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Changes in aggregate-associated organic carbon and nitrogen after 27 years of fertilization in a dryland alfalfa grassland on the Loess Plateau of China

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Abstract: Changes in the distribution of soil aggregate sizes and concentrations of aggregate-associated organic carbon (OC) and nitrogen (N) in response to the fertilization of grasslands are not well understood. Understanding these changes is essential to the sustainable development of artificial grasslands. For understanding these changes, we collected soil samples at 0–20 and 20–40 cm depths from a semi-arid artificial alfalfa grassland after 27 years of applications of phosphorus (P) and nitrogen+phosphorus+manure (NPM) fertilizers on the Loess Plateau of China. The distribution of aggregate sizes and the concentrations and stocks of OC and N in total soils were determined. The results showed that NPM treatment significantly increased the proportions of >2.0 mm and 2.0–0.25 mm size fractions, the mean geometric diameter (MGD) and the mean weight diameter (MWD) in the 0–20 cm layer. Phosphorous fertilizer significantly increased the proportion of >2.0 mm size fractions, the MGD and the MWD in the 0–20 cm layer. Long-term application of fertilization (P and NPM) resulted in the accumulation of OC and N in soil aggregates. The largest changes in aggregate-associated OC and N in the 0–20 cm layer were found at the NPM treatment, whereas the largest changes in the 20–40 cm layer were found at the P treatment. The results suggest that long-term fertilization in the grassland leads to the accumulation of OC and N in the coarse size fractions and the redistribution of OC and N from fine size fractions to coarse size fractions.

Keywords: alfalfa grassland; long-term fertilization; nitrogen; organic carbon; semi-arid Loess Plateau

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Alfalfa plants can improve soil quality by increasing aboveground and belowground biomass while providing canopy coverage, thus decreasing erosion and soil nutrient loss caused by water and wind (Blanco and Lal, 2004). Due to its high N content, alfalfa is high-quality forage for livestock and has been extensively used for stockbreeding around the world (Badaruddin and Meyer, 1989). Alfalfa is widely planted on the Loess Plateau, where soil erosion is a serious problem. The management of artificial alfalfa grassland is thus important for the prevention of soil erosion and for the development of local agriculture. The most widely used management practice for alfalfa grassland is the application of phosphorus (P) and manure fertilizers, which can significantly increase plant biomass (Fan et al., 2011). Although an understanding of the effects of fertilization on soil structure and on the amounts of organic carbon (OC) and nitrogen (N) in loess is essential for the sustainable development of the artificial grasslands, these effects have not yet been studied.

The distribution of soil aggregates can provide important information on changes in the structure and physical properties of soil (Cammeraat and Imeson,

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1998). Soil organic matter, OC and N can indicate soil fertility (Gregorich et al.. 1997; Lal et al., 1997). Soil structure and organic matter are interrelated; organic matter acts as a binding agent to facilitate aggregation, and aggregation mediates many biological and physical processes that in turn affect the accumulation of organic matter in soils (Elliott, 1986; Beare et al., 1994; Six et al., 2000).

Many experiments conducted in croplands have indicated that soil organic matter and the stability of aggregates are sensitive to tillage and the management of fertilization (Beare et al., 1994; Pinheiro et al., 2004; Madari et al., 2005). The continuous application of fertilizers can increase soil OC and N contents, aggregate stability and soil biological activities, all of which are associated with soil aggregation (Reeder et al., 1998; Schunman et al., 2002). In addition, OC and N contents of soil aggregates and the stability of soil aggregates are significantly enhanced after the long-term application of manure (Whalen and Chang, 2002; Whalen et al., 2003). These increases in soil OC and N mainly occurred in macro-aggregates (Aoyama et al., 1999). Previous studies have suggested that fertilization may influence the interaction between soil organic matter and aggregation and thus prevent the loss of soil OC and N (Blanco and Lal, 2004). Most of these studies, however, have been conducted on croplands. Grassland ecosystem is sensitive to climatic change, and it has significant impact to the global carbon cycle. Nevertheless, studies of soil OC in soil aggregates of grassland is relatively few (He et al., 2008; Shrestha and Stahi, 2008), and little is known about the responses of soil aggregate sizes distributions or OC and N concentrations within aggregates to grassland fertilization, particularly artificial alfalfa grassland fertilization, which differs greatly from cropland fertilization.

