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Research Article

Soil Organic Carbon, Nitrogen, and Phosphorus Levels and Stocks After Long-Term Nitrogen Fertilization

The effects of fertilizers and tillage on soil organic carbon (SOC) storage have been tested in many field experiments worldwide. However, conflicting findings have been reported in the literature about the long-term effects of nitrogen (N) fertilization on the profile distribution of SOC, N, and phosphorus (P) concentrations and stocks. To determine the carbon (C), N, and P changes occurring after long-term N fertilization at different levels, SOC, soil total N (TN), soil total P (TP), and soil bulk density were measured in a long-term N fertilization experiment (2004–2013) using two winter wheat cultivars and five N application rates. The findings showed that (i) N fertilization affected the C and N distribution at the 0–120 cm soil depth, whereas the effect on P was mainly in the 0–30 cm soil layer; additionally, C, N, and P levels were positively correlated in the 0–30 cm layer, but the relationship weakened as the soil depth increased. (ii) N fertilization had a large influence on C, N, and P stocks and these influences varied for the different wheat cultivars. Wheat cropping with an N fertilizer rate $<270\,\mathrm{kg\,ha^{-1}}$ resulted in C fixation in the soil after long-term N fertilization, but when N was applied in excess of the crop requirements for the maximum yield, the soil became a C source; moreover, after long-term N fertilization, the stock of N was decreased, and the stock of P was increased.

Keywords: Ecosystems; Fertilizers; Northwest China; Nutrients; Winter wheat

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1 Introduction

Carbon (C), nitrogen (N), and phosphorus (P) are essential elements for plant growth. N and P are the two major elements that limit terrestrial ecosystem productivity and play important roles in limiting or regulating plant growth and development. Thus, the levels of these elements in soil ultimately affect the stability of the ecosystem [1]. Human activities, such as fertilizer use and tillage, have altered global nutrient cycles and caused nutrient enrichment, especially in the N and P levels, in different ecosystems [2, 3]. Due to the strong coupling relationship among C, N, and P in ecological systems, any changes in N and P concentrations substantially affect C fixation in the ecosystem [1]. Traditional research on soil organic carbon (SOC) content has focused on its impact on the productivity and sustainability of agriculture [4]. More recently, with the increase in carbon dioxide (CO_2) in the global atmosphere during the past several decades, the possibility of raising SOC levels to counteract anthropogenic emissions of CO_2 into the atmosphere has been discussed [5]. Thus, studies on changes in SOC, TN, and TP stocks under different fertilizer use and tillage conditions are important. A better understanding of the distribution of C, N, and P and their stocks in the soil might therefore not only help to understand the active region in soil but also aid in the selection of appropriate field management practices.

Fertilizers contribute to soil nutrient levels depleted by crop production and, hence, sustain productivity [6]. The effects of N fertilizer and tillage systems on SOC storage have been tested in several field experiments worldwide [7]. Changes in SOC levels occur over several decades, and many long-term experiments around the world have provided valuable information on SOC dynamics [4]. N fertilizers have been observed to increase SOC levels [8], although their influence is strongly dependent on the management regime [9], soil type and climate [7]. Alvarez [7] concluded that N fertilizers increased SOC only when crop residues were returned to the soil. The classic conventional tillage experiments performed at Rothamsted, England, showed that the addition of animal manure at fairly high rates to long-term arable soils can lead to substantial increases in SOC levels, whereas fertilization with straw removal leads to only a small increase in SOC [10]. However, Jagadamma et al. [11] have reported that N fertilizers significantly increased the stocks of organic C at a soil depth of 0–30 cm. Changes in fertilizer practices also affect soil N, and it has been well documented that >90% of N in the surface soil occurs in organic forms coupled with SOC [12]. However, other studies have reported opposite results. For

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Abbreviations: **CH**, Changhan No. 58; **CK**, control; **SOC**, soil organic carbon; **SON**, stock of TN; **SOP**, stock of TP; **SSOC**, stock of SOC; **TN**, total nitrogen; **TP**, total phosphorus; **ZM**, Zhengmai No. 9023 example, Diovisalvi et al. [13] have reported that after 10 years of application, N fertilization caused no change in soil total nitrogen (TN) in the 0–5 and 0–20 cm soil layers in southeastern Buenos Aires Province, Argentina. Current interest in soil total phosphorus (TP) is primarily focused on P fertilization, which can lead to eutrophication of water bodies through P runoff and leaching [14], although few studies have examined the ecological impact of P addition on terrestrial ecosystems [15]. However, the concentration and stocks of TP are also important, because they can affect plant growth and alter soil properties and microbial activity, which in turn affect SOC and TN. Moreover, the TP distribution in soil can also be influenced by SOC and TN dynamics. In young soils, P is primarily found in the form of stable primary calcium phosphate minerals, which are unlikely to be altered by changes in soil physicochemical conditions over short timescales (seasons) [16]. As soils age, geochemical weathering can release P to the soil solution, where it becomes available to plants and microbes, transfers to surface water, is sorbed to secondary soil minerals, or becomes occluded in soil aggregates [17]. Because TP concentrations and stocks are seldom studied in farmland ecological systems, the effects of N fertilization on P distribution remain uncertain. Although many long-term experiments have examined the effects of fertilization on SOC and TN in soil, they have primarily focused on the surface soil and have rarely studied concentrations below a depth of 100 cm. Moreover, such studies frequently neglect the effects of plant variety or growth.

