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Changes in soil carbon and nitrogen pools in a Mollisol after long-term fallow or application of chemical fertilizers, straw or manures

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ABSTRACT

Appropriate fertilizer management can effectively restrain decreases in soil carbon (C) and nitrogen (N) in agricultural Mollisols in northeast China. A 22-yr-old field experiment was employed to explore the effects of fallow, chemical fertilizer N, phosphorus (P), potassium (K), or straw or manure combined with NPK (SNPK, MNPK), and 1.5 times the N rate of MNPK (1.5MNPK) application on soil C and N pools. Soil organic C (SOC), total N (TN), inorganic C (IC), and mineral N (NO_3-N, NH_4-N) to 100 cm soil depth and C and N in particulate organic matter (POC, PON), soil microbial biomass (MBC, MBN), and dissolved organic matter (DOC, DON) to 40 cm soil depth were determined. In all treatments, the 1.5MNPK treatment had the significantly highest ($P < 0.05$) SOC and TN concentrations above 40 cm soil depth and the SNPK treatment had the significantly highest ($P < 0.05$) IC concentration at 40–60 and 60–80 cm soil depths. Comparing the other treatments to 40 cm soil depth, in most cases 1.5MNPK and MNPK treatments at each depth had significantly higher ($P < 0.05$) POC, PON concentration, POC/SOC and PON/TN. SNPK treatment had significantly higher ($P < 0.05$) MBN and MBN/TN at 10–20 and 20–40 cm soil depths and the fallow treatment had significantly higher ($P < 0.05$) MBC and DOC concentrations and higher DOC/SOC ratio at each soil depth. The optimum combination of manure, straw and NPK therefore favored C and N storage in this agricultural Mollisol.

1. Introduction

Soil carbon (C) and nitrogen (N) stocks have become a subject of unprecedented concern in the desire to mitigate increasing global atmospheric greenhouse gas emissions (Sainju et al., 2008; Gattinger et al., 2012; MacBean and Peylin, 2014; Shcherbak et al., 2014) because soil C storage benefits C trading and decreases CO_2 emissions and also improves soil quality. Increasing soil N immobilization can also reduce the application rate and cost of fertilizer N and protect the environment from the negative effects of reactive N species. Soil C and N are more vulnerable and complex in agricultural systems than in natural ecosystems due to disturbance resulting from management practices such as fertilizer applications and tillage practices. The accumulation and dynamics of soil C and N are long-term processes and their study requires long-term fertilization experiments (Qiu et al., 2010; Mazzoncini et al., 2011; Meng et al., 2014; Tripathi et al., 2014).

Fertilization practices used in agricultural systems include the application of chemical N, phosphorus (P), and potassium (K) fertilizers, organic fertilizers and animal manures or straw, or combinations of chemical fertilizers and organic sources of nutrients. Nitrogen, P, and K are macronutrients necessary for crop growth and the application of the chemical fertilizers can affect soil C and N accumulation through increasing biomass production (Tang et al., 2008; Lu et al., 2009; Mazzoncini et al., 2011; Qiu et al., 2014; Zhao et al., 2014). Application of organic

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fertilizers or combinations of chemical and organic fertilizers can further increase soil organic C (SOC) and total N (TN) concentrations because of enrichment with C sources and additional macronutrients and micronutrients present in organic fertilizers and especially in manures (Watts et al., 2010; Zhao et al., 2014). Overall, the storage of SOC or TN depends on the input and output balance of C or N when other nutrients are present at concentrations that do not limit crop growth. Some studies have reported that unbalanced nutrients (van Groenigen et al., 2006) or excessive nutrient application (Qiu et al., 2010) may constrain soil C and N storage, for example soil acidification due to excessive N application (Guo et al., 2010) can result in the loss of soil carbonates and further decrease soil total C storage (Qiu et al., 2010). Moreover, excessive N application will increase the risk of N loss under the limitation of available C sources (Qiu et al., 2013, 2015b). Regardless of the contribution of crop yield for food demand, the conversion of agricultural soil to fallow may increase SOC and TN storage in the surface soil due to vegetation recovery and annual plant residue return to the surface soil (Zhou et al., 2013). However, croplands in developing countries are required to be used for intensive food production to meet the needs of an increasing population and it is difficult to include fallow land in the intensively managed arable systems used. The selection of rational fertilization practices in arable areas therefore plays a vital role in controlling soil C and N storage but little information of soil C and N storage (including carbonates) is available and long-term field experiments are required to explore the relationships between fertilization practices and soil organic matter dynamics.

Soil active C and N pools respond sensitively to fertilization practices and affect nutrient supply or storage (Wander, 2004; Andruschkewitsch et al., 2013). These active pools can be denoted by C and N in particulate organic matter (POC, PON), microbial biomass (MBC, MBN), and dissolved organic matter (DOC, DON). Particulate organic matter (POM) is a nucleus for aggregates (Marriott and Wander, 2006a,b), represents a substantial fraction of the fresh plant residues, and is readily accessible for microbial decomposition (Yang et al., 2012). Soil microorganisms turn over the soil organic matter (SOM), and the soil microbial biomass (SMB) represents a nutrient reservoir that contributes to maintaining the sustainability of cropland. Dissolved organic matter (DOM) can be derived from decomposed SOM, the intermediate compounds of microbial metabolism or the death of microorganisms, plant debris, and root excretion (Kalbitz et al., 2000). POM also provides C or N substrates for microorganisms (Wander, 2004; Marriott and Wander, 2006a,b) and may be bound by polysaccharides and hyphae from SMB or DOM (Mendham et al., 2004; Wander, 2004; Qiu et al., 2010). Thus, the differences in the quantity and quality of these active pools after different long-term fertilization practices or fallow can effectively indicate the mechanisms of C and N storage in soils.

The C/N ratio is a key parameter in the evaluation of the quality of soil organic matter or different soil active pools and further reflects soil C and N cycling or status in different soil pools. Application of different chemical or organic fertilizers can affect the soil C/N. A low C/N ratio (<20:1) of exogenous substrates will promote net N mineralization (Stevenson and Cole, 1999) and a higher C/N ratio (>30:1) will lead to transient competition for N between soil microorganisms and crops and increase soil N immobilization. Soil C/N ratio is also under the influence of climatic conditions (temperature, precipitation) and crop rotations from year to year and long-term field experiments are necessary for the study of soil C/N ratio responses to different fertilization practices. Long-term experiments can be used to identify which factors are associated with changes in C/N ratio and which fertilization practices contribute to C and N storage in different soil pools.

