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Soil carbon and nitrogen fractions in the soil profile and their response to long-term nitrogen fertilization in a wheat field

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ABSTRACT

Ecosystems receive elevated inputs of nitrogen (N) from anthropogenic sources, and understanding the effects of such N addition on the soil carbon (C) and N cycles are important. Soils contain many different C and N fractions that have diverse physical and chemical compositions, and these fractions can be used as early and sensitive indicators of C and N cycling. In this study, we investigated the composition of different C and N fractions and their sensitivities in the upper 200 cm of soils planted with winter wheat in areas that had received different N fertilization rates since 2004. Our results indicate that N enrichment may affect C and N cycling by changing certain fractions of soil organic matter. Light fraction of carbon (LFOC), light fraction of nitrogen (LFON), heavy fraction of carbon (HFOC), and heavy fraction of nitrogen (HFON) were constant among the different N rates. In contrast, dissolved organic carbon (DOC) decreased in the 180, 270 and 360 kg ha^{-1} treatments in the 20–200 cm soil layer, and easily oxidizable organic C (EOC) was the highest in the 90 kg ha^{-1} treatment and decreased in the high-N treatments in the 0–20 cm soil layer. Both dissolved organic nitrogen (DON) and inorganic N (NO_3^- and NH_4^+) increased as N input increase. Nitrate (NO_3^-) is the fraction most sensitive to N fertilization. The LFOC, LFON and EOC are more sensitive than other fractions in the surface soil layer, but dissolved organic C and N may be better indicators in the subsurface layers. Our results demonstrate how the C and N fractions respond to different N fertilization rates in the top 200 cm of the soil profile and further our understanding of the physical protection mechanisms of soil organic carbon (SOC) and total nitrogen (TN), which will aid in the adoption of appropriate management practices for C and N accumulation and stabilization in the field.

1. Introduction

Worldwide, great quantities of N are annually applied directly to soils in the form of fertilizer (Fierer et al., 2012), and understanding the effects of such N addition on the soil carbon (C) and N cycles are important. Farmland ecosystems are the most active C sinks, and the C fixation capacity in these systems is largely dependent on fertilization and field management (Fluck, 2012). Therefore, understanding the effects of N fertilization on the C and N cycles benefits both soil productivity and environmental quality (Lal, 2004).

Many studies have shown that field management has a strong effect on soil C and N cycles (Liang et al., 2012b). However, changes in total C and N caused by management practices have been difficult to detect because these changes occur slowly and are relatively small compared to the abundant soil organic carbon (SOC) and total nitrogen (TN), which vary both spatially and temporally (Purakayastha et al., 2008). Therefore, determining whether alternative C or N fractions could be used as indicators of changes in total C and N concentrations would be useful. In general, the labile C/N pool has a greater turnover rate (or shorter mean residence time in soils) of several weeks to months or years, compared with more recalcitrant pools (Paul et al., 2001).

The process of separating soil C and N into different physical components (i.e., light fractions of C (LFOC) and N (LFON), dissolved organic C (DOC) and N (DON)) and chemical components (i.e., easily oxidizable organic C (EOC)) and evaluating their individual responses to field management practices are useful ways to detect soil C and N changes (Davidson and Janssens, 2006). These fractions, which feature different stabilities and turnover rates, are influenced by agricultural management practices (Silveira et al., 2008) and can be used as early and sensitive indicators of changes in SOC and TN (Haynes, 2000).

The light fractions of the C and N pools largely represent degraded plant material together with microbial tissues and products that are not associated with mineral soil particles (Six et al., 2002). These fractions represent an unprotected pool of soil organic matter (SOM) that responds more rapidly to agricultural management than the total SOM pool. Thus, these light fractions can serve as early indicators of the effects of management practices (Bending et al., 2004; Janzen et al.,

Abbreviations: LFOC, light fraction of carbon; HFOC, heavy fraction of carbon; LFON, light fraction of nitrogen; HFON, light fraction of nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; EOC, easily oxidizable organic carbon; DOM, dissolved organic matter; NO_3^-, nitrate nitrogen; NH_4^+, ammonium nitrogen; SI, sensitivity index.

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1992; Leifeld and Kögel-Knabner, 2005). Dissolved organic matter (DOM) is widely known to play a dominant role in several soil processes (Jardine et al., 1989) and is more sensitive than the total SOM pool. Microorganisms mediate DOM formation and decay. A number of abiotic parameters govern the DOM dynamics in soil (Zsolnay, 2003). EOC is composed of amino acids, simple carbohydrates, a portion of microbial biomass and other simple organic compounds (Zou et al., 2005). Nitrate and ammonium are the major forms of inorganic N in soil, and, as the sources of N absorbed by crops, help determine the fertility of the soil. However, nitrate can easily leach into deeper soil and escape as runoff with precipitation, resulting in environmental problems (Kunrath et al., 2015).

Currently, large amounts of urea are applied to farmland soil, resulting in nitrate leaching, increased soil acidity and other environmental issues (Guo et al., 2010). The alteration of soil properties leads to changes in C and N cycling, but the effects are inconsistent. Certain studies have reported that inorganic fertilizer application resulted in significant increases in SOC and its fractions due to the positive effects of the fertilizer on crop growth and, in turn, crop C return (Gong et al., 2009a; Purakayastha et al., 2008). However, in other studies, inorganic fertilizer application, either balanced or unbalanced, was not associated with any significant effects on SOC or its fractions (Lou et al., 2011b; Manna et al., 2006; Rudrappa et al., 2006). However, these studies primarily focused on surface soil or the top 100 cm, yet N fertilization can have considerable effects on deeper soil via leached nitrate (Di and Cameron, 2002). Therefore, understanding how the distributions of the C and N fractions respond to N fertilization throughout the soil profile is necessary. Additionally, long-term fertilization studies with multiple nitrogen treatments with the goal of detecting C and N changes are lacking.