This study investigated the concentrations and stocks of C, N, and P at depths of 0–200 cm in soil planted with two different water-sensitive cultivars of winter wheat that had received different N fertilization rates since 2004. The objectives of the study were to (i) investigate the effects of long-term N fertilization on the profile distribution of SOC, N, and P concentrations and their relationships under different N application rates in the two cultivars and (ii) evaluate the changes in the cumulative stocks of SOC, TN, and TP after nine years of nitrogen fertilization. The results may shed light on how SOC, TN, and TP change under long-term nitrogen fertilization and provide guidelines for the development of effective strategies for the management and sustainability of farmland ecosystems under nutrient addition.

2 Materials and methods

2.1 Experimental site and climatic conditions

The study was conducted in an experimental field of the Institute of Soil and Water Conservation of Northwest A&F University, Yangling, Shaanxi (34°17′56″N, 108°04′7″E) beginning in October 2004. The experimental site, located on the southern boundary of the Loess Plateau, has 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 occurs 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, and this mode of land use resulted in the development of Lou soil (Eum-Orthic Anthrosols). Selected soil physical and chemical properties recorded in the 0–20 cm layer before fertilization are presented in Table 1.

Table 1. Selected soil physical-chemical properties measured in the 0–20 cm soil layer before fertilization

Property	Value
Taxonomy	Eum-orthic anthrosols
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	8.25
Water-holding capacity (%)	23.6
Available N (mg kg^{-1})	25.10
Available P (mg kg^{-1})	7.90

2.2 Experimental design

The study used a randomized block design and involved ten treatments with three replicates and two winter wheat cultivars: Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH). ZM is a water-sensitive and drought-intolerant cultivar, and CH is a drought-tolerant cultivar that is suitable for growth in drought-prone environments. The thousand-kernel weights of the ZM and CH cultivars were very similar, 43.58 and 43.61 g, respectively. The two cultivars exhibit different water and nutritional absorption capacities based on previous findings [18]. N was applied at five rates: 0, 90, 180, 270, and 360 kg ha^{-1} (termed N0, N90, N180, N270, and N360, respectively). The plots had an area of 2 × 3 m^2, 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. The seeding rate was 130 kg ha^{-1}. 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}). No potassium fertilizer was applied in this study. In addition, the experimental land was plowed to a depth of 20 cm before sowing. During the study, the soil was never irrigated, weeds were regularly removed, and there was no tillage during the growth stage. Three blank plots without fertilization and crops were established as controls (CK), while other field management practices were the same as in the treatment plots.

2.3 Measurements

Wheat was harvested in early June every year by cutting the aboveground parts of the straw while leaving the roots in the soil. After the harvest in 2013, three soil samples were randomly collected from each treatment plot down to a depth of 200 cm and were divided into 15 sections (every 10 cm in the 0–100 cm soil layer and every 20 cm from 100 to 200 cm) using a soil drilling sampler (5 cm inner diameter). The three samples from each plot were mixed to form one measurement sample. In total, 450 soil samples were collected. Each sample was air-dried and stored at room temperature for the determination of soil chemical properties. All samples were sieved through a 2 mm screen, and the roots and other debris were removed. The soil bulk density (g cm^{-3}) of the different soil layers (0–10–20–30–50–70–100 cm) was measured using a soil bulk sampler with a 5 cm diameter and a 5 cm high stainless steel cutting ring ($n = 3$) for each N fertilization plot. The original volume of each soil core and its dry mass after oven-drying at 105°C were measured. Because soil bulk density remains constant under fallow soil layers and plowing was never performed below a 30 cm depth in this study, and it was estimated that the soil bulk density of 100–200 cm layer was the same as that of the 70–100 cm layer. The soil bulk density under each N fertilization level is shown in Table 2. The average

Table 2. Soil bulk density ($g\,m^{-3}$) across soil profile

Soil depth (cm)	CK	N0	N90	N180	N270	N360
0–10	1.250	1.224	1.180	1.180	1.150	1.140
10–20	1.431	1.427	1.410	1.376	1.340	1.315
20–30	1.570	1.560	1.540	1.530	1.410	1.316
30–50	1.624	1.610	1.570	1.550	1.530	1.523
50–70	1.639	1.615	1.576	1.550	1.550	1.528
70–100	1.640	1.620	1.573	1.551	1.549	1.523

wheat grain yield, aboveground biomass for nine years and nutrient content are shown in Table 3.

The SOC content was assayed via dichromate oxidation [19]. The soil TN content was assayed using the Kjeldahl method [20]. The soil TP content was determined after digestion of the soil with $HClO_4$–H_2SO_4 [21]. Each analysis was performed in duplicate.

2.4 Calculation of soil C, N, and P stocks and statistics

For each soil depth interval in each plot, C, N, and P storage was calculated using the following equation:

$$S = \mathrm{EC} \times \mathrm{BD} \times H \times 10^{-1} \qquad (1)$$

where S is the stock of the element ($Mg\,ha^{-1}$), EC is the concentration of the element ($g\,kg^{-1}$), BD is the bulk density ($g\,cm^{-3}$), and H is the thickness of the soil layer (cm).

A 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 test was used to perform multiple comparisons. All statistical analyses were conducted using the software program SPSS, ver. 17.0 (SPSS, Chicago, IL, USA).

3 Results

3.1 SOC, TN, and TP concentrations in the soil profile and their relationships

The SOC concentration varied with the soil depth at all N application rates. The highest SOC concentration was obtained for the 0–10 cm soil depth and then decreased with depth for both wheat cultivars (Fig. 1). From soil depths of 0–30 cm, the SOC concentration decreased significantly, and it then decreased slowly from 30 to 120 cm. However, between 120 and 200 cm, the SOC concentration increased slightly in all treatments, including CK.