The spring maize region in Jilin province in northeast China is predominantly under rain-fed agriculture with highly fertile Mollisol soils and produces 12.6% of the total Chinese maize yield (Qiu et al., 2014; 2015a). However, the soil organic carbon content in this region has decreased markedly since the 1980s (Huang and Sun, 2006) and this situation has further affected soil N storage and buffering capacity. Numerous studies have reported soil C and N pools in surface soils after long-term fertilization practices in China (Cong et al., 2012; Meng et al., 2014) but there is little information regarding the response of C and N pools deeper in the soil profile to different fertilization practices. The upper 100 cm of the soil profile can reflect soil C and N storage because 70% of SOC is located in this zone (Eswarran et al., 1993) and it is also regarded as the available soil depth for optimum N rate recommendations in the NO_3-N test method (Chen et al., 2011). Furthermore, DOM and NO_3-N can easily leach to the deeper soil but the upper 40 cm of the soil profile can indicate changes in C and N in different active soil pools due to the presence in this zone of >80% of crop roots and the effects of agricultural management practices on the growing roots (Peng et al., 2010; Qiu et al., 2010).

The objectives of the present study were therefore to compare soil total C and N storage in the upper 100 cm of the soil profile under different fertilization practices and fallow in northeast China and to explore the changes in C and N in different soil active pools in the upper 40 cm of the soil after different long-term fertilization practices and fallow in this region.

2. Materials and methods

2.1. Site

The field experiment was established in 1990 at Jilin Academy of Agricultural Science's Gongzhuling experiment station (43°30′N, 124°48′E, 200 m above sea level), Gongzhuling county, Jilin province, northeast China. This region has a typical continental monsoon climate, is predominantly under rain-fed agriculture and is a major maize producing region. The annual mean temperature is 4–5 °C and the annual cumulative mean temperature for days with mean temperatures above 10 °C is 2000–3600 °C. The annual frost-free period is 125–150 d. The annual precipitation in the maize production area is 500–900 mm with 60% of rainfall occurring during the summer (July–September). The soil is classified as a Haplic Phaeozem by the FAO-UNESCO classification system and as a Mollisol in the USDA classification (Boerma et al., 1995). The top 20 cm of the soil profile was sampled at the start of the field experiment and after the maize harvest in October 1989 and the basic soil properties are shown in Table 1.

2.2. Experimental design

The field experiment was composed of seven treatments with three replicates in a completely randomized block design. The treatments were (1) fallow, no nutrient application or tillage; (2) control (CK), no nutrient application; (3) application of fertilizer nitrogen only (N); (4) combined application of fertilizer N, phosphorus (P) and potassium (K) (NPK); (5) combined application of fertilizer NPK plus straw return (SNPK), with a ratio of chemical fertilizer N to straw N of 7:3; (6) combined application of fertilizer NPK plus horse manure application (MNPK), with a ratio of chemical fertilizer N to manure N of 3:7; (7) MNPK treatment at 1.5 times each fertilizer rate (1.5MNPK). The equivalent fertilizer rate in treatments (2)–(6) was 165 kg N ha^{-1} as urea, 36 kg P ha^{-1} as triple superphosphate, and 68 kg K ha^{-1} as potassium chloride. The area of each plot was 220 m^2. All the treatments except for the fallow treatment were planted with maize. Treatments (2)–(4) were chemical fertilizer applications and the control treatment

Table 1
Selected soil properties at 0–20 cm soil depth after spring maize harvest at the end of September 1989.

pH	Soil density ($g\,cm^{-3}$)	TC ($g\,kg^{-1}$)	SOC ($g\,kg^{-1}$)	TN ($g\,kg^{-1}$)	alkali-hydrolyzable N ($mg\,kg^{-1}$)	Olsen-P ($mg\,kg^{-1}$)	Exchangeable K ($mg\,kg^{-1}$)
7.2	1.18	15.0	13.0	1.42	131.5	23.3	160.0

was regarded as zero chemical fertilizer application. Treatments (5)–(7) were applications of C sources and treatments (6) and (7) were manure applications. Manure, straw, one-third of the N rate in chemical fertilizers and SNPK treatments, P fertilizer and K fertilizer were broadcast as basal fertilizers before the soil was ploughed, and the remaining two-thirds of the N rate in chemical fertilizers and SNPK treatments or chemical fertilizer N in manure treatments was applied at the maize jointing stage. Other details regarding weed control, pesticide application, maize density and other agronomic management practices in plots planted with maize can be found in Qiu et al. (2014, 2015a). Locally dominant weeds and shrubs were prevalent in the absence of fertilizer or pesticide applications or anthropogenic disturbance in the fallow plots.

2.3. Soil sampling

Spring maize was usually harvested at the end of September or at the beginning of October (Qiu et al., 2014). Soil samples were similar to Qiu et al. (2014). On October 22, 2011, soil cores from three points in each plot were taken from the top 100 cm of the soil profile. Each core was separated into 0–10, 11–20, 21–40, 41–60, 61–80, and 81–100 cm depth categories and mixed thoroughly to obtain composite samples from each depth category. At 0–20 cm soil depth, soil density was determined every three years after the maize harvest during the 22 years of the experiment, and the average values in fallow, CK, N, NPK, SNPK, MNPK and 1.5MNPK treatments were 1.21 ± 0.05, 1.29 ± 0.03, 1.31 ± 0.03, 1.26 ± 0.02, 1.16 ± 0.02, 1.18 ± 0.02 and 1.15 ± 0.02 $g\,cm^{-3}$, respectively. The fresh soil samples were immediately passed through a 2-mm sieve. A portion of sieved soil was air-dried for the analysis of soil total C (TC), TN, SOC, and C and N in POM and the remaining portion of fresh sample was stored at 4 °C for subsequent analysis for mineral N (N_{min}), MBC, MBN, DOC and DON. Total C, TN, and SOC were determined to 100 cm soil depth, and C and N in POM, SMB, and POM were determined to 40 cm soil depth.

2.4. Soil analysis

Soil samples for SOC analysis were pretreated with excess 0.4 M HCl to remove carbonates and then the slurries were washed repeatedly with deionized water until the solution pH was >6 and the samples were oven-dried at 60 °C (Micwood and Boutton, 1998). Particulate organic matter was determined as described by Bronson et al. (2004). Briefly, 25 g air-dried soil (<2 mm) was dispersed with sodium hexametaphosphate solution (1:4 w/v) for 2 h on a reciprocal shaker, the suspension was passed through a 53 μm mess sieve and rinsed thoroughly with deionized water, and the remaining materials on the sieve were oven-dried in a beaker at 60 °C before visible stones and roots were removed. Total C, TN, SOC, POC and PON were determined with a CN analyzer (Macro cube, Elementar, Hanau, Germany) after the soil samples had been passed through a 0.15 mm sieve. Soil inorganic carbon was calculated by the difference between TC and SOC.