The present study investigated the C and N fractions at a depth of 0–200 cm in soil planted with winter wheat in areas that had received different N fertilization rates since 2004. The objectives of the study were to (1) investigate the effects of long-term N fertilization on the profile distribution of different C and N fractions; (2) evaluate the sensitivity of each C and N fraction to N fertilization; and (3) understand the relationships between N fertilization and changes in the C and N fractions. The results will help clarify how the C and N fractions respond to different N fertilization rates and provide insight into the physical protection mechanisms of SOC and TN, facilitating the development of appropriate management practices for C and N accumulation and stabilization in the field.

2. Materials and methods

2.1. Experimental site and climatic conditions

Beginning in October 2004, the study was set up in an experimental field at the Institute of Soil and Water Conservation of Northwest A&F University, Yangling, Shaanxi (34°17′56″N, 108°04′7″E). The experimental site, located on the southern boundary of the Loess Plateau, features a temperate, semi-humid climate, with a mean annual temperature of 13 °C and a mean annual precipitation of 632 mm, approximately 60% of which falls between July and September. The location was managed under a stubble-free winter wheat–corn rotation with a chisel plow tillage system before the application of the experimental design. Taxonomically, the soil is a Udic Haplustalf in the United States Department of Agriculture (USDA) system and a Eum-Orthic Anthrosol in the Chinese Soil Taxonomy system (Liang et al., 2012a). Selected soil physical and chemical properties of the 0–20 cm layer before fertilization (based on 10 randomly selected replicates) are presented in Table 1.

2.2. Experimental design

We used a randomized block design with six 3-replicate treatments with winter wheat cultivars (Changhan No. 58). N was applied at five rates: 0 kg ha^{-1}, 90 kg ha^{-1}, 180 kg ha^{-1}, 270 kg ha^{-1} and 360 kg ha^{-1} (termed N0, N90, N180, N270 and N360, respectively). The plots had an area of 2 m × 3 m, and each plot included twenty 15-cm-spaced rows of wheat, in which 90 seeds were sown. Wheat was sown in early October and harvested in early June of the following year. Immediately prior to wheat sowing, fertilizer was evenly spread on the soil surface and then incorporated into the upper 15 cm of soil via chiseling. N was applied in the form of urea, and P was applied in the form of superphosphate (33 kg P ha^{-1}). During the study, the soil was never irrigated, weeds were regularly removed, and no tillage was performed during the growth stage. Three blank plots, used as controls (CK), received no fertilization or crops, but the other field management practices were the same as in the treatment plots.

Table 1
Selected physical–chemical properties of the uppermost 20 cm soil layer before fertilization.

Property	Value
Taxonomy	Udic Haplustalf
Texture	
2000–50 μm (g kg^{-1})	64
50–2 μm (g kg^{-1})	694
<2 μm (g kg^{-1})	342
Bulk density (g cm^{-3})	1.23
pH (H_2O)	8.25
Water-holding capacity (%)	23.6
Soil organic carbon (g kg^{-1})	8.79
Soil total nitrogen (g kg^{-1})	0.96
Available N (mg kg^{-1})	25.10
Available P (mg kg^{-1})	7.90

The values are the mean of ten replicates that were randomly collected before fertilization.

2.3. Measurements

After the harvest in 2014, three soil samples were randomly collected from each treatment plot down to a depth of 200 cm and were divided into 10 sections (every 20 cm for 0–200 cm) using a soil-drilling sampler (5 cm inner diameter). Three samples per plot were mixed as one measurement sample. Each sample was air-dried and stored at room temperature for chemical analysis.

The light fractions and heavy fractions of C and N were separated by density fractionation (von Lützow et al., 2007). The light fractions with low density (<1.7 g cm^{-3}) consisted of partially decayed plant and animal products, whereas heavy fractions with high density (>1.7 g cm^{-3}) included humic substances that are generally associated with mineralization (Aanderud et al., 2010; Six et al., 1998).

The soils were suspended in water (1:2 soil:water) for 30 min and filtered through 0.45-μm membranes to determine the DOC and DON contents. The organic C in the extracts was determined using an automated total organic C (TOC) analyzer (Shimadzu, TOC-Vwp, Japan), and the N was detected via the Kjeldahl method.

The EOC was determined according to Vieira et al. (2007). Finely ground air-dried soil samples were oxidized using 25 ml of 333 mM $KMnO_4$. The suspensions were horizontally shaken at 60 r min^{-1} for 1 h and centrifuged at 2000 r min^{-1} for 5 min. The supernatants were diluted and measured at 565 nm with a spectrophotometer (UV2300).

Ammonium and nitrate were extracted by vigorously shaking the sample with 50 ml of 1.0 mol l^{-1} KCl for 30 min. The extract was then filtered, and the NH_4^+ and NO_3^- nitrogen concentrations were measured using a Continuous Flow Analytical System (Autoanalyzer 3, Bran + Luebbe, Germany).

The SOC content was assayed via dichromate oxidation (Black et al., 1965). The soil TN content was assayed using the Kjeldahl method (Bremner and Mulvaney, 1982). The soil pH was measured using a pH meter after shaking the soil water (1:2.5 w/v) suspension for 30 min. Each analysis was performed in duplicate.