As expected, the TN concentration followed a pattern similar to that of the SOC concentration. TN levels differed with both soil depth and fertilizer treatment, ranging from 0.40 to 1.03 $g\,kg^{-1}$ (Fig. 2), and showed a significant decrease between the depths of 0 and 30 cm. For both wheat cultivars, the TN concentration was lower in N0 than in the other N treatments. However, below a soil depth of 30 cm, TN presented statistically similar values across all N application rates and in CK, and there was no significant difference between the two cultivars.

Interestingly, TP showed a different dynamic change from that observed at the SOC and TN levels. At soil depths of 0–200 cm, TP gradually decreased with increasing depth across all treatments and in both wheat cultivars (Fig. 3), and the concentration of P below 120 cm was very low.

As expected, the concentrations of C, N, and P were all significantly correlated in both wheat cultivars. Because of the existence of two turning points in the soil C, N, and P concentrations, the relationship among SOC, TN, and TP was also assessed in the different soil layers (Table 4). SOC was positively correlated with TN with a high correlation coefficient (R). However, differences were also detected. TN explained 87 and 94% of the observed SOC variation for the ZM and CH cultivars, respectively. In addition, TP was found to be positively correlated with both TN and SOC but was not strictly correlated with the TN and SOC levels measured among all N fertilization treatments.

3.2 Stocks of organic C, N, and P under different treatments

In this study, there were two obvious turning points in the measured soil SOC and TN concentrations (Figs. 1 and 2), at depths of 30 and 120 cm, respectively. Therefore, the soil was divided into four parts, consisting of 0–30, 30–120, 120–200, and 0–200 cm layers, to calculate the C, N, and P stocks.

At the 0–30 cm depth, the stock of SOC (SSOC) for the ZM cultivar in N0 (31.5 $Mg\,ha^{-1}$) was significantly higher than in N360 (28.5 $Mg\,ha^{-1}$), whereas there was no significant difference in SSOC between N0 and the other N application rates (Fig. 4). Similarly, for

Table 3. Wheat yields and C, N, and P concentrations in grain and straw in each treatment

Variety	Treatment	Average grain yield ($kg\,ha^{-1}$)	Average aboveground biomass ($kg\,ha^{-1}$)	Average 0–20 cm root biomass ($Mg\,ha^{-1}$)	Aboveground total nitrogen ($kg\,ha^{-1}$)	Aboveground total phosphorus ($kg\,ha^{-1}$)
ZM	N0	4274a	11219a	0.89a	99.6a	3.23a
	N90	6299bc	14574bc	1.19b	133.5b	3.98a
	N180	7131bc	16951bc	1.58c	176.1c	3.12a
	N270	6990bc	14513bc	1.61c	183.6c	3.09a
	N360	7293cd	17628d	1.60c	194.3c	3.11a
CH	N0	4019a	10208a	0.90a	96.6a	3.44a
	N90	5666bc	12946bc	1.04b	127.3b	4.33a
	N180	6558bc	14991bc	1.26b	169.6c	3.45a
	N270	6399bc	15731bc	1.21b	187.9c	3.50a
	N360	7375cd	16499d	1.24b	198.8c	3.56a

Yield and aboveground biomass values are the means of three replicates for each treatment for 2004–2013. Root biomass and TN and TP content are the means of three replicates for each treatment for 2011–2013. Different letters within the same column indicate statistically significant differences at $p < 0.05$.

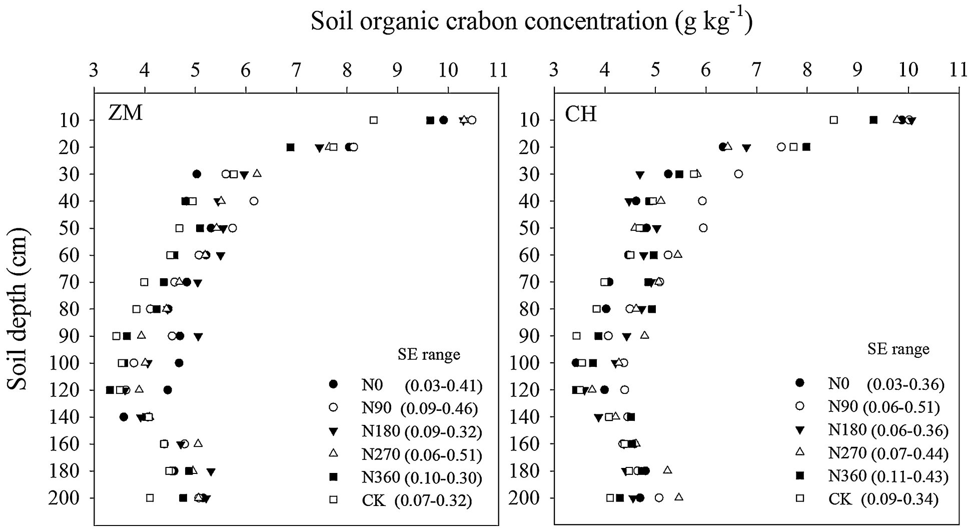

Figure 1. Effect of nitrogen fertilization on the distribution of soil organic carbon concentrations in two wheat cultivars—Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH)—and in the control treatment (CK) across the 0–200 cm soil profile.

the CH cultivar, at the 0–30 cm soil depth, SSOC was lower in N0 (29.3 Mg ha^{-1}) than in N90 (32.6 Mg ha^{-1}) but showed no difference under the other N application rates. In the 30–120 cm sample, the changes in SSOC between N treatments differed in the two wheat cultivars. The SSOC associated with ZM decreased with increasing N application rates. However, CH presented the highest SSOC in the N90 treatment. For the 120–200 cm depth, there was no difference in SSOC levels detected between the two wheat cultivars.