Mineral N was extracted with 1 M KCl solution (1:5 soil: water, w/v) and determined with a continuous flow analyzer (FIAstar 5000, FOSS, Hillerød, Denmark). For soil microbial biomass the 2-mm sieved soil was incubated for 7 d at 25 °C and 45% WHC in the dark and the incubated soil was subjected to the $CHCl_3$ fumigation—0.5 mol L^{-1} K_2SO_4 extraction method at a soil:water ratio of 1:4 (w/v) (Brookes et al., 1985). The fumigated and unfumigated C in 0.5 mol L^{-1} K_2SO_4 solution was determined with a TOC analyzer (LiquiTOC II, Elementar, Hanau, Germany) and their N concentrations were determined with a continuous flow analyzer (FIAstar5000, FOSS, Hillerød, Denmark) following the alkaline persulfate oxidation method as described by Cabrea and Beare (1993). The C and N in SMB were calculated as: MBC or MBN = (C or N in fumigated solution—C or N in unfumigated solution)/K_E, where K_E is 0.45 for MBC (Wu et al., 1990) and 0.57 for MBN (Jenkinson, 1988). Dissolved organic C was the value of total organic C in unfumigated solution and DON was the difference between total N and NH_4-N in unfumigated solution (Cookson et al., 2007; Ghani et al., 2007). Soil alkali-hydrolyzable N was determined using the Mason jar diffusion method (Bremner, 1996). Soil Olsen-P was extracted with 0.5 M $NaHCO_3$ and determined using the molybdenum blue colorimetric method at a wavelength of 880 nm (Kuo, 1996). Soil exchangeable K was extracted with 1 M NH_4OAc and determined by atomic absorption spectrophotometry (Helmke and Sparks, 1996). Soil pH was determined in water (1:2.5). Soil bulk density was calculated using the dry weights and the volumes of soil sampled.

2.5. Statistical analysis

Data are expressed on oven-dried basis. One-way analysis of variance was conducted using the SAS version 9.0 software package and mean values of the variables in each treatment were compared using least significant difference at the 5% level.

3. Results

3.1. Soil pH

At each soil depth (Fig. 1) soil pH in the SNPK treatment was significantly ($P < 0.05$) higher than that in the N and NPK treatments except at 80–100 soil depth, and the pH in the fallow treatment was significantly ($P < 0.05$) higher than that in the N and NPK treatments except for fallow versus N treatments at 40–60 cm

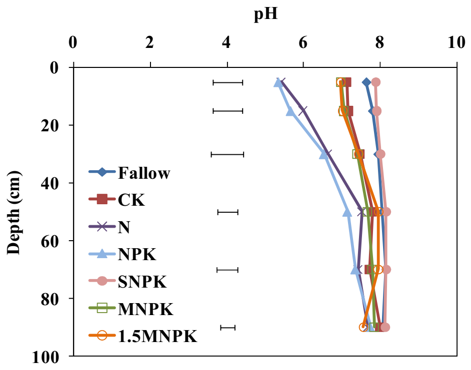

Fig. 1. Change in soil pH at 0–100 cm soil depth after 22 years of different management practices. CK: control, no fertilizer application; N, nitrogen; P, phosphorus; K, potassium; S, straw; M, manure. Values at each depth are the means of three replicates. The horizontal lines denote the least significant difference values at the 0.05 level ($LSD_{0.05}$).

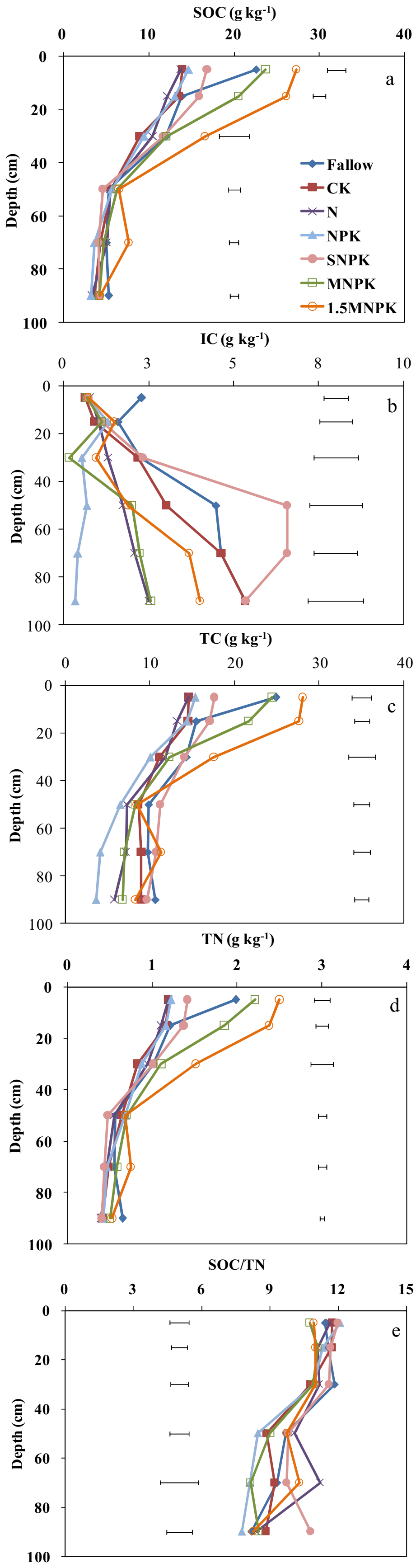

Fig. 2. Soil organic C concentration (SOC), inorganic C concentration (IC), total C concentration (TC), total nitrogen concentration (TN), and ratio of SOC to TN (C/N) at 0–100 cm soil depth after 22 years of different management practices. CK: no fertilizer application; N, nitrogen; P, phosphorus; K, potassium; S, straw; M, manure. Values at each depth are the means of three replicates. The horizontal lines denote the least significant difference values at the 0.05 level ($LSD_{0.05}$).

soil depth. At 0–10 and 10–20 cm soil depths soil pH in MNPK and 1.5MNPK treatments was significantly ($P < 0.05$) higher than in the N and NPK treatments, and they were significantly ($P < 0.05$) lower than in the fallow and SNPK treatments.