2.4. Calculations and statistics

The sensitivity index (SI) related to fertilizer treatments for C/N fractions was calculated using Eq. (1) (Liang et al., 2012b):

$$\mathrm{SI} = (\mathrm{C/N\ fractions\ in\ N\ treatments} - \mathrm{C/N\ fractions\ in\ N0}) \times 100/\mathrm{C/N\ fractions\ in\ N0}. \quad (1)$$

The Kolmogorov–Smirnov test was used to test for data normality. A one-way ANOVA was performed to test significance. Significant differences were evaluated at the 95% confidence level. When significance was observed at the $P < 0.05$ level, a post hoc least significant difference (LSD) test was used to perform multiple comparisons. All statistical analyses were conducted using the software program SPSS, ver. 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Soil carbon and nitrogen fraction concentrations

After long-term N fertilization, the SOC in all treatments exhibited similar trends in the 0–200 cm soil layer: The SOC values were highest in the 0–20 cm layer and gradually decreased as soil depth increased (Fig. 1A). No significant differences in SOC, LFOC or HFOC were observed among the N treatments, thus we combined N treatments data (N90, N180, N270 and N360) in the same layers to show the major trends in Fig. 1A, B and C. LFOC was the highest in the 0–20 cm layer and

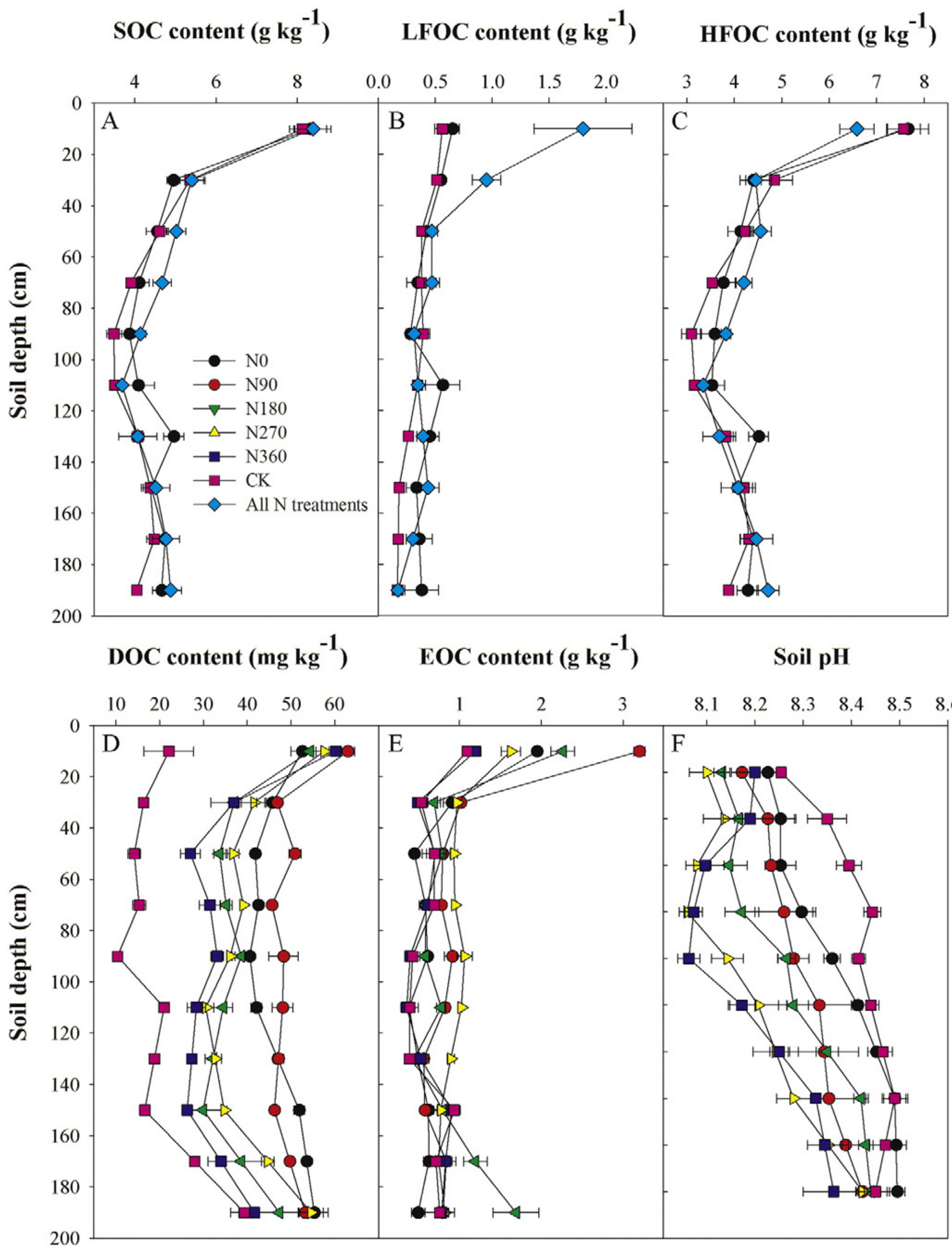

Fig. 1. Effects of nitrogen fertilization on the distribution of soil organic carbon (SOC), its fractions (LFOC, HFOC, DOC and EOC) and soil pH in a 200 cm soil profile. The values are the mean ± SE. N0, N90, N180, N270, and N360 represent the applied nitrogen rates of 0 kg ha^{-1}, 90 kg ha^{-1}, 180 kg ha^{-1}, 270 kg ha^{-1} and 360 kg ha^{-1}, respectively. LFOC: light fraction of carbon; HFOC: heavy fraction of carbon; DOC: dissolved organic carbon; and EOC: easily oxidizable organic carbon. In Figures A, B and C, data from the N treatments (N90, N180, N270 and N360) were combined for the same soil layers to show the major trends because no significant differences were observed among the N treatments.

decreased with depth (Fig. 1B). In the 0–20 cm layer, the LFOC content was the highest in the N270 treatments (2.3 g kg^{-1}), and the LFOC concentrations of the N treatments were significantly higher than those of N0 (0.65 g kg^{-1}) and CK (0.56 g kg^{-1}). Below 40 cm, no significant differences were observed in the N rate. The HFOC trends were similar to those of SOC, i.e., no significant differences were observed among the N treatments and CK, except in the 0–20 cm layer (Fig. 1C). The DOC content in all N treatments was significantly higher than in CK (Fig. 1D). The DOC content was higher in the N90 treatment than in other N treatments in the 0–120 cm soil profile. Interestingly, N0 did not have the lowest DOC content among the N treatments. The EOC content was highest in the surface soil (Fig. 1E), and the highest value was observed in the 0–20 cm range of N90. However, N270 had the highest EOC concentration in the 60–140 soil layer.