In this study, we investigated the distribution of soil aggregate sizes, as well as the concentrations of OC and N in total soils and separated aggregates in a semi-arid, artificial alfalfa grassland on the Loess Plateau of China after applying P and nitrogen+phosphorus+ manure (NPM) fertilizers annually for 27 years. The objective of this study was to understand the responses of soil structure and the accumulation of OC and N in aggregates to long-term fertilization in artificial alfalfa grasslands. This knowledge may provide a useful guide for the management of alfalfa and other legumes in grasslands on the Loess Plateau, or similar regions.

1 Materials and methods

1.1 Study area

A long-term field experiment was initiated in September 1984 at the Agro-ecological Experimental Station of the Chinese Academy of Sciences, Changwu county, Shaanxi province, China (35°12′N, 107°40′E). The experiment was arranged on dry farmland that did not require irrigation. The study site has a warm-temperate, semi-arid and continental climate. During the period 1984 to 2011, the annual average temperature was 9.1°C, and the average frost-free period was 171 days. The annual accumulation of temperatures higher than 0°C and 10°C were 3,866°C and 3,029°C, respectively. The average annual precipitation was 584 mm. Rainfall occurs mainly between June and September, with a large variation in intensity within and between years. The soil is known as Heilu soil, which corresponds to Calcarid Regosol, according to the FAO/UNESCO classification system (FAO, 1998). The soil properties are shown in Table 1. Soil loss due to water and wind erosion is very low.

Table 1 Main chemical properties of the top soil layer (0–20 cm) at the start of the experiment in 1984 (Wei et al., 2006)

Soil chemical property	Content
Organic matter (g/kg)	10.5
Total nitrogen (g/kg)	0.80
Available nitrogen (mg/kg)	37.0
Total phosphorous (g/kg)	0.7
Available phosphorous (mg/kg)	3.0
Available potassium (mg/kg)	129.3
$CaCO_3$ (g/kg)	108.4

1.2 Experimental design and soil sampling

A long-term fertilization experiment on artificial alfalfa grassland was conducted in plots of 10.3 m×6.5 m. The experiment included a control (CK), a P and a NPM treatment. Plots were randomly arranged with three replicates each. The alfalfa plant was sown in 1984. In mid-April of each year, fertilizers were broadcasted on the surface of soil. The land was then tilled with a moldboard plough to a depth of approximately 10 cm to mix the fertilizers with the soil. The CK treatment was also tilled so as to minimize the effects of tillage on the experimental results. The alfalfa plant was cut and removed in mid-June and mid-October each year. Previous studies demonstrated that no significant changes in soil OC and N occurred in the CK treatment between 1984 and 2011 (Wei et al., 2006; Fan et al., 2011). This suggests that tillage has negligible effects on the three treatments, and any changes in the distribution of aggregate size fractions OC and N levels in total soils and associated with aggregates were due to the fertilization.

Urea and superphosphate were used as the source of N and P. The manure came from cattle. In the P treatment, the P addition was 26 kg/(hm^2·a). In the NPM treatment, the N addition was 120 kg/(hm^2·a), the P addition was 26 kg/(hm^2·a) and the manure addition was 75 t/(hm^2·a), respectively. Manure OC and N contents were 17.67 and 1.97 g/kg, respectively, and available N was 91 mg/kg. Total P content of the manure was 0.97 g/kg and available P was 115 mg/kg.

Soil bulk density was measured at depths of 0–20 and 20–40 cm for each plot using a stainless steel cutting ring 5.0-cm long and 5.0-cm in diameter. The soil cores were dried at 105°C for 24 h to a constant weight. Soil samples were collected in September 2011. Five random cores were taken at 0–20 and 20–40 cm depths for each plot using a tube auger 5-cm in diameter. The five cores samples from each soil layer were mixed. Visible pieces of organic material were removed by hand. Soil samples were brought to the laboratory, air dried and prepared for the analysis of soil aggregates, OC concentrations and N concentrations.

1.3 Laboratory analysis

Four size fractions of aggregates were separated by wet sieving each sample through 2, 0.25 and 0.053 mm sieves following the procedures described by Cambardella and Elliott (1994). The aggregate size samples were dried in an oven at 50°C–60°C, weighed and stored at room temperature.