The stock of TN (SON) in the 0–30 cm sample was lower in N360 than in N0 for both cultivars (Fig. 5). In the 30–120 and 120–200 cm depth samples, there was no significant difference in SON across all N application rates, suggesting that this parameter was not affected by N application.

In the 0–30 cm depth samples, the stock of TP (SOP) decreased with increasing N application rates for the ZM cultivar (Fig. 6), whereas for CH, there was no significant difference in SOP associated with increasing N treatment levels. Between depths of 30 and 120 cm, SOP increased with increasing N application and then decreased when the N rate exceeded N180. In the 120–200 cm depth samples, SOP showed no obvious difference. Overall, across the entire measured soil depth of 0–200 cm, SOP increased with increasing levels of N application from N0 to N180.

3.3 Changes in cumulative SOC, TN, and TP stocks in the 0–200 cm soil layer after long-term N fertilization

The cumulative SSOC, SON, and SOP stocks in the 0–200 cm soil layer after nine years of application of different nitrogen fertilization rates were estimated based on a comparison with the stocks in the control treatment (Fig. 7). Overall, after nine years of fertilization and wheat cropping, SSOC was increased, except in the N360 treatments, whereas SON was decreased and SOP was increased in all

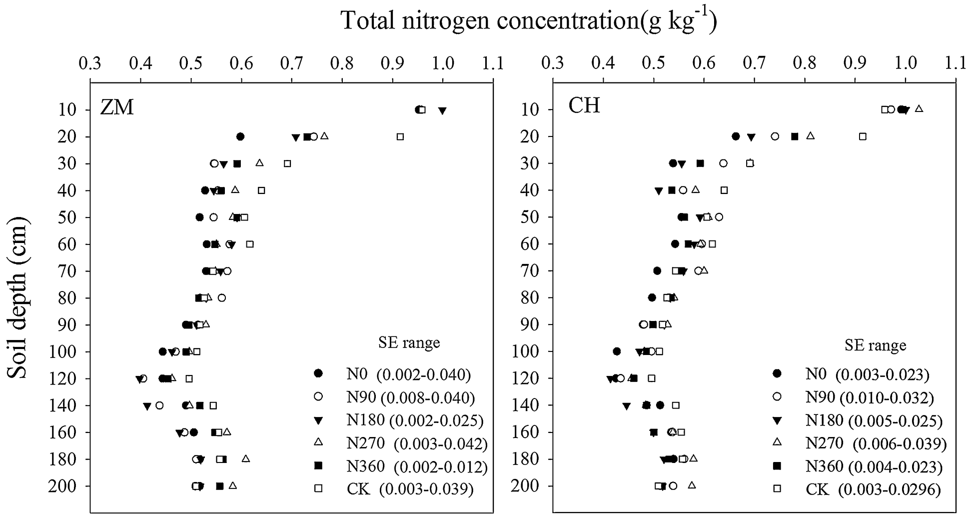

Figure 2. Effect of nitrogen fertilization on the distribution of soil total nitrogen (N) concentrations in two wheat cultivars—Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH)—and in the control treatment (CK) across the 0–200 cm soil profile.

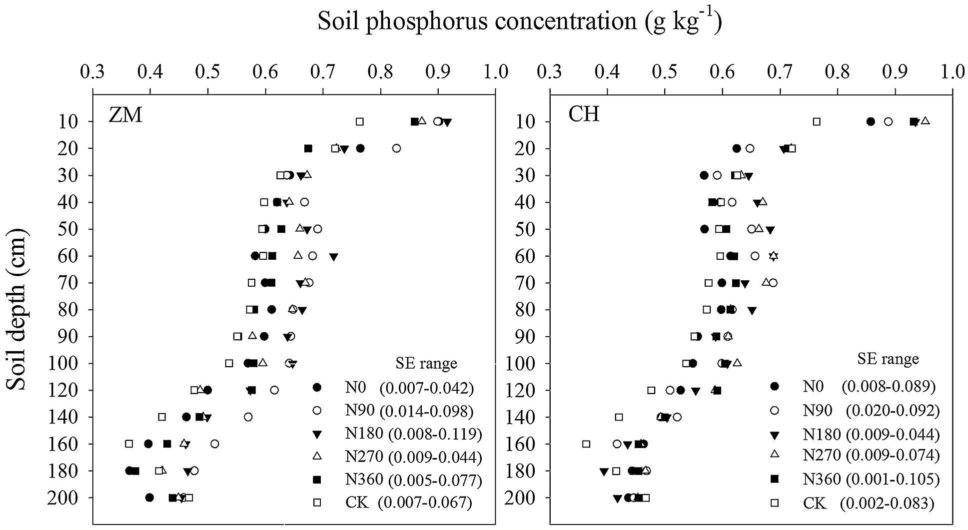

Figure 3. Effect of nitrogen fertilization on the distribution of soil total phosphorus (P) concentrations in two wheat cultivars—Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH)—and in the control treatment (CK) across the 0–200 cm soil profile.

treatments. SSOC showed a decreasing trend in the ZM cultivar as the N fertilizer rate increased, and CH presented a higher stock in the N90 treatment, which reached 13.4 Mg ha^{-1}, although this difference was not significant compared with N0 and N180. The SSOC under ZM and CH decreased by 7.5 and 2.7 Mg ha^{-1}, respectively, compared with CK. SON decreased in all treatments, with the decrease for ZM greater than for CH. In the N360 treatment, both cultivars showed large decreases in SON. SOP increased with an increasing N rate, with the largest stock observed in the N180 treatment for both cultivars, followed by decreases at higher N application rates.