The soil pH values in all treatments ranged from 8.05 to 8.5 (Fig. 1F). The soil pH decreased as the N rate increased, and the pH values of all of the N treatments were significantly lower than those of CK. In the 0–20 cm layer, the lowest pH values were in the N270 and N360 treatments, but there are no significant differences between treatments.

TN content was highest in the 0–20 cm depth and decreased as soil depth increased in all treatments (Fig. 2A). In Fig. 2A, B, and C, we combined the TN, LFON and HFON data from the N treatments (N90, N180, N270 and N360) for the same soil layers to show the major trends because no significant differences were observed among the N treatments. LFON represented only a small portion of the total nitrogen (0–0.16 g kg^{-1}), and its content was highest in the 0–40 cm soil layer of the N treatments (Fig. 2B). The HFON content showed a similar dynamic change as that of TN (Fig. 2C). The DON content was lower in

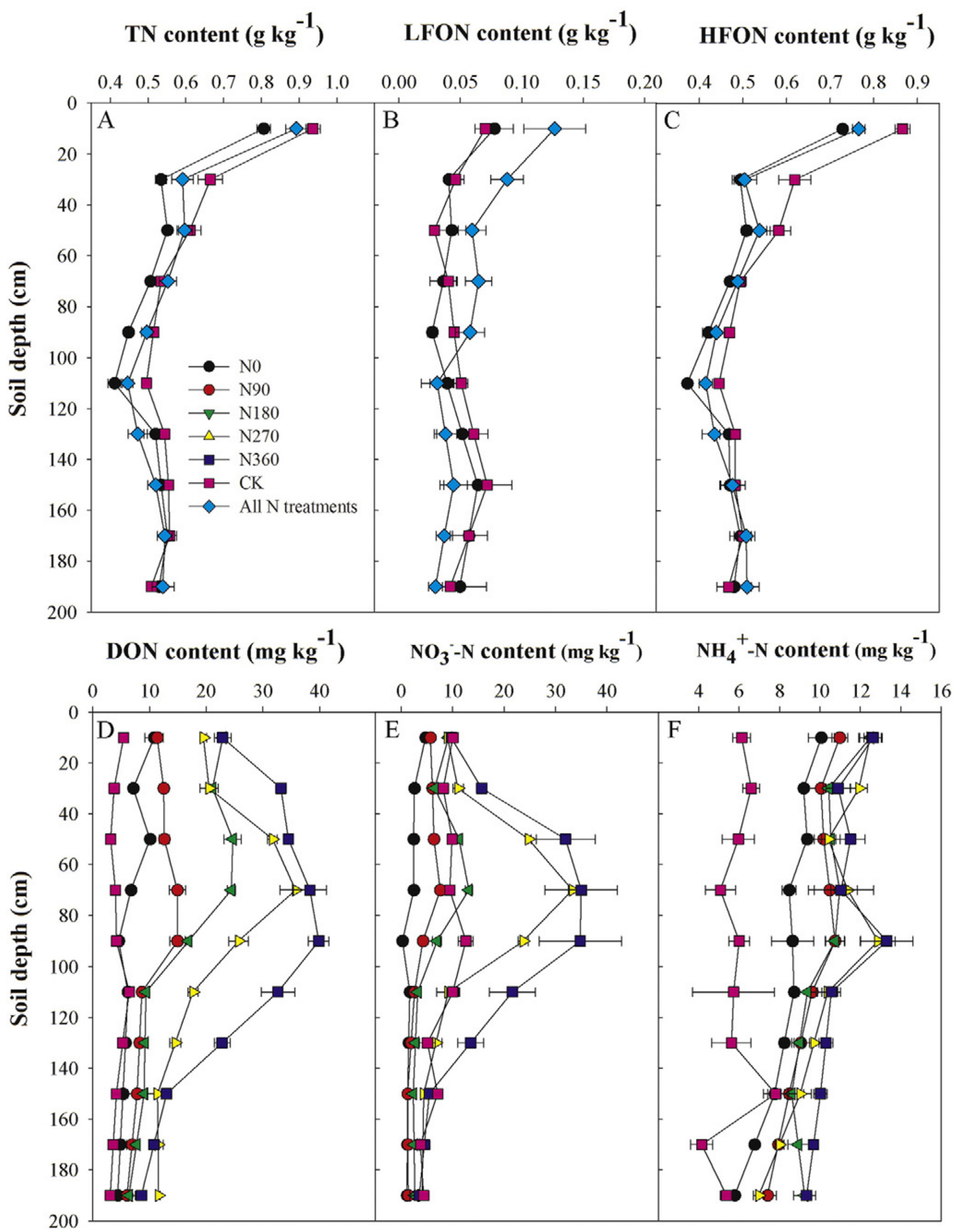

Fig. 2. Effects of nitrogen fertilization on the distribution of soil total nitrogen (TN) and its fractions (LFON, HFON, DON, NO_3^- and NH_4^+) in a 200 cm soil profile. The values are the mean ± SE. LFON: light fraction of nitrogen; HFON: light fraction of nitrogen; DON: dissolved organic nitrogen; NO_3^-: nitrate nitrogen; and NH_4^+: ammonium nitrogen. N0, N90, N180, N270, and N360 represent the applied nitrogen rates of 0 kg ha^{-1}, 90 kg ha^{-1}, 180 kg ha^{-1}, 270 kg ha^{-1} and 360 kg ha^{-1}, respectively. In Figures A, B and C, data from the N treatments (N90, N180, N270 and N360) were combined for the same soil layers to show the major trends because no significant differences were observed among the N treatments.