The mean weight diameter (MWD) and mean geometric diameter (MGD) were used to evaluate soil structure and were calculated as follows (Bavel, 1950; Pinheino et al., 2004):

$$MWD=\sum X_i W_i, \quad (1)$$

$$MGD=\exp(\sum(W_i \times \log X_i/100)). \quad (2)$$

Where X_i and W_i are the mean diameter (mm) and proportion (%) of each size fraction of the aggregate, respectively.

A subsample of air-dried, undisturbed soil from each plot was ground to pass through a 0.25-mm sieve to measure OC and N in total soils. OC and N for total soils and different aggregate size fractions were analyzed using a C/N/H/S-analyzer (Vario ElementarIII, Germany).

Stocks of soil OC (or N) (Mg C (or N)/hm^2) in total soils were calculated as follows:

$$\text{Stocks of OC (or N)} = D \times BD \times OC \text{ (or N)}/10. \quad (3)$$

Where D is the thickness of the soil layer (cm), BD is the bulk density (g/cm^3), and OC (or N) is the concentration of OC (or N) in the soil layer (g/kg).

Stocks of OC (or N) (Mg C (or N)/hm^2) in each size fraction were calculated as follows:

$$\text{Stocks of } OC_i \text{ (or } N_i) = D \times BD \times M_i \times OC_i \text{ (or } N_i)/10000. \quad (4)$$

Where M_i is the aggregate mass in the i^{th} size fraction (g/kg), and OC_i (or N_i) is the OC (or N) concentration of the i^{th} size fraction (g/kg aggregate) for the selected soil layer.

1.4 Statistical analysis

A two-way analysis of variance was conducted using SAS version 8 to test the effects of fertilization and soil depth on soil aggregate size distribution, MWD, MGD and OC and N concentrations and stocks in total soils and aggregates. The values of each variable were compared at the $P<0.05$ level of significance using the least significant difference for the variance.

2 Results

2.1 The effects of long-term fertilization on the distribution of soil aggregate sizes

The <0.053-mm fraction comprised the largest proportion of soil aggregates and accounted for 40% of the total soil mass in both layers, whereas the >2.0-mm fraction accounted for the smallest percentage of soil mass at both soil depths (13% and 7%, respectively) (Fig. 1). NPM treatment of 27 years significantly increased the proportions of >2.0-mm and 2.0–0.25-mm fraction and decreased the proportion of

<0.053-mm fraction in the 0–20 cm layer. Phosphorous fertilizer, however, increased the proportion of >2.0-mm fraction and decreased the proportion of <0.053-mm fraction in the 0–20 cm layer, but did not significantly change the distribution of soil aggregates in the 20–40 cm layer. Similarly, the NPM treatment increased the proportion of MGD and MWD by 58% and 98%, respectively, in the 0–20 cm layer (P<0.05), whereas the P treatment increased the proportion of MGD and MWD by 21% and 22%, respectively, in the 20–40 cm layer (P>0.05) (Fig. 2). These results suggest that the effects of long-term fertilization on the distribution of soil aggregate fractions varied with fertilizer types and soil depths.

2.2 The effects of long-term fertilization on OC and N in total soils

Long-term fertilization increased OC in non-sieved soils in the 0–20 cm layer (Fig. 3). The P treatment increased OC concentrations and stocks by 16% and 15% (P>0.05), respectively, whereas the NPM treatment increased them by 50% and 35% (P<0.05), respectively. Soil N concentrations and stocks were not significantly increased by the P treatment but were