3.2. SOC, IC, TC and TN

At each soil depth (Fig. 2a) the SOC concentration in the 1.5MNPK treatment was highest except for 80–100 cm soil depth, and a significant ($P < 0.05$) difference was found between 1.5MNPK and N, NPK, and the control except for 40–60 cm soil depth. At 0–10 cm soil depth (Fig. 2b), the IC concentration in fallow treatments was significantly ($P < 0.05$) higher than the other treatments; at 40–60 and 60–80 cm soil depth (Fig. 2b), the IC concentration in SNPK treatments was significantly ($P < 0.05$) higher than the other treatments. At each soil depth above 40 cm (Fig. 2c) the TC concentration in the 1.5MNPK treatment was significantly ($P < 0.05$) higher than the other treatments. At each soil depth below 40 cm (Fig. 2c) the TC concentration in the SNPK treatment was the highest or second highest among all treatments. Total N concentration (Fig. 2d) showed a similar trend to SOC concentration among the different treatments at each soil depth. However, the TN concentration in the fallow treatment was significantly ($P < 0.05$) higher than in the other treatments at 80–100 cm soil depth. The overall SOC/TN (Fig. 2e) trend was a decrease from 10.7–12.1 at 0–10 cm to 7.8–10.8 cm at 80–100 cm soil depth. At 0–10 cm soil depth, SON/TN in the SNPK treatment was significantly ($P < 0.05$) higher than in the MNPK and 1.5MNPK treatments.

After 22 years of the different fertilization practices (Table 2), the order of the significant ($P < 0.05$) differences SOC, TC and TN sequestration at 0–20 cm soil depth was 1.5MNPK > MNPK > fallow > SNPK, CK, NPK, N. Total N sequestration in CK, N, NPK, and SNPK treatments at 0–20 cm soil depth showed a slight decrease after 22 yr of maize production. The IC content significantly ($P < 0.05$) decreased after 22 years maize cropping comparing with the fallow treatment, and in the fallow treatment at 0–20 cm soil depth remained more or less unchanged.

3.3. NO_3-N and NH_4-N

At each soil depth (Fig. 3) the NO_3-N concentration in the 1.5MNPK treatment was significantly ($P < 0.05$) higher than in the other treatments above 40 cm soil depth, and in the MNPK treatment was significantly ($P < 0.05$) higher than in the fallow, CK, or SNPK treatments above 20 cm soil depth. At each soil depth below 40 cm the NO_3-N concentrations in the N and NPK treatments were the highest and the NO_3-N concentration in 1.5MNPK was significantly ($P < 0.05$) lower than in the N and NPK treatments at 40–60 cm soil depth. Overall, NH_4-N concentrations in all treatments in the upper 100 cm soil depth were <3 mg kg^{-1} except for the NPK treatment at 0–10 cm soil depth and fallow treatment at 0–10 and 10–20 cm soil depth. At 0–10 cm soil depth the NH_4-N concentration in the fallow treatment was significantly ($P < 0.05$) higher than in the other treatments.

3.4. Active C and N pools

3.4.1. POC and PON

At each soil depth above 40 cm (Fig. 4a & b) the POC and PON concentrations in the 1.5MNPK treatment were significantly ($P < 0.05$) higher than in the other treatments except for the

Table 2

Estimation of C and N sequestration in soil organic C (SOC), total carbon (TC), inorganic C (IC), and total nitrogen (TN) pools at 0–20 cm soil depth under different management practices from 1989 to 2011. CK: control, no fertilizer application; N, nitrogen; P, phosphorus; K, potassium; S, straw; M, manure. Values at each depth are the means of three replicates. Negative values denote the loss of C or N. Lower letters in each column denote the least significant difference values at the 0.05 level ($LSD_{0.05}$).

Treatment	SOC		TC		IC		TN	
	Total (t ha^{-1})	Δ (kg ha^{-1} yr^{-1})	Total (t ha^{-1})	Δ (kg ha^{-1} yr^{-1})	Total (t ha^{-1})	Δ (kg ha^{-1} yr^{-1})	Total (t ha^{-1})	Δ (kg ha^{-1} yr^{-1})
Fallow	13.5c	613.4c	13.5c	613.8c	0.01a	0.4a	0.5c	23.0c
CK	4.9d	221.3d	2.2d	97.8d	−2.7b	−123.5b	−0.3d	−14.5d
N	3.4d	153.9d	1.1d	48.1d	−2.3b	−105.8b	−0.4d	−16.1d
NPK	4.3d	194.7d	2.1d	95.2d	−2.2b	−99.5b	−0.4d	−16.6d
SNPK	7.4d	336.0d	4.8d	219.4d	−2.6b	−116.6b	−0.1d	−5.9d
MNPK	21.6b	979.6b	19.0b	862.9b	−2.6b	−116.7b	1.4b	65.7b
1.5MNPK	30.8a	1399.6a	28.6a	1301.8a	−2.2b	−97.7b	2.3a	102.6a

NB: soil C or N sequestration was calculated by the multiplication between the mean of C or N concentration at 0–10 and 10–20 cm soil depths and the mean of soil bulk density every three years during the 22 years of the experiment minus the multiplication between C or N concentration and soil bulk density at the start of the experiment.

POC concentration in both the manure treatments (MNPK and 1.5MNPK) at 0–10 cm soil depth. At 0–10 cm soil depth POC and PON in the control was significantly ($P < 0.05$) lower than in the other treatments. At 10–20 cm soil depth the POC and PON in the SNPK treatment were significantly ($P < 0.05$) lower than in the MNPK treatment and significantly ($P < 0.05$) higher than in the fallow, CK, N and NPK treatments.

At each soil depth above 20 cm (Fig. 4c & d) the POC/SOC and PON/TN in MNPK and 1.5MNPK treatments were significantly ($P < 0.05$) higher than in the other treatments, and POC/SOC and PON/TN in the control were significantly ($P < 0.05$) lower than in the other treatments except at 10–20 cm soil depth.

At each soil depth above 40 cm (Fig. 4e) the POC/PON in MNPK and 1.5MNPK treatments were significantly ($P < 0.05$) lower than in the other treatments except for the NPK treatment at 20–40 cm soil depth, and POC/PON in the control was significantly ($P < 0.05$) higher than in the other treatments at each soil depth above 20 cm.

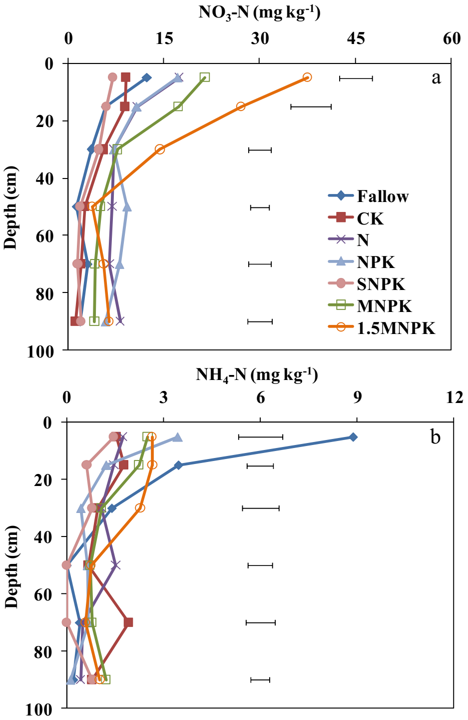

Fig. 3. Concentrations of NO_3-N and NH_4-N at 0–100 cm soil depth after 22 years of different management practices. CK: no fertilizer application; N, nitrogen; P, phosphorus; K, potassium; S, straw; M, manure. Values at each depth are the means of three replicates. The horizontal lines denote the least significant difference values at the 0.05 level ($LSD_{0.05}$).