CK than in the other treatments and was the highest in the N360 soil profile. The profile of DON first increased and then decreased with soil depth in the high N treatments (Fig. 2D). Nitrate concentrations were indicative of obvious leaching in the N270 and N360 treatments, and the highest nitrate concentrations were observed in the 0–100 cm soil layer in N360 (34.8 mg kg^{-1}) (Fig. 2E). Ammonium nitrogen concentrations were higher in all N treatments than in CK and increased as the N rate increased: N0 had lower ammonium nitrogen values than the other N treatments (Fig. 2F).

3.2. Percentages of each C and N fraction to SOC and TN

Based on the trends of the C and N fractions in the soil profiles in Figs. 1 and 2, we divided the soil layers into three main layers to investigate the percentages of C and N fractions to total SOC and TN: 0–20 cm, the surface layer, which is the most active layer in soil; 20–120 cm, the middle soil layer, which is closely associated with wheat roots; and 120–200 cm, the deep soil layer, which is less affected by cropping and fertilization. The percentages of LFOC were highest in the 0–20 cm layer (Fig. 3A) and were significantly higher in the N fertilization treatments than in CK (6.9%) or N0 (6.6%). N270 had the highest percentages (35.5%), but no significant differences were observed among the N treatments. The LFOC percentages were lower in the 20–200 cm soil layer than in the 0–20 cm. For the full profile (0–200 cm), the LFOC percentages increased as the N rate increased and were higher in the N treatments than in CK. In contrast, the HFOC percentages exhibited the opposite trend (Fig. 3B): For the full profile, the HFOC percentages decreased as the N rate increased. The DOC percentages decreased as the N rate increased in the 20–200 cm soil layers, and all treatments were significantly higher than CK (Fig. 3C). EOC was higher in the 0–20 cm layer and significantly higher in the 0–200 cm layers of the N90, N180 and N270 treatments (Fig. 3D).

The LFON percentage was also higher in the surface soil layer. The percentages in N180 (14.9%), N270 (16.6%) and N360 (15.6%) were significantly higher than N0 (9.6%), N90 (8.7%) and CK (7.5%) (Fig. 4A). As expected, the HFON percentage exhibited a trend opposite to that of LFON (Fig. 4B). DON significantly increased in the 20–200 cm soil layers in the N treatments, similar to DOC (Fig. 4C). Nitrate and ammonium were combined as inorganic N, and this percentage significantly increased as the N rate increased (Fig. 4D).

3.3. Sensitivity of each C and N fraction

The sensitivity of each C and N fraction was evaluated as the sensitivity index (SI) relative to N0 (Figs. 5 and 6). The SI of LFOC was the highest in the 0–20 cm soil layer, reaching 256% in N270, and as the N rate increased, sensitivity increased, except in N360 (Fig. 5B). However, in the soil layers below 20 cm, the SI of LFOC was low. The SI of HFOC (Fig. 5C) was similar to that of SOC (Fig. 5A). DOC and EOC had higher SI values below the 20 cm soil layer, especially at N inputs greater than 180 kg ha^{-1} (Fig. 5D, E). The SI of DOC was highest in N360, but the SI of EOC was low in the same treatment.

TN and HFON were the least sensitive fractions and exhibited inconsistent trends as the N rate increased (Fig. 6A, C). The most sensitive N fraction was NO_3^-, which reached 1395% under the N360 treatment in the 20–120 cm soil layer and increased significantly as the N rate increased (Fig. 6E). DON was the second most sensitive N fraction and was most sensitive in the 20–120 cm soil layer. Its sensitivity significantly increased as the N rate increased. The SI of ammonium did not differ significantly among the N180, N270 and N360 treatments (Fig. 6F).

4. Discussion

C and N fractions with different physical and chemical compositions and qualities respond differently to the addition of N, potentially influencing the response of the total soil C and N contents (Song et al., 2014). In the present study, no significant changes were detected in soil total C or N contents, even after 10 years of N addition. This lack of significant changes may be due to the large size of the C and N pools because significant changes in total soil C and N concentrations in response to N fertilization are usually difficult to detect over short periods of time (Song et al., 2012). Alternatively, the lack of significant differences in the C and N contents may have resulted from the low C input and enhanced fertilizer-induced decomposition of C and N (Wu et al., 2004). Our results are consistent with previous studies (Lee and Jose, 2003; Reid et al., 2012) that have reported that N fertilization did not increase the soil C content. In contrast, Huang et al. (2011) reported a 14.6% increase in C following N addition. The applied inorganic N may not have significantly affected the total N level for two possible reasons. A portion of the mineral N applied may have been lost via ammonia volatilization (44.1% of applied N), leaching (14.8%) and/or denitrification