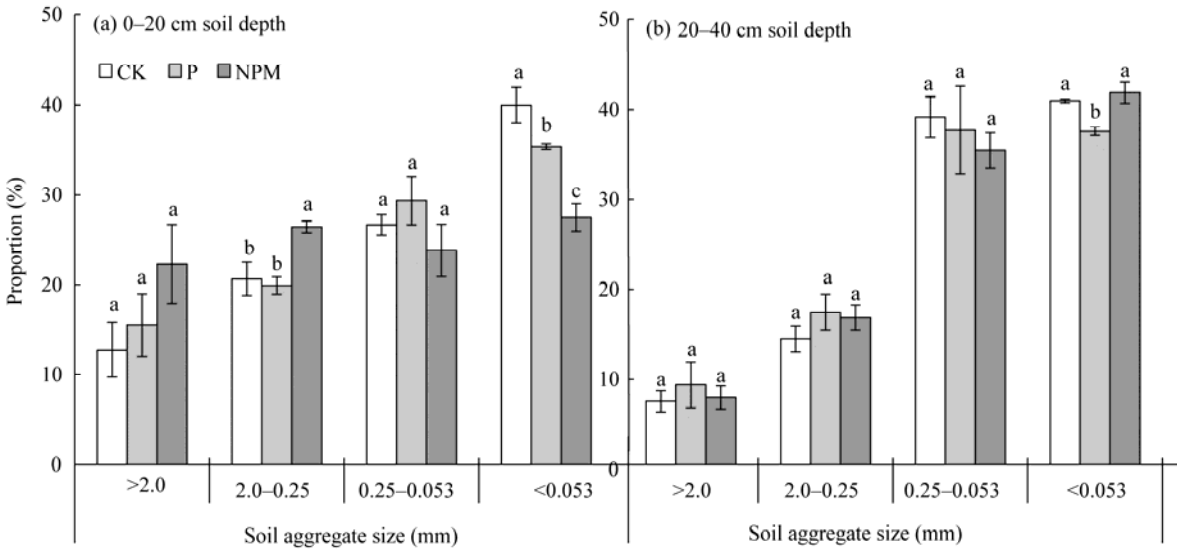

Fig. 1 The effects of fertilization on the distribution of soil aggregate sizes. Error bars are the standard error of the mean (n=3). Means with different lowercase letters within same aggregate size fractions and soil depths are significantly different (P<0.05). CK, control; P, phosphorus; NPM, nitrogen+phosphorus+manure. The symbols represent the same below.

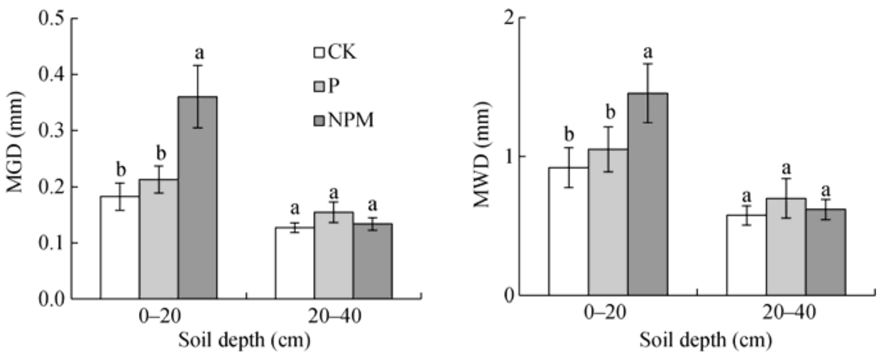

Fig. 2 The effects of fertilization on the mean geological diameter (MGD) and mean weight diameter (MWD) of soil aggregate sizes. Error bars represent the standard error of the mean (n=3). Means with different lowercase letters within the same soil layer are significantly different (P<0.05).

significantly increased by NPM treatment in the 0–20 cm layer (39% and 25%, respectively) (Fig. 3). On the other hand, the concentrations and stocks of OC and N in the deep soil layer (20–40 cm) were unaffected by long-term fertilization, indicating that the effects of fertilization on total soil OC and N contents are limited to the top 20 cm soil depth in the semi-arid alfalfa grassland.

