#### Catena 135 (2015) 38-46

Contents lists available at ScienceDirect

### Catena

journal homepage: www.elsevier.com/locate/catena

# 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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#### ARTICLE INFO

#### ABSTRACT

Article history: Received 11 March 2015 Received in revised form 16 June 2015 Accepted 26 June 2015 Available online 25 July 2015

Keywords: Nitrogen fertilization Soil carbon fraction Soil nitrogen fraction Sensitivity

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<sup>-1</sup> treatments in the 20–200 cm soil layer, and easily oxidizable organic C (EOC) was the highest in the 90 kg ha<sup>-1</sup> treatment and decreased in the high-N treatments in the 0-20 cm soil layer. Both dissolved organic nitrogen (DON) and inorganic N (NO<sub>3</sub><sup>-</sup> 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.

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#### 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

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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<sub>3</sub><sup>-</sup>, nitrate nitrogen; NH<sub>4</sub><sup>+</sup>, 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<sup>-1</sup>, 90 kg ha<sup>-1</sup>, 180 kg ha<sup>-1</sup>, 270 kg ha<sup>-1</sup> and

#### 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 <sup>-1</sup> )	64
50–2 μm (g kg <sup>-1</sup> )	694
$<2\mu m(gkg^{-1})$	342
Bulk density (g cm <sup>-3</sup> )	1.23
pH (H <sub>2</sub> O)	8.25
Water-holding capacity (%)	23.6
Soil organic carbon (g kg <sup>-1</sup> )	8.79
Soil total nitrogen (g kg <sup>-1</sup> )	0.96
Available N (mg kg <sup>-1</sup> )	25.10
Available P (mg kg <sup>-1</sup> )	7.90

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

360 kg ha<sup>-1</sup> (termed N0, N90, N180, N270 and N360, respectively). The plots had an area of 2 m  $\times$  3 m, and each plot included twenty 15cm-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<sup>-1</sup>). 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.

#### 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<sup>-3</sup>) consisted of partially decayed plant and animal products, whereas heavy fractions with high density (>1.7 g cm<sup>-3</sup>) 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<sub>4</sub>. The suspensions were horizontally shaken at 60 r min<sup>-1</sup> for 1 h and centrifuged at 2000 r min<sup>-1</sup> 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<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> 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.

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