3.4.2. MBC and MBN

At each soil depth above 40 cm (Fig. 5a) the fallow treatment had a significantly ($P < 0.05$) higher MBC concentration than the other treatments except for the control at 20–40 cm soil depth. At 0–10 cm soil depth (Fig. 5b) the fallow treatment had significantly ($P < 0.05$) higher MBN concentration than the other treatments. Compared to the exogenous C source application treatments (SNPK, MNPK and 1.5MNPK treatments) (Fig. 5b), the chemical fertilizer application treatments (N, NPK) had significantly ($P < 0.05$) lower MBN concentrations at each soil depth above 40 cm.

At each soil depth above 40 cm (Fig. 5c) the fallow treatment had significantly ($P < 0.05$) higher MBC/SOC than the other treatments except for the control at 20–40 cm soil depth. At 0–10 cm soil depth (Fig. 5 d) the fallow treatment had significantly ($P < 0.05$) higher MBN/TN than the other treatments, and at 10–20 and 20–40 cm soil depths the SNPK treatment had significantly ($P < 0.05$) higher MBN/TN than the other treatments. At each soil depth above 40 cm the MBN/TN in the N and NPK treatments was significant ($P < 0.05$) lower than the other treatments at 0–10 and 10–20 cm soil depths.

At 0–10 cm soil depth (Fig. 5e) the chemical fertilizer treatments had significantly ($P < 0.05$) higher MBC/MBN than the other treatments. Moreover, MBC/MBN in the N treatment was significantly ($P < 0.05$) higher than that in the NPK treatment. At 20–40 cm soil depth the MBC/MBN in the fallow treatment was significantly ($P < 0.05$) lower than that in the control, and MBC/MBN in the fallow treatment was significantly ($P < 0.05$) higher than that in the other fertilizers applied treatments.

3.4.3. DOC and DON

At each soil depth above 40 cm (Fig. 6a) the fallow treatment had significantly ($P < 0.05$) higher DOC concentration than the other treatments. At 0–10 and 10–20 cm soil depths the N, NPK, and SNPK treatments had significantly ($P < 0.05$) lower DOC concentrations than the manure application treatments. At each soil depth above 40 cm (Fig. 6b) the 1.5MNPK treatment had the highest DON concentration and became significantly different at 10–20 cm soil depth compared with the other treatments.

Above 40 cm soil depth (Fig. 6c) the DOC/SOC in all treatments ranged from 0.4 to 3.2% with the fallow treatment showing significantly ($P < 0.05$) higher DOC/SOC than the other treatments at each soil depth. Above 40 cm soil depth (Fig. 6d) the DON/TN ranged from 1.4 to 2.5%, the DON/TN in the NPK treatment was the highest in all treatments and significant ($P < 0.05$) higher than in MNPK and 1.5MNPK treatments at 0–10 and 10–20 cm soil depths.

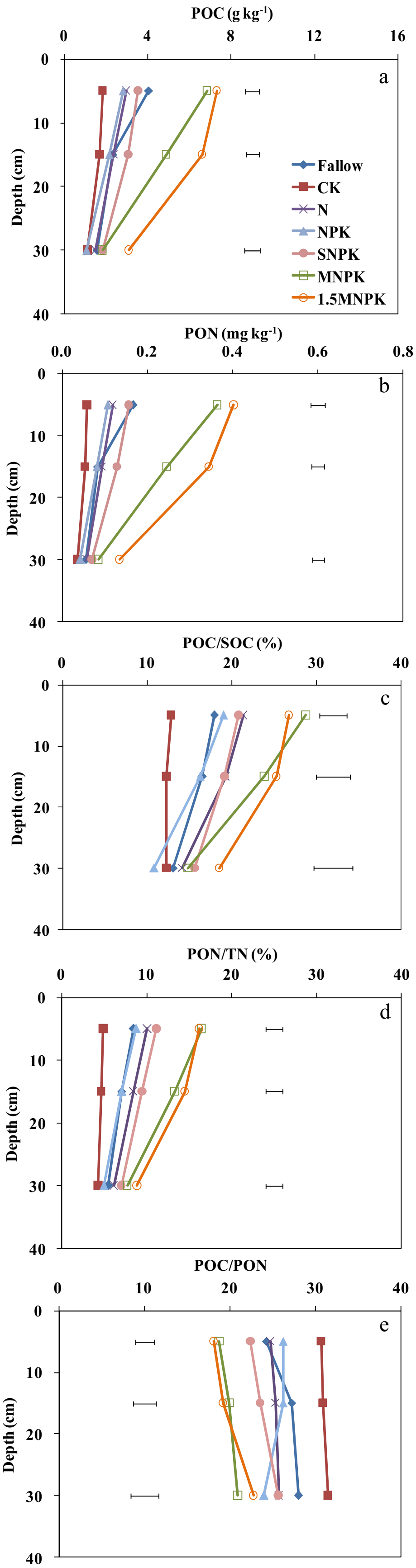

Fig. 4. Particulate organic C and N (POC, PON) concentrations, POC/PON, and the percentage of POC to soil organic C (POC/SOC) or PON to total nitrogen (PON/TN) at 0–40 cm soil depth after 22 years of different management practices. CK: no fertilizer application; N, nitrogen; P, phosphorus; K, potassium; S, straw; M, manure. Values at each depth are the means of three replicates. The horizontal lines denote the least significant difference values at the 0.05 level ($LSD_{0.05}$).

At each soil depth above 40 cm (Fig. 6e) the fallow treatment had significantly ($P<0.05$) higher DOC/DON than the other treatments. In all maize treatments the DOC/DON ranged from 2.9 to 6.6 above 40 cm soil depth.

4. Discussion

4.1. SOC, TN IC, and TC

At 0–20 cm soil depth after 22 years of different fertilization practices from 1989 to 2011 (Table 2) there was no significant difference in SOC sequestration among the CK, N, NPK, and SNPK treatments, but SOC sequestration in the SNPK treatment was slightly higher than in the CK, N, and NPK treatments. On one hand, this indicates that chemical fertilizer applications (N and NPK treatments) produced little increase in SOC sequestration compared to the control; on the other hand, SOC sequestration among CK, N, NPK, and SNPK treatments was closely related to the change in soil bulk density after 22 years of maize production. Jung et al. (2011) reported that soil macroaggregates and porosity decreased with decreasing root biomass and length under conditions of sufficient N supply, and soil bulk density increased further.