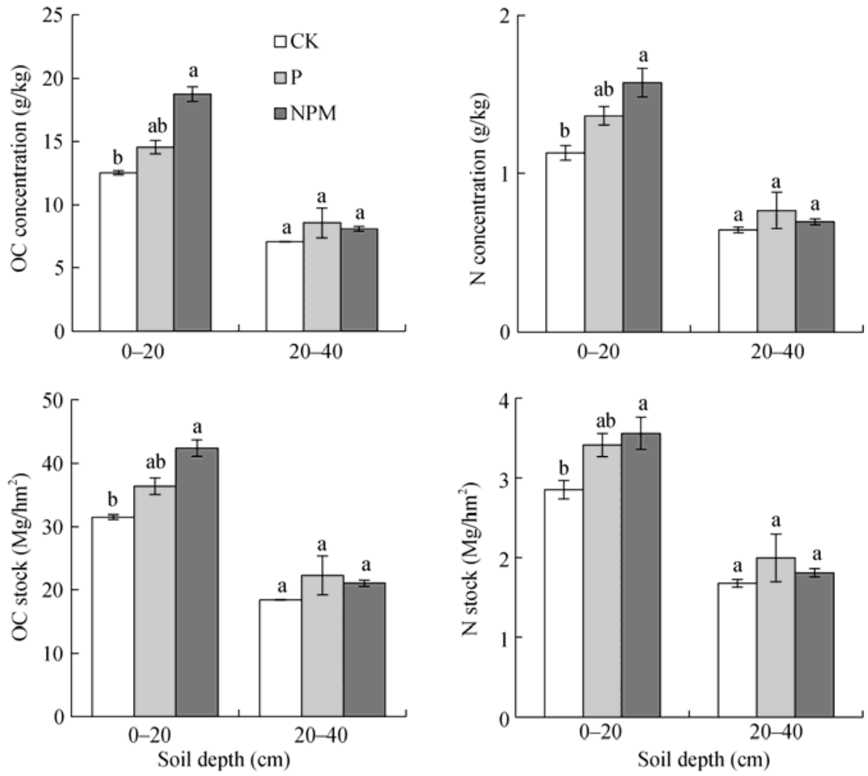

Fig. 3 The effects of fertilization on concentrations and stocks of organic carbon (OC) and nitrogen (N) in total soils. Error bars represent the standard error of the mean (n=3). Means with different lowercase letters within the same soil layer are significantly different (P<0.05).

2.3 The effects of long-term fertilization on OC and N in aggregate size fractions

The largest increase of soil OC and N concentration in the 0–20 cm layer occurred with the NPM treatment, whereas the largest increase in the 20–40 cm layer occurred with the P treatment (Fig. 4). For example, in the 0–20 cm layer, the P treatment increased OC concentrations by 4%, 11%, 11% and 3% in the >2.0-mm, 2.0–0.25-mm, 0.25–0.053-mm and <0.053-mm size fractions, respectively, and the NPM treatment increased OC concentrations by 18%, 27%, 74% and 18%, respectively. Increases in N concentrations within aggregate size fractions from the 0–20 cm layer followed the same pattern. However, in the 20–40 cm layer, the increases in OC concentrations for each aggregate size fractions (35%, 26%, 29% and 8%, respectively) were greater caused by the P treatment than that resulted from the NPM treatment (16%, 14%, 25% and 6%, respectively).

Long-term fertilization decreased the stocks of OC and N associated with the <0.053-mm size fraction but increased those associated with the >2-mm, 2–0.25-mm and 0.25–0.053-mm size fractions. These increases varied with fertilization types and soil depths (Fig. 5). As with the changes in OC and N concentrations, the largest changes in OC and N stocks associated with aggregate size fractions in the 0–20 cm layer occurred in the NPM treatment (Fig. 5). These results represent that OC and N were concentrated in the coarse size fractions and redistributed from the fine size fractions to coarse size fractions during the 27 years of fertilization.

