore et al., 2000; Bastida et al., 2008) and also by root density, root depth and root exudates (Moore et al., 2000). These factors may contribute to the lower SMBC/SMBN ratio in the greenhouse system in comparison with the conventional system, as shown in Tables 1 and 4.

DOM (DOC and DON) plays an important role in soil biogeochemical processes (Kalbitz et al., 2000; Kalbitz and Geyer, 2002; Park et al., 2002). Lower DOM values in our study (Table 5) compared to those in grassland soils reported by Ghani et al. (2007) may have resulted from differences in climate, disturbance through tillage, and fertilizer applications. The higher DOC the in greenhouse system and significant difference in DOC/SOC (P < 0.05) below 10 cm soil layer in both production systems (Table 5) may have been due to excessive manure applications (Borken et al., 2004) and the acidification of the soil (Table 1) Thus low soil pH can intensify the leaching of bridging cations (e.g. Ca²⁺, Mg²⁺) and further enhance the solubilization of organic matter (Zech et al., 1994). DON release would have been affected by the inorganic N pool (Kalbitz and Geyer, 2002) and so the combined

effects of NO_3^- leaching and root capture may have contributed to the occurrence of the maximum DON at 10–20 cm depth in the soil profiles.

The DOC/DON ratio is important in predicting gross N mineralization (Kalbitz et al., 2000) and Ghani et al. (2007) reported DOC/DON ratios ranging from 5 to 6 in most soils. In the present study the ratio ranged from 2.5 to 6.0 (Table 5) due to the exogenous substrate amendment. However, this may still have contributed to the differences in DOC/DON between soil depths in both production systems because N fertilization would have increased DON concentrations while leaving DOC concentrations unchanged (Neff et al., 2000).

5. Conclusions

Shifting from the conventional cereal production system to highly intensive greenhouse vegetables has resulted in higher accumulated NO₃-N, higher TN, and lower SOC/TN ratios and total C stocks due to a dramatic decline in carbonates in the soil caused by intensive ammonium fertilization and massive irrigation, which involved high nitrification rates and a large release of protons as well as nitrate leaching. There were no significant differences between the two production systems in the isotopic signatures of SOC and TN (P > 0.05) but the SOC in surface soil contained much more newly formed C sources (POM-C) and the δ^{15} N of POM-N responded sensitively to the effects of the exogenous substrates (P < 0.05). Soil quality may deteriorate with low SOC/TN ratios and very low SMBC/SOC and SMBN/TN ratios (P < 0.05) in the greenhouse system. In addition, large accumulation of NO₃-N poses a considerable threat to the quality of groundwater and may even be harmful to local residents. In conclusion, the potential conservation of soil C and N stocks will be greater on the North China Plain if the negative effects of excessive N applications and massive irrigation can be avoided in intensive greenhouse vegetable production systems.

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