Similarly, soil TN sequestration in CK, N, NPK, and SNPK treatments was negative because aboveground N uptake by maize was much greater than N immobilization in the soil, though aboveground N uptake differed among CK, N, NPK and SNPK treatments (Duan et al., 2011; Gao et al., 2011; Zhang et al., 2012). In addition, under the equivalent amounts of N applied in the N, NPK, and SNPK treatments, the low availability of straw N and N immobilization induced by straw C can decrease aboveground N uptake, which might contribute to the N balance (only 0.1 N t ha^{-1} loss) in the SNPK treatment after 22 years of maize production (Table 2). Manures with high available C sources can provide energy for microorganisms and increase C and N immobilization (Watts et al., 2010; Zhao et al., 2014), therefore SOC and TN sequestration at 0–20 cm soil depth after 22 years of maize cropping significantly ($P<0.05$) increased in the MNPK and 1.5MNPK treatments (Table 2).

At 0–20 cm depth from 1989 to 2011 (Table 2), some soil N or straw C was removed from the field when the grain was harvested in CK, N, NPK, and SNPK treatments, while more N in the fallow treatment was assimilated from atmospheric deposits of N (Liu et al., 2013) and subsoil N entered soil N cycling concomitantly with the enriched decaying plant C in the surface soil every year. SOC and TN sequestration in the fallow treatment were therefore significantly ($P<0.05$) higher than in the CK, N, NPK, and SNPK treatments.

In addition, at 0–100 cm soil depth among all the treatments the availability and amount of C source application, root biomass or root exudation, DOM leaching from manure, plant or straw residue decomposition, root biomass and microbial C immobilization in the subsoil together contributed to the change in SOC concentration (Fig. 2a). Similarly, N removal by grain, N immobilization induced C source application, NO_3-N leaching, subsoil N assimilation by roots, and decayed root N transformation to soil N altogether explain the changes in TN concentration (Fig. 2d).

Inappropriate long-term chemical fertilizer application can result in soil acidification (Guo et al., 2010) as indicated by the changes in pH (Fig. 1). The 0–10 cm to 40–60 cm soil depths (Fig. 1) in the N and NPK treatments showed pH decreases because of the substantial removal of mineral nutrients with grain and straw removal at harvest (Guo et al., 2010). Below 20 cm soil depth

(Fig. 2b, c), soil acidification promoted the loss of soil IC and TC concentration in the chemical fertilizer treatments, while the application of exogenous C sources (SNPK, MNPK, 1.5MNPK) decreased the IC loss, especially in the SNPK treatment because manure (Meng et al., 2014) and straw substantially promote the application/return of mineral nutrients to the soil. At 0–20 cm soil depth after 22 years of the experiment (Table 2), significant ($P < 0.05$) lower IC loss in all maize treatments than fallow treatment, and the lack of difference in IC loss in all maize treatments was possibly due to the removal of the assimilated mineral nutrients by maize aboveground from the soil, or the leaching of activated mineral nutrients by applied fertilizers and maize roots to the deeper soil (Guo et al., 2010; Ciampitti et al., 2013). For example, root exudation, NO_3^-, and soil moisture can induce the dissolution of $CaCO_3$ and the free Ca^{2+} and HCO_3^- further leach to the deeper soil when they encounter heavy rainfall, and then they can re-precipitate deeper in the soil (Wu et al., 2009; Tirado-Corbalá et al., 2013). This might result in the significantly ($P < 0.05$) higher IC concentration in SNPK treatment than the other treatments at 40–60 and 60–80 cm soil depths (Fig. 2b). Liu et al. (2014) also reported that farmland showed clear IC accumulation at 70–80 cm soil depth on the Chinese Loess Plateau.

Clearly, the MNPK and 1.5MNPK treatments increased the SOC and TC concentrations above 40 cm soil depth (Fig. 2 a,c), straw returned to the soil increased the IC and TC concentrations at 40–60 and 60–80 cm soil depths (Fig. 2b, c), and therefore the combination of NPK, manure and straw return to the soil will further increase C storage throughout the top 100 cm of the soil profile.

The SOC/TN ratio reflects the interaction of soil C and N cycling and the stability of soil organic matter (Russell et al., 2005; Tong et al., 2009). The availability and amount of C source application and N fertilizer may affect the SOC/TN ratio. For example, large amounts of active C sources and N sources in manure are responsible for the increase in N immobilization in the MNPK and 1.5MNPK treatments; chemical fertilizer N can promote soil mineralization (Malhi and Lemke, 2007) but the mineralized nutrients cannot be re-immobilized immediately with C source limited conditions and this might explain the lower SOC/TN ratio in the manure treatments and higher SOC/TN ratio in the chemical fertilizer treatments (Fig. 2e). The range of the SOC/TN ratio increased with soil depth among all the treatments because of soil NO_3-N leaching, as indicated by the NO_3-N concentration at 0–100 cm soil depth (Fig. 3a).

4.2. Soil active C and N pools

Particulate organic matter can respond sensitively to management practices and acts as a nucleus of soil aggregation (Wander, 2004). Carbon source application in our study increased C and N concentrations in POM and their percentage in SOC or TN (Fig. 4a–d). In particular, in the 1.5MNPK and MNPK treatments they were significantly ($P < 0.05$) higher than in the other treatments and this may be ascribed to the quantity and quality of the manures. Hai et al. (2010) reported that manure application increases soil aggregates and decreases the turnover of soil organic matter, further strengthening the physical protection of POM. The lower POC and PON and their percentages in SOC and TN (Fig. 4a–d) among CK, N, and NPK treatments may be attributed to the shortage of C sources because chemical fertilizer did not substantially increase SOC and TN sequestration after 22 years of different fertilization practices as shown in Table 2.