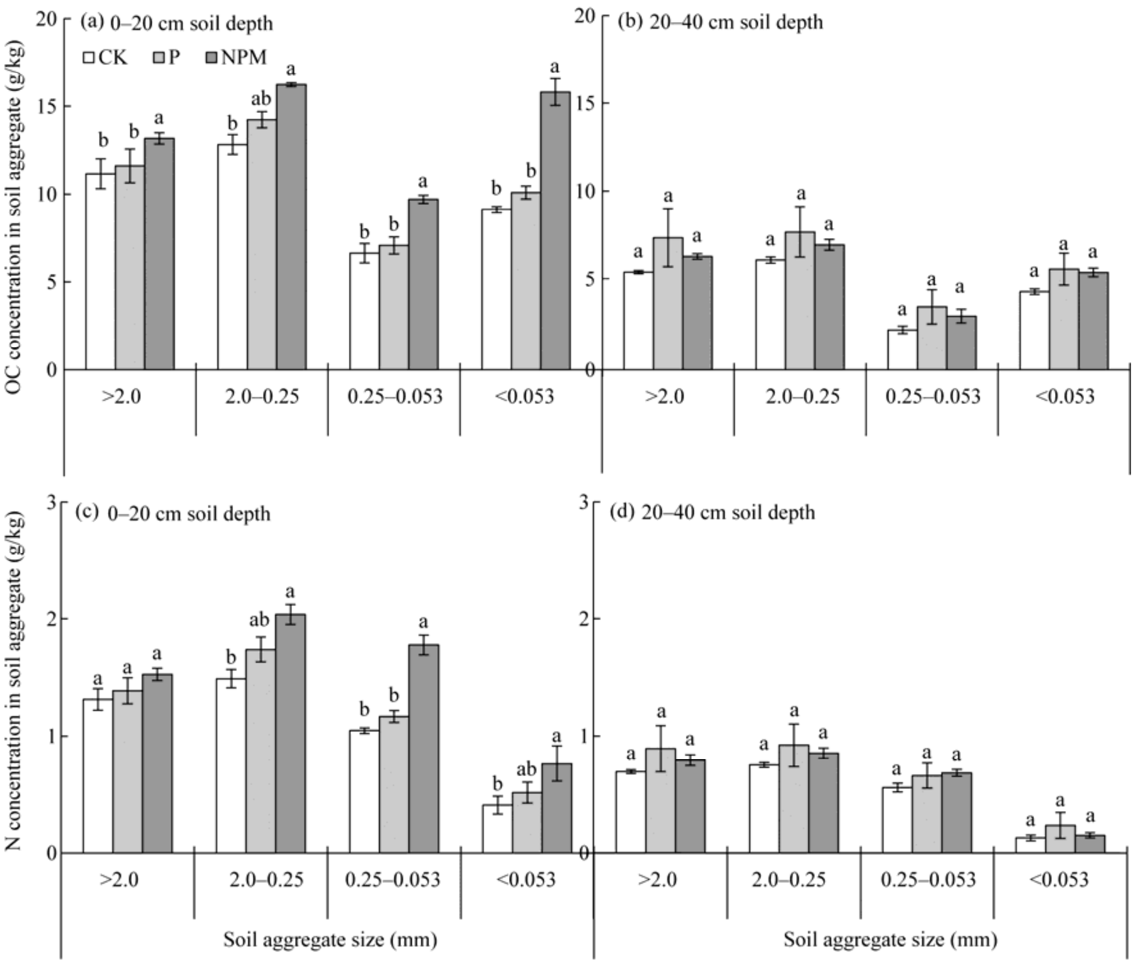

Fig. 4 The effects of fertilization on concentrations of organic carbon (OC) and nitrogen (N) in different soil aggregates. Error bars represent the standard error of the mean (n=3). Means with different lowercase letters within the same aggregate size fraction and soil layer are significantly different (P<0.05).

3 Discussion

3.1 The effects of long-term fertilization on soil aggregate size fractions

We observed a significant influence of long-term fertilization fractions on the distribution of soil aggregate size fractions with NPM fertilization in the 0–20 cm layer and with P fertilization in the 20–40 cm layer. The increase in coarse aggregates and the decrease in fine size aggregates in the 0–20 cm layer as a result of NPM treatment accelerated the integration of fine particles into the coarse elements (Yu et al., 2012). This observation is similar to the results reported by Manna et al. (2006). The P fertilizer was also expected to increase the proportion of coarse size fractions in relation to fine size fractions (Rasool et al., 2008; Lugato et al., 2010); however, tillage of the surface soil each year breaks up the aggregates, thereby decreasing the coarse size fractions and increasing the fine size fractions (Wang et al., 2009). Tillage during the study period may have offset the effects of the P fertilizer on soil aggregation in the surface soil (0–20 cm). On the other hand, the long-term application of P fertilizer can significantly increase the P content in the subsurface soil (20–40 cm). For example, Wei et al. (2006) found that the application of P fertilizers over 18 years increased the available P by 530% at a depth of 20–32 cm at the same site of alfalfa grassland, which could increase the coarse size fractions and decrease the fine size fractions in this layer. For the soils in the 20–40 cm layer were unaffected by tillage, and significant