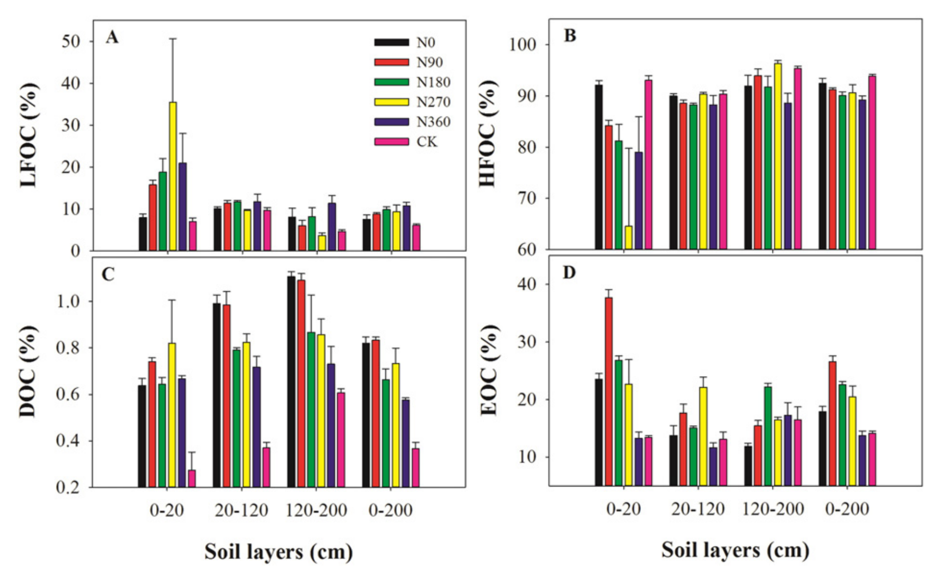

Fig. 3. Effects of nitrogen fertilization on the percentages of the soil organic carbon (SOC) fractions (LFOC, HFOC, DOC and EOC) to SOC in different soil layers. The values are the mean ± SE. LFOC: light fraction of carbon; HFOC: heavy fraction of carbon; DOC: dissolved organic carbon; and EOC: easily oxidizable organic carbon. N0, N90, N180, N270, and N360 represent the applied nitrogen rates of 0 kg ha^{-1}, 90 kg ha^{-1}, 180 kg ha^{-1}, 270 kg ha^{-1} and 360 kg ha^{-1}, respectively.

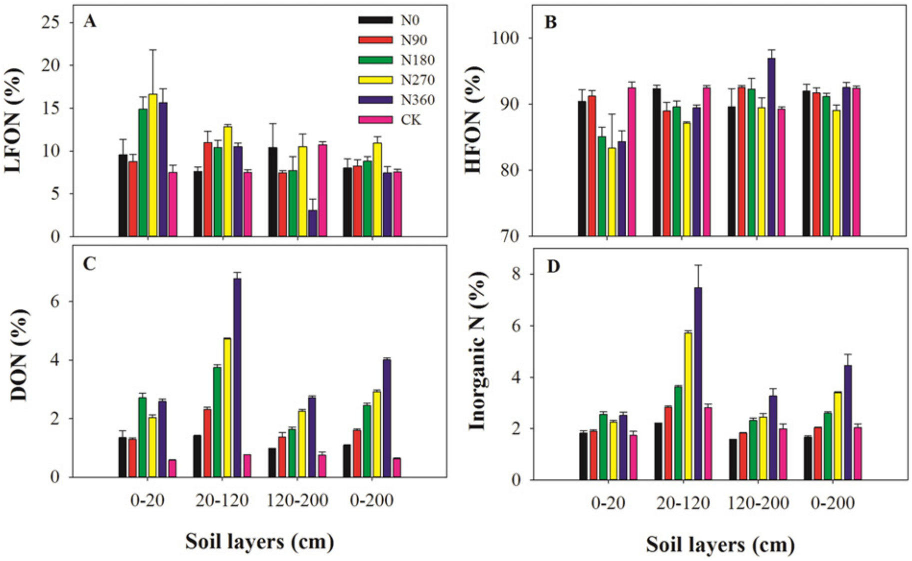

Fig. 4. Effects of nitrogen fertilization on the percentages of the soil nitrogen fractions (LFON, HFON, DON and inorganic N) to total nitrogen (TN) in different soil layers. The values are the mean ± SE. LFON: light fraction of nitrogen; HFON: light fraction of nitrogen; and DON: dissolved organic nitrogen. Inorganic N is the sum of nitrate nitrogen and ammonium nitrogen. N0, N90, N180, N270, and N360 represent the applied nitrogen rates of 0 kg ha^{-1}, 90 kg ha^{-1}, 180 kg ha^{-1}, 270 kg ha^{-1} and 360 kg ha^{-1}, respectively.

(4.4%), as was reported by Ju et al. (2009), or the increased amount of N fertilizer and root decomposition may have been used by microbes, which can accelerate the turnover of organic nitrogen in the soil.

Density fractionation has been widely used for more than 50 years as an effective way to assess light and heavy pools of SOM that have different sensitivities to environmental changes (Crow et al., 2007). The heavy soil fractions of C and N are relatively stable (Song et al., 2012) and accounted for approximately 90% of the total C and N in our study (Figs. 3B and 4B). The trends of SOC and TN were similar, but the proportions of HFOC and HFON relative to total C and N, respectively, decreased significantly with increasing N additions (Figs. 3B and 4B). These results indicated that the stability of C and N in the soil was affected by long-term N addition. N addition altered the heavy fractions of the soil C and N, but these fractions were less sensitive than other fractions, consistent with Song et al. (2012), who reported that the heavy C and N fractions were stable in response to field management practices.

The light fraction is a short-term reservoir of plant nutrients and the primary fraction for soil carbon formation and serves as a readily decomposable substrate for soil microorganisms (Gregorich et al., 1994; Neff et al., 2002). The size of the light fraction pool is a balance between residue inputs and decomposition (Gregorich and Janzen, 1996). The light fractions of C and N increased in N treatments in this study, especially in the 0–40 cm soil layer (Figs. 1B and 2B). The LFOC and LFON values in the 0–20 cm soil layer were significantly higher in the N treatments than in N0 and CK (Fig. 3B). Because we removed the aboveground biomass from the field plots, the input of organic matter was primarily through root biomass (Ding et al., 2012). Increased N fertilization may directly enhance soil microbial activities and accelerate soil carbon and nitrogen decomposition, increasing the light fractions of C and N. The impact of N addition on the light C fraction is currently debated and has been reported to be positive (Hagedorn et al., 2003), neutral (Reid et al., 2012) or negative (Cusack et al., 2011). These conflicting results are largely due to the differing amounts of N addition in the different studies. However, both light C and N percentages decreased in surface soil of the N360 treatment. This response implies that too much N addition may actively decrease C and N contents by suppressing