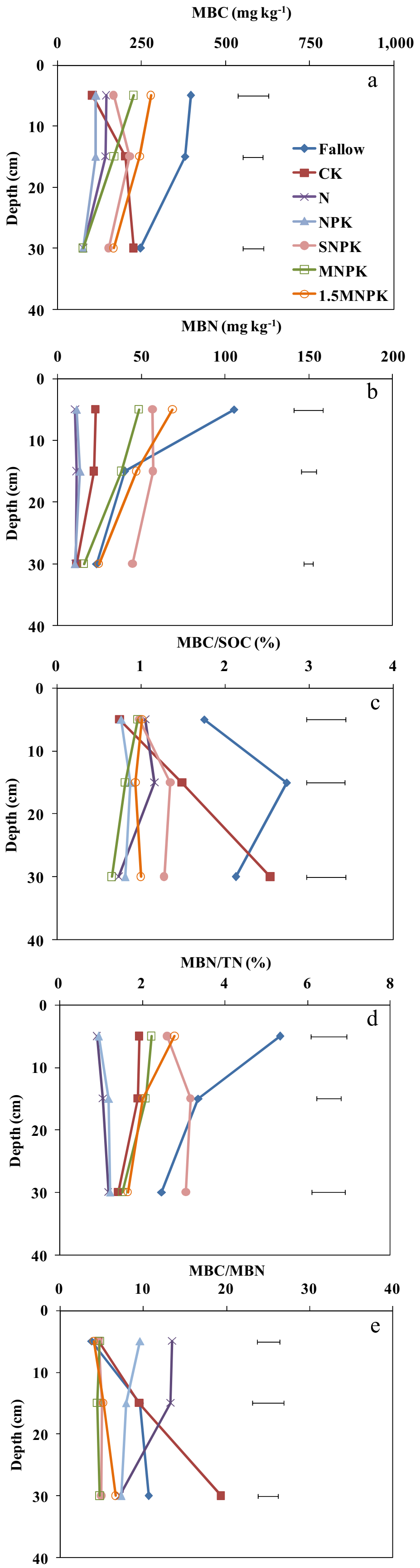

Fig. 5. Soil microbial biomass C and N (MBC, MBN) concentrations, MBC/MBN, and the percentage of MBC to soil organic C (MBC/SOC) or MBN to total nitrogen (MBN/TN) at 0–40 cm soil depth after 22 years of different management practices. CK: no fertilizer application; N, nitrogen; P, phosphorus; K, potassium; S, straw; M, manure. Values at each depth are the means of three replicates. The horizontal lines denote the least significant difference values at the 0.05 level ($LSD_{0.05}$).

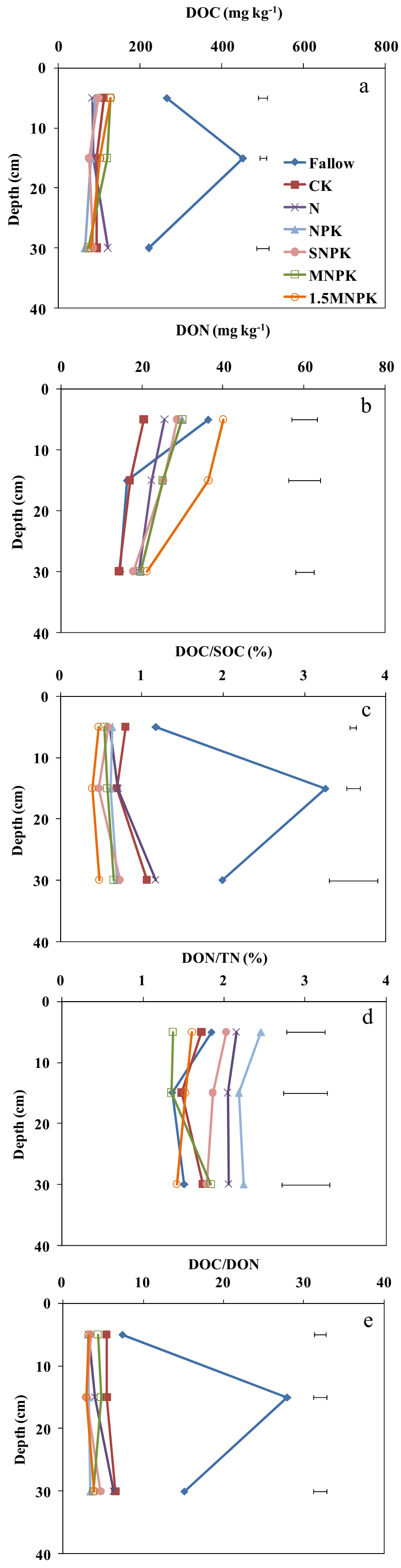

Fig. 6. Dissolved organic C and N (DOC, DON) concentrations, DOC/DON, and the percentage of DOC to soil organic C (DOC/SOC) or MBN to total nitrogen (DON/TN) at 0–40 cm soil depth after 22 years of different management practices. CK: no fertilizer application; N, nitrogen; P, phosphorus; K, potassium; S, straw; M, manure. Values at each depth are the means of three replicates. The horizontal lines denote the least significant difference values at the 0.05 level ($LSD_{0.05}$).

The ratio of POC/PON reflects the original C/N ratio of organic materials incorporated into the soil and their degree of decomposition (Gregorich et al., 1996; Christensen, 2001) and this agrees with the characteristics of low C/N in manure (Fig. 4e) in comparison with straw. At 0–10 and 10–20 cm soil depths the relative shortage of C sources and relative abundant N supply in the N and NPK treatments resulted in higher POC/PON in comparison with the straw and manure application treatments, while POC/PON in the SNPK treatment was higher than that in the manure treatments and lower than that in the chemical fertilizer treatments because the high C:N ratio in straw induced chemical fertilizer N immobilization and further promoted the decomposition of straw. The N depletion in the control because of the removal of aboveground production further increased the ratio of POC/PON (Fig. 4e).

Soil microbial biomass C is closely related to the amount and availability of C sources (Perelo et al., 2006). In most cases at each soil depth (Fig. 5a & c) the significantly ($P < 0.05$) higher MBC and higher MBC/SOC in the fallow treatment than the other treatments and the lowest MBC and MBC/SOC in the chemical fertilizer treatments might be attributable to (1) the higher amount and availability of grass C input in the fallow treatment (Yang et al., 2010; Raphael et al., 2016) than the maize root systems, as shown by the highest DOC and DOC/SOC in the fallow treatment (Fig. 6a & c); (2) the ploughing practice in all the maize treatments accelerating SOM oxidation because of increased soil aeration and stimulated C loss from the exposed SOM by microbial attack (Bini et al., 2014); (3) long-term fallow improving soil structure and maintaining the integrity of fungal hyphae; and (4) the copious supply of active salts in applied manure being disadvantageous to microorganisms and further affecting the size of the microbial biomass, though manure has a great deal of available C and N (Meng et al., 2014). In the present study the control plots were nutrient-deficient, especially in the surface soil; maize roots can assimilate nutrients deeper in the soil by root development (Wang et al., 2006), root secretions can activate soil nutrients to meet plant demand (Wang et al., 2006), and fungi can also assimilate soil degradation-resistant P and transfer available P to the roots via their hyphae (Bucher, 2007). Until the maize harvest the decomposed root residues deeper in the soil may further provide C sources for microorganisms. Therefore, MBC and MBC/SOC in the control (Fig. 5a & c) were lowest at 0–10 cm soil depth and had the second highest or the highest value at 20–40 cm soil depth in all treatments. This explanation is also supported by the second highest or the third highest DOC/SOC and DON/TN (Fig. 6c, e) of the control in all treatments at each soil depth.