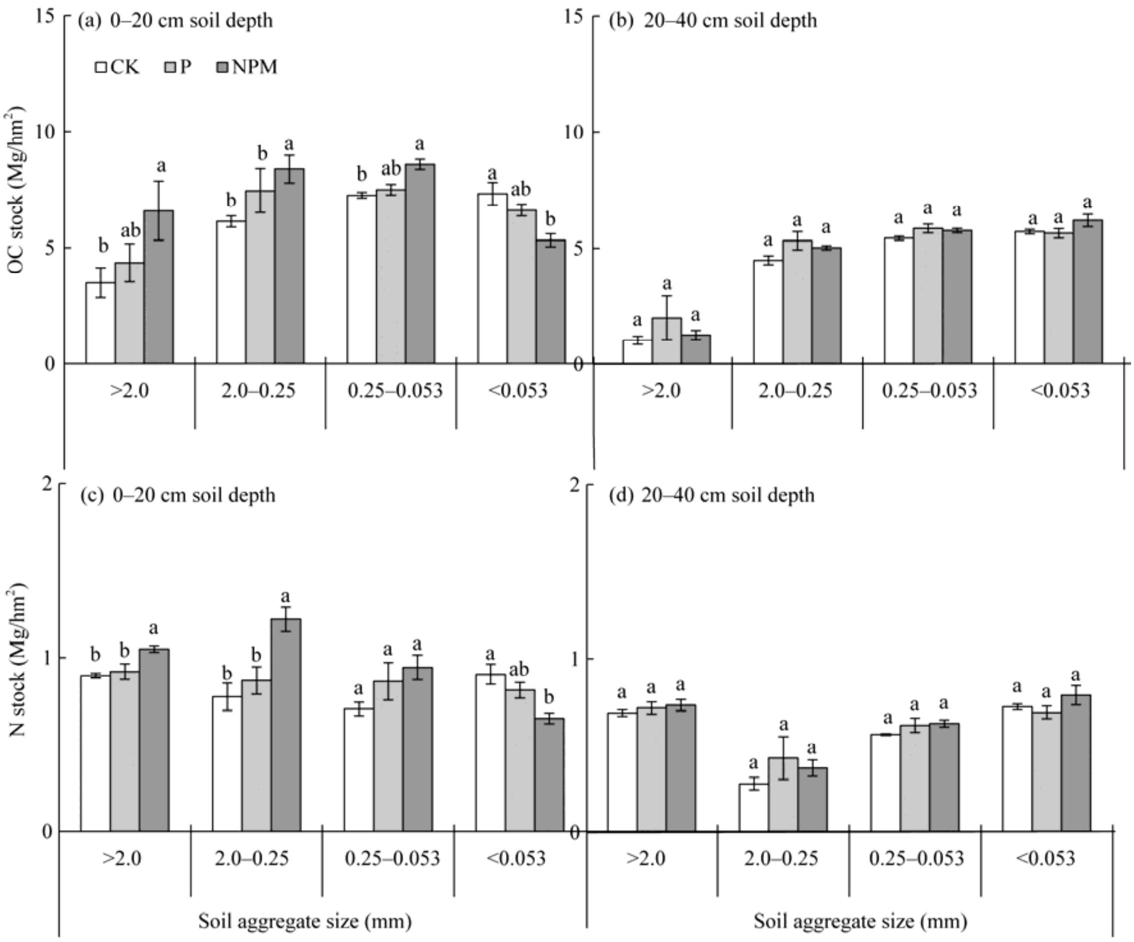

Fig. 5 The effects of fertilization on organic carbon (OC) and nitrogen (N) stocks in different soil aggregate size fractions. Error bars represent the standard error of the mean (n=3). Means with different lowercase letters within the same aggregate size fraction and soil layer are significantly different (P<0.05).

increased coarse size fractions and decreased fine size fractions were found in that layer after the P treatment. Our observations suggested that NPM treatment significantly improved the structure of surface soil and that P treatment improved the structure of subsurface soil at the study site.

The effects of fertilization on the distribution of soil aggregate sizes could also be influenced by the enhancement of plant root biomass, which could accelerate the formation of coarse aggregates (Schuman et al., 2002) and thus increase their mean weight diameter (Elliott, 1986).

3.2 The effects of long-term fertilization on soil OC and N

Fertilization of 27 years resulted in the accumulation of OC and N in the soil aggregate size fractions, though this accumulation varied with fertilizer types and soil depths (Fig. 4). P and NPM treatments increased concentrations of OC and N in all aggregate size fractions.