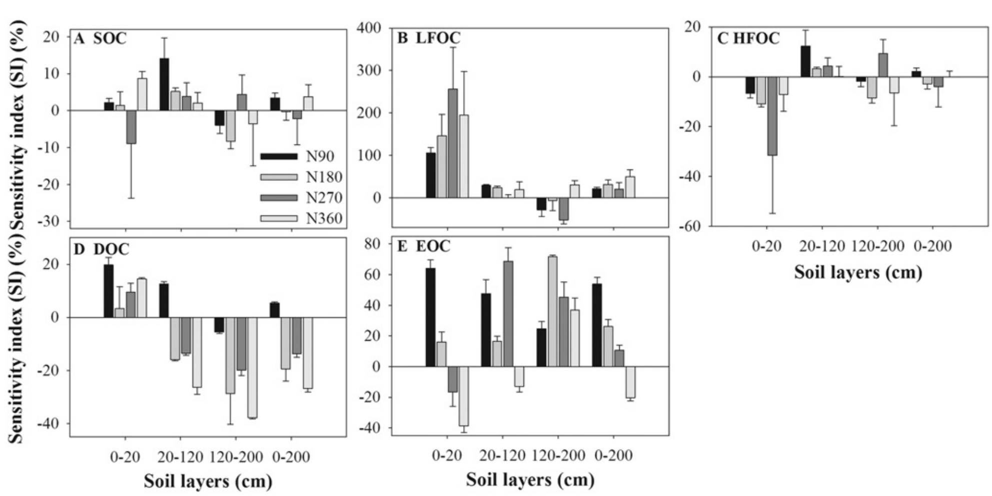

Fig. 5. Sensitivity index (SI) values for soil organic carbon (SOC) and its fractions under different N treatments relative to N0. The values are the mean ± SE. A: SOC, soil organic carbon; B: LFOC, light fraction of carbon; C: HFOC, heavy fraction of carbon; D: DOC, dissolved organic carbon; and E: EOC, easily oxidizable organic carbon.

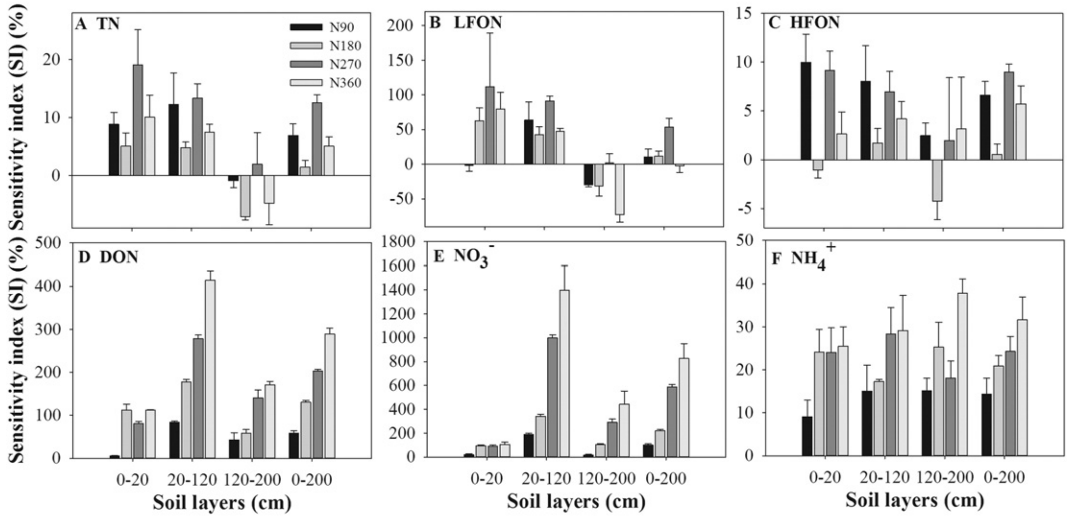

Fig. 6. Sensitivity index (SI) values for total nitrogen (TN) and its fractions under different N treatments relative to N0. The values are the mean ± SE. A: TN, total nitrogen; B: LFON, light fraction of nitrogen; C: HFON, light fraction of nitrogen; D: DON, dissolved organic nitrogen; E: NO_3^-, nitrate nitrogen; and F: NH_4^+, ammonium nitrogen.

soil microbial activity (Song et al., 2014). The SI of LFOC was significantly higher in the 0–20 cm soil layer, increased as the N rate increased, and was higher than that of LFON. However, the LFOC SI values were lower than those of LFON in the 20–200 cm soil layers. Poeplau and Don (2013) reported that C was less sensitive in subsoil layers. Additionally, Chen et al. (2009) reported that SOC was easily affected by management practices in the upper 15 or 30 cm and that accumulation of SOC in the subsoil was unaffected for one to two decades. These results indicate that LFON has a large range of sensitive soil layers because N is also greatly affected in deeper soil layers because nitrate leaches into the deeper soil.

Dissolved organic C and N play an important role in several soil processes. DOC is a vital component of ecosystem carbon cycling, has a relatively rapid turnover rate, and serves as a source or a sink of labile nutrients (Song et al., 2012). DOC is the primary energy source of soil microorganisms, controlling nutrient turnover and the development of microbial populations (Gong et al., 2009b). The DOC in all of the N treatments was significantly higher than in the CK soil profile because the wheat planted in the treatments increased C input to the soil. The higher DOC content and percentage in the N0 and N90 treatments may have been due to higher microbial activity. Multiple previous studies have reported that N addition can decrease microbial biomass and activity (Song et al., 2014). In the 20–200 cm layer, DON concentrations significantly increased as N addition increased. The DON content and percentage increased with increasing soil depth in all treatments, similar to that observed in other studies (Jinbo et al., 2006; Liang et al., 2012b). Therefore, illuviation or leaching increased the relative proportion of DON in the subsurface layers and increased the sensitivity of DOC and DON in the deeper soil layers. DON had higher sensitivity values than DOC and increased as the N rate increased, consistent with Liang et al. (2012b), who reported that the DON concentration increased with the application of inorganic fertilizer.