Similarly, the size of the MBN depends on C availability, the percentage of N and the C/N of organic amendments (Ocio et al., 1991; Stevenson and Cole, 1999) while competition for N can occur between plants and microorganisms under conditions of low N supply. At a total N rate of 165 kg N ha^{-1} with 3:7 of straw N to chemical fertilizer N, the highest MBN and MBN/TN in the SNPK treatment (Fig. 5b & d) at 10–20 and 20–40 cm soil depths may be due to the combined effects of chemical fertilizer N immobilization after the ploughing in of crushed straw into the soil, plant N uptake, and root growth into the deeper subsoil under conditions of soil N shortage. In the fallow treatment at 0–10 cm soil depth the explanation for the highest MBN and MBN/TN may also be the same as that for TN, MBC and MBC/SOC (Fig. 5).

The ratio of MBC/MBN often describes soil microbial community structure and status (Moore et al., 2000) and most bacteria have a lower MBC/MBN than fungi (Sarathchandra et al., 1988).

Fungi can increase plant P uptake (Bucher, 2007) and this might explain the highest MBC/MBN (Fig. 5e) in the N treatments at 0–10 and 10–20 cm soil depths under conditions of low P, the highest MBC/MBN (Fig. 5e) in the control at 20–40 cm soil depth, and the increasing trend of MBC/MBN in the CK and fallow treatments with increasing soil depth under low N and P conditions (Fig. 5e). Bacteria are usually more abundant than fungi in organic materials applied to soils (Melero et al., 2006), thus MBC/MBN in the SNPK, MNPK and 1.5MNPK treatments was lower than that in the N, NPK, and CK treatments (Fig. 5e).

Long-term manure application can increase DOC and DON concentrations and their leaching (Long et al., 2015). Correspondingly, the increase in DOC can provide available C sources for microorganisms, further increasing DON immobilization (Fig. 6a, b). Thus, similar results were usually found in the MNPK and 1.5MNPK treatments at each soil depth in our study. However, manure application treatments showed relatively low DOC/SOC and DON/TN compared to the N, NPK or SNPK treatments (Fig. 6c, d). On one hand, soil aggregate improvement by manure application further promoted DOC and DON incorporation into soil aggregates (Wander, 2004), but on the other hand, soil acidification induced by chemical fertilizers (Fig. 1) or the activation of mineral nutrients by root exudation further enhanced the solubilization of soil organic matter (Kalbitz et al., 2000). In the fallow treatment at each depth to 40 cm similar factors for MBC and MBN can explain the highest DOC and DOC/SOC and the changes in DOC, DON, DOC/SOC and DON/TN with soil depth (Fig. 6).

The DOC/DON ratio is important in predicting gross N mineralization (Kalbitz et al., 2000) and so the DOC/DON in the fallow treatment showed higher N immobilization than the other treatments (Fig. 6e). The difference in DOC/DON in all maize growth treatments may be attributed mainly to the different DOC or DON concentrations (Fig. 6a, b) induced by the integrated effects of maize root exudation, the different fertilizer applications, and C and N turnover induced by microorganisms (Neff et al., 2000; Long et al., 2015).

Furthermore, the climatic conditions when our samples were collected may have affected soil active C and N pools. At the end of October at our experimental site the air temperature generally decreases below 0 °C at night and increases above 10 °C during the daytime according to the meteorological data (weather data were accessed from the China Meteorological Data Sharing Service System, http://cdc.cma.gov.cn/home.do). Alternating freezing and thawing can disrupt soil aggregates, destroy microbial cells, and release DOM (Kalbitz et al., 2000; Koponen et al., 2006). The subsequently released nutrients may be highly available to the surviving microorganisms. Thus, the climatic conditions in October and the undisturbed soil structure may be responsible for the significantly ($P < 0.05$) highest NH_4-N concentration of the follow treatments at 0–10 cm soil depth in all treatments (Fig. 3b).

According to our results the combination of manure, straw and NPK will benefit C and N storage in Mollisols. Yan et al. (2007) also reported that in a long-term experiment chemical fertilizer, straw return and manure combined benefited SOC and TN storage. In our study significant ($P < 0.05$) NO_3-N leaching occurred (Fig. 3a) when the manure N rate increased from 115.5 to 173.2 kg ha^{-1} with a total N rate from 165 to 247.5 kg ha^{-1} (MNPK and 1.5MNPK treatments), and the optimum N rate is 140 − 210 kg ha^{-1} in this region (Qiu et al., 2015a). The rate of straw return to the soil was approximately 5.0 t ha^{-1} in the SNPK treatment according to the stover N concentration documented by Qiu et al. (2015a). In agricultural Mollisols in Heilongjiang province (to the north of Jilin province), total C and N in the top 20 cm of the soil profile shows a slight decrease when straw return to the soil increases from 2250 to 4500 kg ha^{-1} yr^{-1} after 21 years (Ding et al., 2013). Thus, the optimum ratio of straw, manure and chemical fertilizer N must be investigated in the field taking full consideration of N immobilization induced by straw C and manure C as well as the decomposition of straw and manure based on the climatic conditions in northeast China.

5. Conclusions

Long-term MNPK and 1.5MNPK treatments in a Mollisol soil in Jilin province (Table 2) significantly ($P < 0.05$) increased SOC and TN sequestration compared with the other treatments in the top 20 cm of the soil profile (Table 2). After 22 years of straw and NPK application combined, the IC concentration was significantly ($P < 0.05$) higher than in the other treatments at 40–60 and 60–80 cm soil depths (Fig. 2b), though SOC and TN sequestration in the SNPK treatment were not significantly ($P < 0.05$) higher than in the chemical fertilizer treatments at 0–20 cm soil depth (Table 2). Soil active C and N pools can well indicate soil quality (Bastida et al., 2008; Qiu et al., 2010). In most plots planted with maize, manure applications significantly ($P < 0.05$) increased POC and PON concentrations and their percentages in SOC and TN compared with the other treatments (Fig. 4), and MBC/SOC in SNPK treatment was not significantly ($P < 0.05$) lower than that in the MNPK and 1.5MNPK treatments (Fig. 5c). Moreover, straw return to the soil provided an advantage for microbial N immobilization because MBN/TN in the SNPK treatment was highest at each soil depth in all maize planted treatments, and it was significantly ($P < 0.05$) higher than in the other treatments at 10–20 and 20–40 cm soil depths (Fig. 5d). Therefore, the combination of manure, straw and NPK will be a good choice to further promote C and N sequestration in Mollisols in arable regions such as northeast China, but their optimum rates and proportions require further testing in long-term field experiments.

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