Our results demonstrated that long-term fertilization led to large increases in OC concentrations and stocks within total soils at the 0–20 cm depth. Soil OC concentrations recorded in 2011 were approximately 2 to 3 times greater than those recorded in 1984. N concentrations in 2011 were approximately 1.4 to 2.0 times greater than those recorded in 1984. The increase in OC associated with the P treatment may be due to the increased input of plant material, additional above- and below-ground biomass and the transport of photosynthetically fixed atmospheric C into the soil (Bronick and Lal, 2005). The increase in OC associated with the NPM treatment, which includes the application of manure fertilizers, may be the result of similar processes. Manure fertilizers increase the proportion of coarse aggregates in topsoil, which could have physically prevented the loss of original OC from the soil (Celik et al., 2004).

In our study, P treatments increased the concentration and total amount of N in the 0–20 cm layer by 21% and 20% (P>0.05), respectively, whereas NPM treatments increased the concentration and total amount of N by 39% and 25% (P<0.05), respectively. However, the annual application of NPM added a total input of 7.2 Mg N/hm^2 into the soil over 27 years. This means that the increase in N in the NPM plot may be attributed to the input of N from the fertilizer rather than from the increased N fixation by alfalfa.

Our results demonstrated that NPM application increased the concentrations of OC and N in aggregate size fractions in the top 20 cm layer, whereas P treatment increased the concentrations of OC and N in aggregate size fractions in the 20–40 cm layer (Fig. 4). Our results further showed that the increases in OC and N concentrations in the coarse size fractions were larger than those in the fine size fractions. These results were consistent with our observation that NPM treatment increased the presence of coarse size fractions and decreased the fine size fractions in the 0–20 cm layer, whereas P treatment had the same effects throughout the 20–40 cm layer. This pattern may be attributed to the often greater organic matter content (and thus C and N) in coarse size fractions compared with fine size fractions (Cambardella and Elliott, 1994).

The accumulation of OC and N in coarse size fractions has been well documented in different soil management systems (Cambardella and Elliott, 1994; Monreal et al., 1995; Six et al., 1998). While the redistribution of OC and N between different aggregate size fractions due to long-term fertilization was not well understood, the integration of fine particles into micro- and macro-aggregates during long-term fertilization led researchers to anticipate this process (Tisdall and Oades, 1982). The increase of macro-aggregates as a result of fertilization (Rasool et al., 2008; Yu et al., 2012) stems from the integration of relatively stable micro-aggregates and the silt+clay particles by plant roots, organic materials and organic binding agents (Onweremadu et al., 2007). Coarse aggregates can physically protect original and recently added OC and N from microbial attack and mineralization (Blanco and Lal, 2004), thereby favoring the accumulation of OC and N in the coarser size fractions. Long-term fertilization thus transfers OC and N from fine size fractions into coarse size fractions and physically protects them. We concluded that the effects of long-term fertilization on soil OC and N was due to the accumulation of OC and N in the coarse size fractions and the redistribution of OC and N from fine size fractions to coarse size fractions.

4 Conclusions

Coarse size fractions are generally highly susceptible to the loss of OC and N from any disturbance when compared with fine size fractions, which are relatively recalcitrant and stable. Additionally, OC and N in deep soils are relatively more stable than those in surface soils. Our results demonstrated NPM treatments lead to the accumulation of OC and N in coarse size fractions and the redistribution of OC and N from fine size fractions to coarse size fractions in the 0–20 cm soil layer. Total 35.8 Mg/hm^2 OC and 7.23 Mg/hm^2 N were added to soils in our experiment through the application of NPM fertilizer annually for 27 years. However, our results showed that the NPM treatments increased soil OC and N by 13.57 and 0.85 Mg/hm^2, respectively, in both soil layers, suggesting that most of the OC and N from the fertilizers were lost. Compared with NPM treatments, P treatments would increase OC and N in coarse size fractions from the subsurface layer (20–40 cm), where OC and N are relatively more stable than in surface soils. NPM and P treatments increased the biomass of alfalfa at the study site. For example, in this experiment, the above-ground biomass of alfalfa over 24 years increased 9% on plots that received the P treatment and 27% on those that received the NPM treatment when compared with the control. Phosphorous fertilization is therefore recommended in the study region.

Acknowledgments

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