The measurement of C released by the oxidation of soil with 333 mM $KMnO_4$ is a rapid and economical method to quantify the labile SOC fraction (Blair et al., 1995; Weil et al., 2003). EOC is a sensitive indicator of changes in SOC caused by different management practices (Weil et al., 2003). In the present study, EOC concentrations changed significantly in the 0–20 cm layer. Previous studies have demonstrated that N additions can increase EOC (Lou et al., 2011a; Pregitzer et al., 2004; Sinsabaugh et al., 2004). Song et al. (2014) reported a positive linear relationship between EOC and N addition rates, but Chen et al. (2012) reported no significant differences associated with the addition of N. Interestingly, the present study showed that EOC proportions were highest in the N90 treatment and decreased as the N rate increased. N360 exhibited particularly low EOC values, similar to those of CK, indicating that an overabundance of N addition decreases the EOC. As N addition increased, the root biomass likely decreased, leading to less C input to the soil (Song et al., 2014). N addition may decrease EOC by suppressing microbial activity because a significant correlation exists between EOC and MBC, as reported by Lou et al. (2011a).

Nitrate and ammonium nitrogen are the main inorganic N fractions in soil. These forms of N are directly absorbed by plants and can represent the N availability in soil (Di and Cameron, 2002). Historically, improving nitrogen availability has been the main driver of improvements in crop yield (Sinclair and Rufty, 2012). However, the use of large quantities of N through nitrogen cascades (Galloway and Cowling, 2002) in intensive agricultural systems has led to severe environmental contamination. In the present study, nitrate increased as the N addition rate increased, and N270 and N360 exhibited significant leaching resulting from too much N fertilization (Di and Cameron, 2002). However, nitrate levels in N0 and N90 were lower than in CK due to wheat absorption. Ammonium nitrogen increased as the N rate increased, but no differences were observed among the N treatments. The ammonium nitrogen in CK was significantly lower than in the N treatments. The concentration of ammonium is usually low because it is easily converted to nitrate. Because nitrate and most soils are negatively charged, nitrate is not retained by the soil and is thus the dominant form of N leached (Di and Cameron, 2002). Our results indicate that any excess ammonium, which cannot be absorbed by plants, is converted to nitrate in the soil. The percentage of inorganic N significantly increased as the N rate increased, and the SI of nitrate and ammonium also increased as the N rate increased.

The soil in the study area was alkaline in all of the treatments, but N fertilization decreased the pH in the soil profile, consistent with previous studies (Guo et al., 2010; Liang et al., 2012b; Song et al., 2014). The pH in the N treatments was significantly lower than CK. The lowest pH was detected in N360 in the 100 cm soil layer, which was consistent with the highest nitrate leaching point. The correlations between soil pH and each C and N fraction are shown in Table 2. Soil pH was significantly related to the soil inorganic N content, which is consistent with many previous studies that found that N addition leads to soil acidification. Soil pH was significantly correlated with most fractions. This correlation suggests that the change in the C and N cycles with N addition may be caused by soil microbial activity because N addition may decrease the MBC (Treseder, 2008), and the soil pH could change the microbial function and activity (Song et al., 2014). These changes in microbial function will lead to changes in C and N turnover, and different N fertilization rates affect the availability of N in soils, which also alters the C and N fractions.

Table 2
Correlations between the carbon fractions, nitrogen fractions and pH for all treatments.

	pH	NO_3^-	NH_4^+	DOC	DON	EOC	SOC	TN	LFOC	LFON	HFOC
NO_3^-	−0.70 **										
NH_4^+	−0.83**	0.47**									
DOC	−0.11	−0.31*	0.35**								
DON	−0.86**	0.85**	0.77**	0.01							
EOC	−0.28*	−0.07	0.34**	0.45**	0.03						
SOC	−0.33*	−0.03	0.29*	0.47**	0.09	0.72**					
TN	−0.34**	0.07	0.20	0.30*	0.09	0.67**	0.94**				
LFOC	−0.45**	0.07	0.49**	0.36**	0.27*	0.51**	0.74**	0.73**			
LFON	−0.43**	0.18	0.40**	0.35**	0.30*	0.43**	0.64**	0.71**	0.71**		
HFOC	−0.23	−0.07	0.16	0.44**	0.01	0.70**	0.96**	0.88**	0.52**	0.52**	
HFON	−0.27*	0.03	0.15	0.26*	0.02	0.67**	0.92**	0.98**	0.66**	0.54**	0.90**

Correlations among soil pH, NO_3^- (nitrate nitrogen), NH_4^+ (ammonium nitrogen), DOC (dissolved organic carbon), DON (dissolved organic carbon), EOC (easily oxidizable organic carbon), SOC (soil organic carbon), TN (total nitrogen), LFOC (light fraction organic carbon), LFON (light fraction organic nitrogen), HFOC (heavy fraction organic carbon) and HFOC (heavy fraction organic nitrogen).

* Correlations are significant at $P < 0.05$.

** Correlations are significant at $P < 0.01$.

In conclusion, all labile fractions responded differently to the addition of N to the 0–200 cm soil profiles after 10 years of N fertilization. Our results indicated that N enrichment may impact C and N cycling by changing certain fractions of soil organic matter. LFOC, LFON, HFOC, and HFON were constant among the different N rates. However, DOC decreased in the 180, 270 and 360 kg ha^{-1} treatments in the 20–200 cm soil layers, and EOC was the highest in 90 kg ha^{-1} treatment and decreased in 0–20 cm layer of the high-N treatments. Both DON and inorganic N (NO_3^- and NH_4^+) increased as the N rates increased. Nitrate was the most sensitive fraction under N fertilization. The light C and N fractions and EOC were the most sensitive fractions in the surface soil layer, and DOC and DON appeared to be good indicators for the subsurface layers. Changes in these fractions were primarily affected by microbial activity, which can be affected by N availability and soil pH. The dynamics of soil C and its fractions are complex under N application, and clarifying these dynamics requires long-term studies and the analysis of many factors and deeper soil layers. Our study provides a clear picture of the distribution and sensitivity of fractions in soil profiles with different N input rates, and our results clarify the C and N cycles under N addition.

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Appendix A. Supplementary data

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