4 Discussion

4.1 Effect of long-term N fertilization on the profile distribution of the SOC, TN, and TP concentrations

In this study, the relationship between SOC levels and N application rates was not linear over a soil depth of 0–30 cm. This result is consistent with the work of Raun et al. [22], who indicated that SOC did not increase when N application excess the optimal amount required by the crop. However, this result differs from the findings of Jagadamma et al. [11], who observed that SOC levels increased with increasing N rates. The main reason for this difference may be that the highest N rate was only 280 kg ha^{-1} in the Jagadamma et al. [11] study. Alvarez [7] concluded that N fertilization increased SOC only if crop residues were returned to soil. Duiker et al. [23] also showed that there was a significant linear relationship between the SOC concentration and the C input from residues and demonstrated that the effect of N fertilizers on SOC levels also depended on the management regime and climate.

Similar to the results for SOC, the TN concentration was highest at the 0–10 cm soil depth across all N application rates and gradually decreased with soil depth, although it showed a slight increase below 120 cm. Several previous studies have reported that SOC and TN increased slightly at soil depths between 50 and 100 cm in semi-arid areas [24–26]. However, in the present study, a slight increase was observed below 120 cm in all N treatments and in CK. This increase may have been caused by the original concentrations of C, N, and P below 120 cm, which were changed little by the cropping and fertilization practices applied here. The roots of the wheat plants occurred primarily from 0 to 120 cm, where they absorbed nutrients and water, leading to changes in SOC and TN in the root zone but causing little change below the 120 cm soil layer.

Interestingly, TP showed dynamic changes differing from those observed for the SOC and TN levels (Fig. 3). The reason for this difference may be that most of the P in soil comes from fertilizers

Table 4. Correlation of SOC, TN, and TP in different soil layers and varieties

		C–N		N–P		C–P	
Soil depth (cm)	Variety	*R*	*p*	*R*	*p*	*R*	*p*
0–30	ZM	0.86	<0.001	0.80	<0.001	0.94	<0.001
	CH	0.92	<0.001	0.90	<0.001	0.83	<0.001
30–120	ZM	0.48	<0.001	0.40	<0.001	0.30	<0.001
	CH	0.71	<0.001	0.60	<0.001	0.30	<0.001
120–200	ZM	NS	–	0.32	<0.001	NS	–
	CH	0.68	<0.001	NS	–	NS	–

R, correlation coefficient; *p*, significance; NS, no significant difference.
Correlation of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) in different soil layers and for two different wheat varieties, Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH).

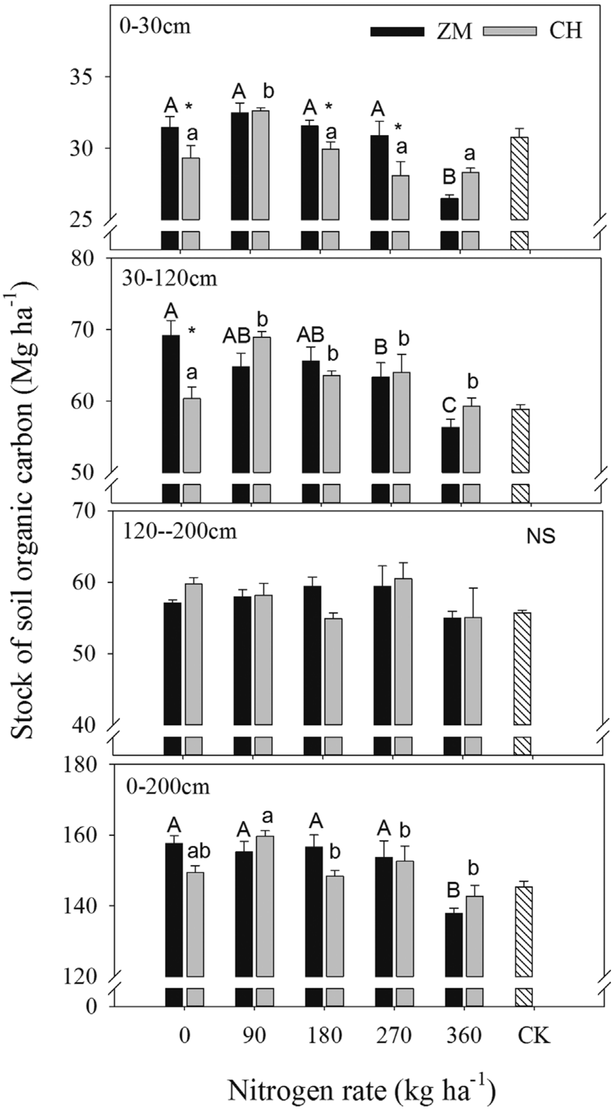

Figure 4. Stocks of soil organic carbon in different soil layers and with varying nitrogen application rates for two wheat cultivars—Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH)—and the control treatment (CK). The values are the mean ± SE. Significant differences between different nitrogen rates are indicated by letters; different letters indicate that there was a significant difference of $p < 0.05$; capital letters are used for ZM, and lowercase letters are used for CH; significant differences between the different varieties are indicated by the symbol *, $p < 0.05$; NS, no significant difference.

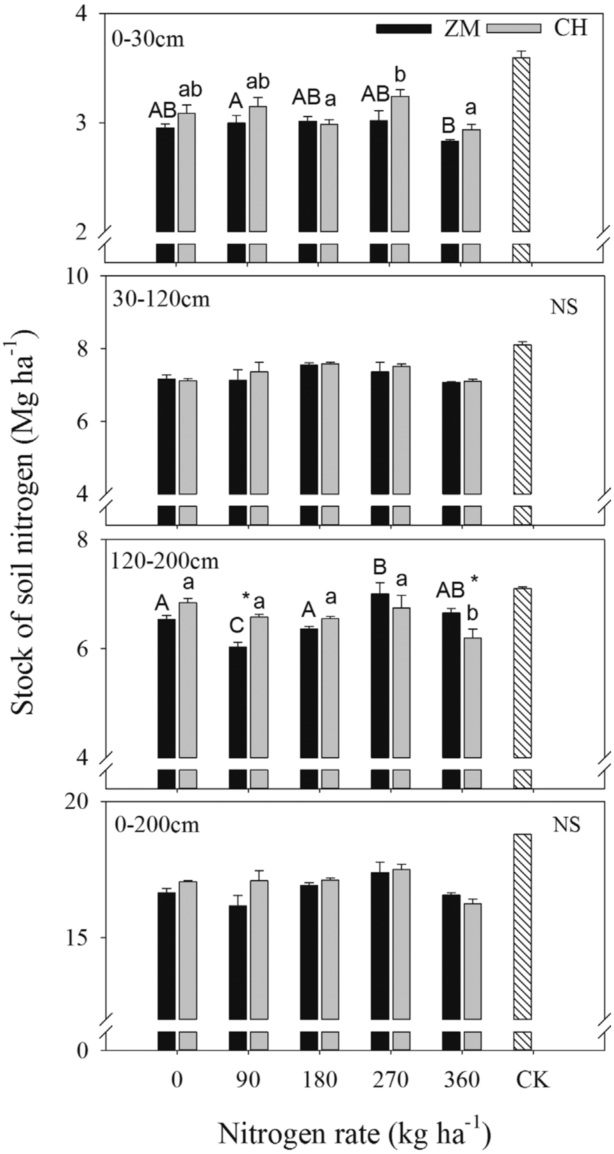

Figure 5. Stocks of total nitrogen in different soil layers and with varying nitrogen application rates for two wheat cultivars—Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH)—and the control treatment (CK). The values are the mean ± SE. Significant differences between different nitrogen rates are indicated by letters; different letters indicate that there was a significant difference of $p < 0.05$; capital letters are used for ZM, and lowercase letters are used for CH; significant differences between the different varieties are indicated by the symbol *, $p < 0.05$; NS, no significant difference.

that are adsorbed or precipitated in soil. In this study, TP did not decrease with increased N application rates. The reason for this result may be that the total amount of P absorbed by wheat did not differ among the N treatments (Table 3). The two wheat cultivars exhibited different P concentrations in soil. This result may be due to the difference in P absorption capacities between ZM and CH. These findings require further studies. Dick et al. [27] reported that soil and vegetation characteristics, species biodiversity, and various other factors can affect TP levels in the soil.

In the 0–30 cm soil layer, there were significant positive correlations among TN, SOC, and TP (Table 4). Zhengchao et al. [28] obtained an *R*-value of 0.88 for the relationship between SOC and total N in the 0–30 cm soil profile following 26 years of treatments on the Loess Plateau. The significant correlation between SOC and TN largely determines the stabilization of soil C/N ratios in the plow layer, as reported by Gál et al. [29]. As soil depth increases, the relationship among the three elements becomes weaker. The relationship between SOC and TN for the ZM cultivar showed a lower *R*-value in the 30–120 cm soil layer and no significant relationship below 120 cm. In contrast, the CH cultivar presented a higher *R*-value in the 30–120 and 120–200 cm layers. This difference may be explained by the physiological differences between the two cultivars. Because CH is a drought-tolerant cultivar and has longer roots than ZM, it can absorb more water from the soil. Hence, the soil region affected by CH is larger than that affected by ZM, as shown by

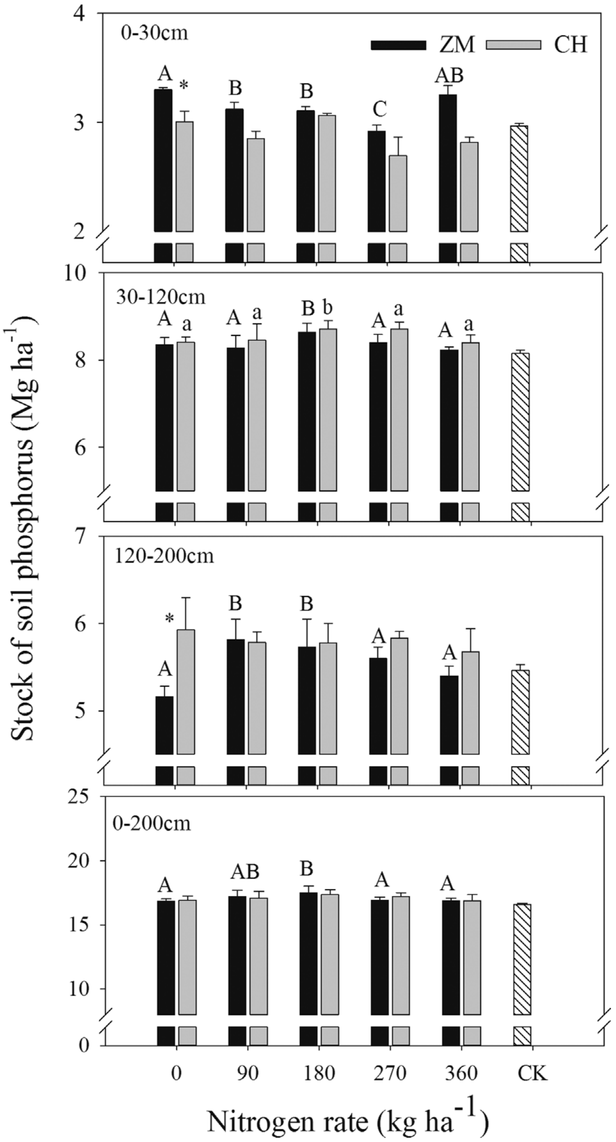

Figure 6. Stocks of total phosphorus in different soil layers and with varying nitrogen application rates for two wheat cultivars—Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH)—and the control treatment (CK). The values are the mean ± SE. Significant differences between different nitrogen rates are indicated by letters; different letters indicate that there was a significant difference of $p < 0.05$; capital letters are used for ZM, and lowercase letters are used for CH; significant differences between the different varieties are indicated by the symbol *, $p < 0.05$; NS, no significant difference.

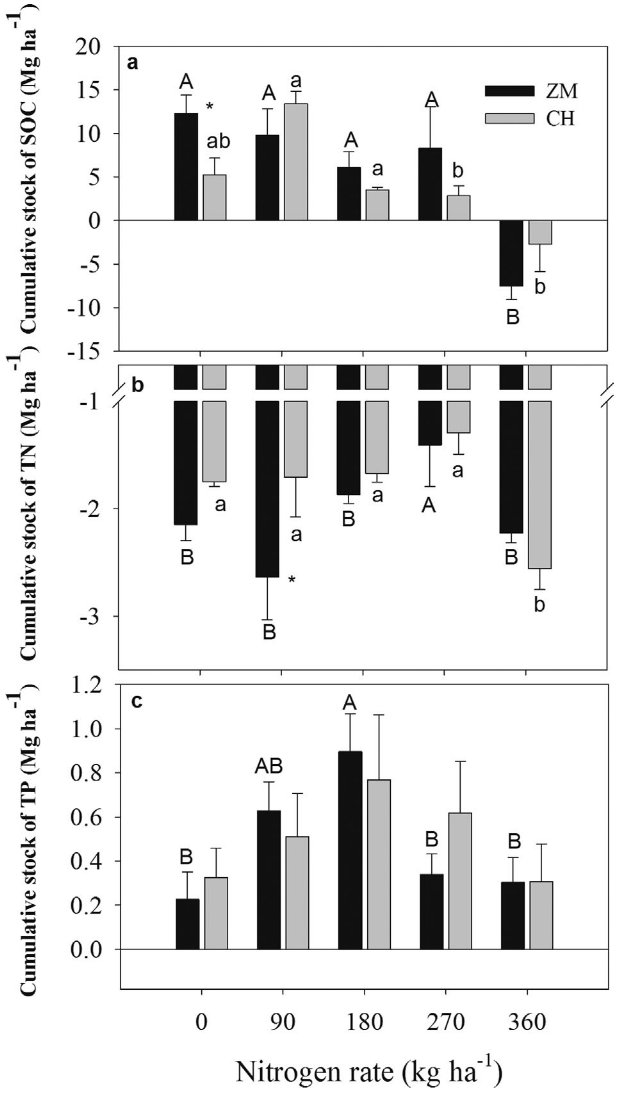

Figure 7. Cumulative stocks of soil organic carbon, total nitrogen, and total phosphorus in the 0–200 cm soil layer compared with CK in the different N treatments and in two cultivars: Zhengmai No. 9023 (ZM) and Changhan No. 58 (CH). The values are the mean ± SE. Significant differences between different nitrogen rates are indicated by letters; different letters indicate that there was a significant difference of $p < 0.05$; capital letters are used for ZM, and lowercase letters are used for CH; significant differences between the different varieties are indicated by the symbol *, $p < 0.05$; the absence of a letter indicates no significant difference.

previous studies [18], resulting in different correlation coefficients between SOC and TN in the CH cultivar and in the ZM cultivar. P exhibited a low *R*-value with C and N in the 30–120 cm soil layer, and no significant relationship was found below the 120 cm soil depth. These results showed that P may be influenced by soil C and N dynamics, but only over a depth of 0–30 cm. The reason for this relationship may be that most P is absorbed or precipitated by the soil and accordingly moves to various depth horizons to a lesser extent than N or C [30]. Zhang et al. [31] reported that the coupling mechanism is influenced not only by the interaction between N and P but also by abiotic factors such as soil temperature, water content, and soil pH. Moreover, N addition leads to soil acidification (data not published), and the volume and timing of N addition are positively correlated with the extent of the decrease in soil pH [32].

4.2 Effects of long-term N fertilization on the stocks of C, N, and P

Several previous studies have shown that the SSOC increases under N fertilizer treatments compared with those without fertilizer application in plow layers [22, 33–35]. The theoretical basis for this result is that N application produces more biomass, thereby increasing the C input. However, in the present study, a different phenomenon could be observed: SSOC in N0 was not significantly lower than in the other N fertilizer treatments at a depth of 0–30 cm

(Fig. 4), and the two cultivars showed no difference except in N360. These results are consistent with the findings of a study by Alvarez [7] that SSOC did not increase without crop residue. The removal of aboveground biomass as cooking fuel and renewable fuel source or for animal feed is a common agricultural practice in the study area. Thus, residues did not remain in this study may be the best explanation for the lack of a significant linear relationship between SSOC and N rates [23]. In the 30–120 cm depth soil samples, the changes in SSOC associated with the N treatments differed between the two wheat cultivars. SSOC under ZM decreased with increasing N application rates, whereas CH exhibited the highest SSOC in the N90 treatment, and both cultivars exhibited a larger stock than CK. This phenomenon indicated that the soil C fixation capacity differs in the two cultivated crops, but its effect is associated with the N fertilization rates. In the 120–200 cm soil layer, there was no difference in SSOC levels between the two wheat cultivars, which indicated that soil C was highly stable between 120 and 200 cm and was affected little by fertilization or the wheat variety. Overall, between 0 and 200 cm soil depths, the changes in SSOC were mainly due to the instability of SOC in the upper soil layers. This instability was caused by biological decomposition and the return of organic residues [4]. However, compared with CK, the cumulative SSOC was increased after nine years of fertilization and cropping except in the N360 treatments (Fig. 7a). This increase occurred mainly because the roots remained in the soil during all study years (Table 3), leading to an increased SSOC in the 30–120 cm soil layers (Fig. 4). However, with increased N fertilization rates, soil respiration also significantly increased, and the N360 treatment had the highest soil respiration as reported by a previous study [36], which may explain the decrease in cumulative SSOC associated with increased N fertilization. Based on a previous study where three years of yield analysis was conducted as part of the same field experiment described here, it could be concluded that the N360 treatment exceeded the requirements of the two wheat cultivars to achieve the maximum yield [18]. When N was applied at rates in excess of those required by the crops for maximum yield, SSOC decreased compared with CK. This finding is consistent with the work of Raun et al. [22]. This phenomenon is mainly caused by microbial activity, as excessive inorganic N remaining in soil leads to an increase in microbial activity, and C emissions caused by respiration are higher than C fixation by wheat. Khan et al. [37] and Mulvaney et al. [2] suggested that N fertilization may enhance the decomposition rate of soil C, and SOC may decline in response to N fertilization. These observations indicate that excessive N fertilization causes farmland carbon sink to become a carbon source. Hence, the findings may provide guidelines regarding the rational application of fertilizers to reduce soil CO_2 efflux in farmland resulting from N fertilizer application, which is important for reducing global CO_2 emissions.

SON in N360 was lower than in N0 in the 0–30 cm sample for both cultivars (Fig. 5), and SON was lower in all N treatments than in CK. This result may have been caused by microbial activity, consistent with the findings of Mulvaney et al. [2], who postulated that an increase in microbial activity was responsible for the depletion of SON. In the 30–120 cm depth samples, there was no significant difference in SON across all tested N application rates, suggesting that this parameter was not affected by N treatment. Similar results were reported by Jagadamma et al. [11], who observed that below a depth of 30 cm, SON was not significantly influenced by N fertilization. In all N treatments, SON was lower than in CK. This result may be attributed to the N absorption capacity during crop growth (Table 3). The applied inorganic N fertilization did not increase the TN level for two reasons: first, inorganic N only accounts for 1–2% of organic nitrogen [38], the increase in inorganic N could not, therefore, increase the TN content; second, the increased amount of N fertilizer and root decomposition could be used by microbes, and microbial function can accelerate the turnover of organic nitrogen in soil. In addition, inorganic nitrogen leaching and nitrogen volatilization from the gaseous phase could cause a decrease in SON [38]. Overall, between soil depths of 0 and 200 cm, there was no difference in the profile of SON among the various N treatments, but this parameter was lower than in the CK treatment (Fig. 7b).

Here, ZM showed differences across N treatments. For CH, however, there was no difference in SOP levels across all applied N treatments. This finding may be a result of the P absorption characteristics of the CH cultivar, as Horst et al. [39] reported that different wheat varieties show different P utilization efficiencies. CH may present a lower P absorption capacity, causing SOP to be influenced only minimally by increased root growth. The P absorption characteristics of the two wheat varieties require further studies. Several researchers have noted that the addition of N reduces the amount of P in soil [40]. In the 0–30 cm depth samples, the SOP decreased with increasing N application rates for the ZM cultivar (Fig. 6), whereas for CH, there was no significant difference in SOP with increasing N treatment levels. Overall, across the entire 0–200 cm soil layer, SOP increased with increasing levels of N application from N0 to N180. The increase in SOP compared with CK in each treatment could be explained by P fertilization applied in every year of the study and the low uptake of P by wheat (Table 3). However, SOP did not always increase as N fertilization increased because when the availability of N increases, plants increase their absorption and utilization of soil P to maintain a stable N/P ratio [41].

The effects of long-term N fertilization with no tillage and without residues on the profile distribution of SOC, N, and P concentrations and stocks and their interrelationships were evaluated for two wheat cultivars. The following conclusions were obtained: (i) N fertilization could affect C and N distribution at the 0–120 cm soil depth, whereas the effect on P was mainly observed in the 0–30 cm soil layer, and C, N, and P levels were positively correlated in the 0–30 cm layer, but this relationship weakened as the soil depth increased. (ii) N fertilization had a large influence on C, N, and P stocks and was affected by the wheat cultivars. Cropping wheat with N fertilization rates under 270 $kg\,ha^{-1}$ could lead to C fixation in soil, and when N was present in excess of the level required by the crop to achieve the maximum yield, the soil became a C source; moreover, after long-term N fertilization, the N stock was decreased and the P stock increased. We suggest that further studies on SOC stocks in farmland should consider soil layers deeper than the surface layer and that crop cultivars, in addition to the P cycle, should also be considered. The results of this study may provide guidelines for farmers in the studied region regarding the choice of an appropriate N application regime to achieve higher soil fertility while becoming more environmentally friendly.

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